Activity of propolis in an experimental model of Pneumocystosis

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ABSTRACT

الأهداف: من أجل التحقق من تأثيرات مضادات تغبر الرئة لوسخ الكوايز على نموذج الجرذ المصاب بتغبر الرئة الكاريني.

الطريقة: أجريت الدراسة على الجرذان بمركز الأبحاث التجريبية والسريرية لجامعة إريسايس – بمدينة كيسيري – تركيا، في يونيو 2007م. من أجل الحصول على التهاب الرئة التلقائي، تم إبقاء الجرذان على علاج كبت المناعة بعقار ديكساميثازون خلال الدراسة. أعطيت الجرذان وسخ الكوايز عبر الفم بجرعات 30mg/kg, 50mg/kg,100mg/kg في اليوم. تم استعمال عقار تريميثوبريم– سولفاميثوكسازول (TMP–SMX 50/250mg/kg) في اليوم كمسيطر إيجابي، وللحيوانات التي لم يتم معالجتها كمسيطر سلبي في هذه الدراسة. كان هنالك ستة حيوانات في كل مجموعة.

النتائج: أظهرت المجموعة التي فيها الحيوانات والتي لم يتم معالجتها الإصابة بتغبر الرئة الكاريني مع كون مستوى الإصابة (± الانحراف المعياري) بلغ عدد الأكياس لكل جرام من نسيج الرئة (4.6±1.6) عند نهاية التجربة. أدى استخدام TMP–SMX 50/250mg/kg في اليوم إلى إنخفاض عدد الأكياس بشكل ملحوظ لكل جرام إلى (1.6±1.8) (p<0.001). لم يتبين وجود تقلص في عدد الأكياس المصابة لدى الحيوانات التي تمت معالجتها بجرعة 30–50-100mg/kg في اليوم من وسخ الكوايز، لذلك لم تكن النتيجة ملحوظة إحصائياً (p>0.05) بالمقارنة مع مجموعة التحكم.

خاتمة: تبين في نموذج الجرذ المصاب بتغبر الرئة، أن كفاءة وسخ الكوايز غير فعالة بالكامل على الرغم من استخدامه في طب الشعوب منذ العصور القديمة.

Objective: To investigate the anti Pneumocystis effects of propolis on *Pneumocystis carinii (P. carinii)* in rat model.

Methods: Rats were obtained, and the study was taken in to place in Erciyes University Clinical and Experimental Research Center, Kayseri, Turkey, in June 2007. In order to obtain spontaneous pneumonia, rats were remained on immunosuppression therapy with dexamethasone throughout the study. Propolis administered orally at doses of 30, 50, and 100 mg/kg/day. Trimethoprim-sulfamethoxazole (TMP-SMX, 50/250 mg/kg/day) was used as positive control and untreated animals as negative control in the study. There were 6 animals in each group.

Results: Untreated animals showed *P. carinii* infection level with a mean (± standard deviation) log number of cysts per gram of lung tissue of 4.6±1.6 at the end of the experiment. Trimethoprim-sulfamethoxazole 50/250 mg/kg/day has significantly reduced the log number of cysts per gram to 1.8±1.6 (p<0.001). There was no reduction found in the number of cysts in infected animals treated with 30, 50, and 100 mg of propolis/kg/day, and so the results were not statistically significant (p>0.05) compared with the control group.

Conclusion: In our rat model of pneumocystosis the efficacy of propolis, this was used in folk medicine since ancient times, found completely ineffective.

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Pneumocystis carinii (*P. carinii*) pneumonia is a life-threatening opportunistic infection of immunocompromised patients, especially malnourished infants, children with immunodeficiency disorders, and patients receiving immunosuppressive therapy with the acquired immunodeficiency syndrome.[1,2] Despite advances in antiretroviral therapy and improved prophylaxis, and a recent decline in acquired immune deficiency syndrome (AIDS) incidence, the rate of human immunodeficiency virus (HIV) infection is increasing, and *P. carinii* pneumonia remains the most common AIDS-defining illness.[3,4] Propolis is a resinous hive product collected by honeybees from plants.[5,6] It has been used in folk medicine since ancient times, due to its many biological properties, such as antimicrobial, antiinflammatory, antioxidant, immunomodulatory activities, among others. In recent years, several studies on the antibacterial, antifungal activity of propolis have been carried out. Although propolis samples of different origins have different compositions, they have similar antimicrobial effects.[7-10] Its antibacterial activity has been well documented but little or nothing is known a both its activity on *P. carinii* pneumonia. The purpose of the study described here was to determine the activity of hydroalcoholic extract of propolis in the corticosteroid-immunosuppressed rat model for *P. carinii* pneumonitis.

