The effect of the bark water extract *Uncaria tomentosa* on the *Pseudomonas aeruginosa* infection in mice

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Abstract

The influence of water extract of Uncaria tomentosa (Willd.) DC. bark on Pseudomonas aeruginosa infection was studied. The preparation was obtained by extraction of bark Uncaria tomentosa with water (37°C, 24 h) and further fractionation. Inbred C57BL/6 mice 7-9 weeks old, ca 20 g of body mass, females, were fed water extract for 7 days (10, 20 or 100 mg/kg), or water (controls). On the 8th day some mice were infected i.p. with P. aeruginosa strain ATCC. Livers were excised after four hours, homogenized and the numbers of viable bacteria were estimated by plating. After administration of Uncaria water extract a significantly decreased number of bacteria in P. aeruginosa infected mice livers, as compared with the control group, was demonstrated. The inhibitory effect of dose 10 and 20 mg/kg was highly statistically significant but highest dose of extract (100 mg/kg) has not evoked important changes in the number of bacteria.

Key words: *Uncaria tomentosa, Pseudomonas aeruginosa, bacterial infection, mice.*

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Introduction

Uncaria tomentosa (Willdenow ex Roemer and Shultes) DC. also known as "vilcacora", "una de gato" or "cat's claw", is a thorny liana, belonging to the family of *Rubiaceae*, growing in humid tropical forests of Middle and South America. It is one of the most popular Peruvian medicinal plants, and preparations of its bark, leaves or roots have been the basis of the local natural medicine for ages. They are used in therapy of viral infections, cancer, inflammations and many other diseases [1, 2]. It is raising more and more interest worldwide, mainly connected with phytotherapy of cancerous diseases.

According to results of numerous biochemical investigations, *U. tomentosa* is very rich in a set of biologically active compounds. The most important group are alkaloids, which are very numerous in that plant and occur in two groups: indole and oxoindole alkaloids [3, 4]. Their contents and mutual proportions vary depending on the plant organ, season or conditions of culture. Apart of alkaloids, which intensively affect the metabolism of live organisms, *U. tomentosa* was found to contain numerous glycosides of quinovic acid [5, 6], triterpenes (ursolic and oleanolic acid) [7] numerous sterols [8], polyphenolic and uncarine acids [9, 10]. Such wealth of biologically active compounds suggests high phytotherapeutical value of the plant.

Apart from isolation and investigation of chemical contents of *U. tomentosa* extracts, some researches of influence of compounds found in extracts of different parts of the plant on animal and human organisms are carried out. Their results obtained so far indicate that the compounds isolated from the plant accelerate phagocytosis [11], show anti-inflammatory [2], [8], [12-14], antimutagenic [15, 16], antiviral [15] and contraceptive [17] action. It was also shown that the extracts have cytoprotective effect against factors inducing oxidative stress in the organism [18]. They act as

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immunostimulators, which is evidenced by raised production of interleukin-1 and -6 by macrophages of alveoli [19]. However, the most important thing is the anti-proliferative effect of these extracts. They induce delayed-type apoptosis and, depending on concentration, strongly inhibit proliferation *in vitro* of human cancer cells: HL-60 leukemia and lymphoma line (Raji) from B cells transformed with EBV virus [20] and breast cancer cells line [21]. Simultaneously, the same preparation applied to rats increased the leukocytosis *in vivo*, and it stimulated *in vitro* the proliferation of healthy lymphocytes isolated from the animals. It also induced higher leukocytosis in healthy people [16], [22]. Additional research on toxicity carried out on experimental animals showed that *U. tomentosa* extracts are not toxic [16], [23].

Pseudomonas aeruginosa is member of the Gamma Proteobacteria class of Bacteria. It is a Gram-negative, aerobic rod belonging to the bacterial family Pseudomonadaceae. *P. aeruginosa* is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. In fact, *P. aeruginosa* is the epitome of an opportunistic pathogen of humans. The bacterium almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. *P. aeruginosa* infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is near 50 percent. *P. aeruginosa* is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its Gram-negative outer membrane. Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics.

The aim of the present work was evaluation of the effect of water extract of *Uncaria tomentosa* on the course of *Pseudomonas aeruginosa* infection in mice.

Material and Methods

Preparation of the extract

The bark of the *U. tomentosa* used for obtaining preparations originated from Peru and was supplied by A-Z Medica Spółka z o.o. Gdańsk, Poland. The voucher material has been deposited at the Laboratory of Phytochemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland. 10g of powdered bark with 100 mL of distilled water was sonicated (2 × 15 min) in a 320 W sonicator (Elecronic Berlin, Germany). The preparation was obtained by extraction of bark Uncaria tomentosa with water (37°C, 24 h). The fractionation of water extract was done according to the method described by A-Z Medica Sp. z o.o Patent pending 2002. Total content of alkaloids in extract was estimated on the level 0.43% dry weight. The dominant alkaloids were uncarine C and isomitraphylline.