Methods. Male Sprague-Dawley rats (weight, 200-250 g) obtained from Hakan Cetinsaya Experimental and Clinical Research Institutions, Kayseri, Turkey were used in this study. Rats were fed regular rodent cow (23% protein), and were immunosuppressed with 2 mg of dexamethasone per liter in the drinking water during 9 weeks. Tetracycline (one mg/lt) was added to the drinking water to minimize bacterial infections.[11,12] All animals remained on immunosuppression therapy with dexamethasone throughout the study. Food and water were supplied ad libitum. Two rats were sacrificed at the initiation of the study to confirm the presence of *P. carinii* pneumonia. The rats were distributed into 5 groups of 6 animals per group in separate cages and labeled. There were 3 propolis derivative groups with different doses (30, 50, and 100 mg/kg/day), one known antipneumocystis agent trimethoprim-sulfamethoxazole (TMP-SMX, 50/250 mg/kg/day) was used as positive control, and no drug administered group called negative control in the study. The drugs to be tested were given by oral gavage on a milligram-per-kilogram basis in a single dose for 10 consecutive days. The rats were observed daily for appearance, activity, and food and water consumption. The protocol of this study was approved by Ethic Committee Faculty of Medicine, University of Erciyes. Turkish poplar type propolis was used in the present study. Propolis sample was collected from honeybee colonies, kept at Erciyes University, The Vocational College of Safiye Cikrikcioglu, Research and Education Apiary in Kayseri. Hand collected propolis was stored in a dark conditions, in a desiccators, until processed. Thirty gram of the propolis sample was kept in 100 ml 70% ethanol at room temperature for a week and ethanol phase evaporated at 50°C. Efficacy was based on a reduction of the organism burden in the lungs rather than survival because the rats sometimes died of causes (for example other opportunistic infections or drug toxicity) unrelated to *P. carinii* pneumonia.

To determine the magnitude of pneumocystosis, after 24 hours from the last dose of the drugs, all animals were sacrificed by an overdose of sodium pentobarbital. The right lung was removed, and cut into small pieces in sterile phosphate-buffered saline solution, and homogenized. Cell debris was removed by filtering the homogenate through sterile gauze. The filtrate was centrifuged at 2,900 x g for 10 minutes, and the pellet was resuspended in phosphate-buffered saline, and stained with cresyl echt violet, which selectively stains the cell wall of the cyst.[1,11,13] The number of cysts was determined by visual assessment under a light microscope (20 microscopic fields). The efficacy of propolis was determined by comparing the *P. carinii* cyst burden of the lungs in the treatment groups with those in the controls. All results expressed as the $\log_{10}$ number of cysts per gram of lung.

The mean log number of cysts per gram of lung in treatment groups were compared with that in the lungs of untreated controls by using the Kruskal-Wallis one way analysis of variance on Rank test (KW). Post-hoc comparisons on parameters were performed using Dunn's procedure. Statistical significance was set at $p<0.05$. All analyses were performed with the statistical package for scientist (SIGMASTAT) Windows version 3.10.

Results. The therapeutic efficacy of propolis was studied in a rat model of pneumocystosis. Corticosteroid-treated rats showed physical signs of *P. carinii* pneumonia (for example loss of weight, cyanosis and so forth). Untreated animals showed *P. carinii* infection level with a mean (± standard deviation) log number of cysts per gram of lung tissue of 4.6±1.6 at the end of the experiment. The therapeutic effect of propolis

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Table 1 - Efficacy of propolis against pneumocystosis in rats.

Compound	Dose (mg/kg/day)	Log_{10} no. of cysts/g of lung	Reduction in log
Control		4.6±0.3*	
TMP-SXT	50/250	1.8±1.6†	2.8
Propolis	30	4.2±0.3*	0.4
Propolis	50	4.2±0.2*	0.4
Propolis	100	4.2±0.1*	0.4
P-value		<0.001	>0.05

*,†: Marked the groups found difference.
TMP-SXT - trimethoprim-sulfamethoxazole

was estimated by the reduction in the number of cysts from the lungs of treated versus untreated rats. Propolis administered at doses of 30, 50, and 100 mg/kg/day by oral gavages at single dose. The TMP-SMX 50/250 mg/kg/ has significantly reduced the log number of cysts per gram to 1.8±1.6. There was significantly difference between TMP-SXT and control group (p<0.001). A reduction in the number of cysts treated with 30, 50, and 100 mg of propolis/kg/day, had no statistically significance when compared with control (p>0.05) (**Table 1**). In rats administered propolis at 30, 50, and 100 mg/kg/day, the reduction in log of cyst level were 0.4 relative to those in control animals.

Discussion. Propolis is a sticky dark-coloured material that honeybees collect from plants, showing a very complex chemical composition.[5] It has been used in folk medicine since ancient times, due to its many biological properties, such as antimicrobial, antiinflammatory, antioxidant, immunomodulatory activities, among others.[6,14,15] Propolis has gained popularity as either an alternative medicine or dietary supplement for health amelioration and diseases prevention in various parts of the world, including United States of America, European Union and Japan.[5] In recent years, several studies on the antibacterial and antifungal activity of propolis was carried out and the results have similarity. Its antifungal and antibacterial activity has been well documented.[7-10,15,16] But there is no study on *P. carinii* pneumonia. *Pneumocystis carinii*, remains an important pathogen for the broad spectrum of immunocompromised individuals, (for example organ transplants, patients with AIDS) despite significant advances in antimicrobial therapy.[3,4] However, treatments for fungal infections are still limited to a few agents. This situation has created a critical need for new approaches on *P. carinii* pneumonia. In order to determine the potential in vivo profile of propolis for the treatment of *P. carinii* pneumonia was evaluated in experimental infection model of pneumonia in immunosuppressed rats. We found that propolis at doses of 30, 50, and 100 mg/kg/day totally ineffective in this model. There was no significantly difference between control and propolis treated groups (p>0.05). Among some investigators, reported that propolis was highly active against fungal species such as *Trichophyton rubrum*, *Trichophyton mentagrophytes*.[7,17] Ghisalberti[18] justifies the antibacterial/antifungal activity of propolis as being due to the presence of poplar compounds, mainly phenols. Recent study of Turkish propolis has shown that its main source is poplar bud exudates.[19,20] Popova et al[19] stated that detailed study of the qualitative chemical differences between sample of poplar (Kayseri, Central Anatolia) and of mixed origin (Adana, Artvin, and Erzurum) was performed by GC-MS and Kayseri sample was confirmed to obtain the typical poplar flavonoid aglycones, phenolic acids, and esters. Koc et al[7] found propolis which collected from Kayseri very active against *Trichophyton rubrum*, *Trichophyton mentagrophytes* in vitro. Investigators also stated that it will be necessary to obtain more clinical data to confirm if this good in vitro efficacy is predictive for clinical outcome. Murad et al[15] studied effects of propolis from Brazil and Bulgaria on fungicidal activity of macrophages against *Paracoccidioides brasiliensis* and they found that an increase in fungicidal activity of peritoneal macrophages from BALB/c mice, by propolis stimulation, independently from its geographic origin. Barros et al[6] studied effect of Brazilian green propolis on experimental gastric ulcers in rats. They found that Brazilian green propolis displays good anti-ulcer activity, corroborating the folk use of propolis preparations, and contributing for its pharmacological validation. The limitation of this study may not to use molecular techniques in detection of *P. carinii*. Even some studies shown effectiveness in vitro and in vivo of propolis we found that on *Pneumocystis carinii* pneumonia propolis is completely ineffective.

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