Animals

Studies were performed on inbred C57BL/6 mice 7-9 weeks old, ca 20 g of body mass, females. All experiments were accepted by the local Ethical Committee.

Bacterial infection

18 mice were fed aqueous *Uncaria tomentosa* extract (10, 20 or 100 mg/kg), and 7 mice were fed water (controls), by Eppendorff pipette, for 7 days. On the 8th day some mice were infected intraperitoneally (i.p.) with *P. aeruginosa* strain ATCC. Four hours after administrations of 0.1 ml of bacteria suspension (3×10^7 CFU) the mice were anesthetized with barbiturates and killed by spinal dislocation after which the livers were isolated. The livers were homogenized and the number of viable bacteria was estimated by plating after 24 hours growth on Cetrymide agar (Merck) [24].

Statistical analysis

All values are expressed as the mean ± SD. The statistical analyses were performed with the Student *t*-test and were verified by a one-way ANOVA analysis of variance.

Results

The results are presented on the graph (Fig. 1). A significantly decreased number of bacteria in livers of infected mice fed water extracts of *Uncaria tomentosa* bark was demonstrated in comparison to the control group. The

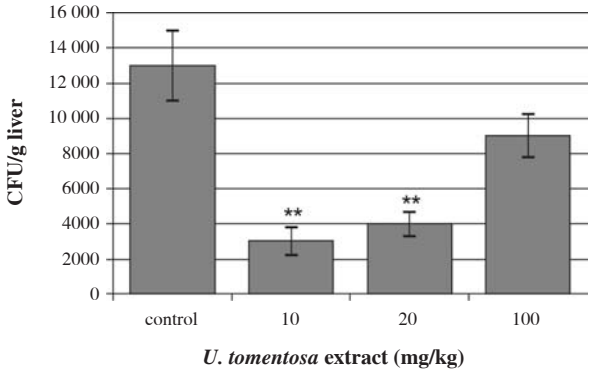

Fig. 1. Number of bacteria in livers of mice fed water extract from *Uncaria tomentosa* bark in various daily doses (mean ± SD). ** $p < 0.001$

inhibitory effect of dose 10 and 20 mg/kg was highly statistically significant. Highest (100 mg/kg) dose of extract has not evoked important changes in the number of bacteria.

Discussion

The water extract of *Uncaria tomentosa* bark is a mixture of many biologically active compounds, therefore it demonstrates many therapeutic properties. The species displayed experimentally immunostimulant, cytotoxic, anti-inflammatory and antioxidant activities [25], [26] and [27]. The discovery of these bioactivities turned the plant into a valuable natural product, leading to commercialization *in natura* or as phytopharmaceutical derivatives [28]. The species contains both oxindole and indole alkaloids, triterpenoid glycosides, sterols and flavonoids, which individually or synergistically contribute to their therapeutic properties. The antimicrobial activity of different concentrations of *Uncaria tomentosa* on different strains of microorganisms isolated from the human oral cavity as *Streptococcus* and *Staphylococcus* was demonstrated. [29]. Isopteropodine (0.3%), a known *Uncaria* pentacyclic oxindol alkaloid exhibited antibacterial activity against Gram positive bacteria [30-31].

In this paper, we present evidence of the beneficial effect of *Uncaria tomentosa* water extract on the bacterial infection in mice. Our results demonstrated that water extract from *U. tomentosa* bark does not directly works on bacteria however it inhibits bacterial infection. That kind of activity is typical for immunomodulating substances. One of the major functions of the immune system is anti-bacterial defence mediated among others by non-specific immunity (macrophages, granulocytes). Similar properties were observed during research on water extracts of *Rhiodiola rosea* [32] which immunostimulating features have been confirmed multiple times before [33]. Moreover the results showed that lower concentrations of the extract exerted most effective inhibitory effect on bacterial developement. Administration of higher dose of extract to the mice did not evoke significant inhibitory effect, what may be connected with activation of some immunosuppressory mechanism by this high dose of *U. tomentosa* extract .

Beneficial effects of *U. tomentosa* extract on animals and human health may be associated with particular combinations of macro- and microelements contained in the bark. It is believed that the great majority of these elements acts as key components of essential enzyme systems and therefore influence all biochemical processes in cells .

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