The *in vitro* effect of *Uncaria tomentosa* water and ethanol extract on the metabolic activity of blood granulocytes in mice

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Abstract

Uncaria tomentosa (una de gato, cat's claw, vilcacora) is a liana belonging to the Rubiaceae family. For hundreds years it has been used by Indians in treating various diseases including cancers. In the present paper we evaluate the in vivo influence of Uncaria tomentosa bark water and ethanol extract on the metabolic activity of blood granulocytes in mice. Mice were fed for 7 days the both of extracts in daily dose 200 μg. The metabolic activity of granulocytes was determined by the measurement of their chemiluminescent activity in scintillation counter, after stimulation by Zymosan. Present results showed that water extract highly stimulated the granulocyte chemiluminescence but ethanol extract slightly decreased the O_2-production and number of leukocytes.

Key words: *Uncaria tomentosa, mice, granulocytes, chemiluminescence.*

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Introduction

In recent years natural preparations of the plant origination have been both more popular and effective. Among them plants originated from rainforests of Middle and South America raise huge interest. It is believed that over 80% of plant species inhabited in mountain areas of Amazon possess medical properties. One of them is *Uncaria tomentosa* (una de gato, cat's claw, vilcacora) of the *Rubiaceae* family [1]. For hundreds years it has been used by Indians in treating various diseases including cancers [1-3]. Wide medical activity of this plant results from many biologically active compounds such as oxindole alkaloids, including pentacyclic (POA, e.g. pteropodine, isopteropodine, speciophylline, uncarine F, mitraphilline, isomitraphilline) and tetracyclic ones (TOA, rhynchophylline, isorhynchophylline) [4]. As shown by Keplinger *et al*., alkaloid content differs in various parts of plant and changes according to seasons of a year [2]. Besides alkaloids many other phytochemicals were identified, including glycosides of quinic acid, triterpenes (ursolic and oleaonlic acids), sterols (stigmasterol and kampesterol), polyphenols, phenolic and catechin tannins and uncaric acids [5-7]. On the basis of different results an extract from *U. tomentosa* was classified as a preparation of antyproliferative or cytostatic [8-10], anti-inflammatory [11, 12], anti-mutagenic [13], antioxidative [14, 15], contraceptive [16], anti-bacterial [17] and anti-viral [2] properties. In addition, preparations of *U. tomentosa* showed immunostimulating activity by increasing rats' leucocytosis *in vivo* and by stimulating proliferation of isolated lymphocytes. It also induced higher leukocytosis in healthy people [18, 19]. It has been shown that *Uncaria* extract would effectively stimulate lymphocyte-dependent immunity [20]. Additional research on toxicity carried out on experimental animals showed that *U. tomentosa* extracts are not toxic [21]. Therefore *U. tomentosa* was legitimated as medicinal plant on the 1st International Conference concerning *U. tomentosa* in Geneva in 1994 year. Due to its wide chemical content and its amazing properties *U. tomentosa* has been more and more popular all over the

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world including Poland. The preparations of *U. tomentosa* have been more widely applied in prevention and treating many diseases e.g. cancers. In this case they are used as a supplement of synthetic medicaments, particularly between radiotherapy and chemotherapy sessions. It was observed that activity of these preparations might work directly, inhibiting cancer cell divisions or indirectly by improving immunology system.

In the present paper we evaluate the *in vivo* influence of *U. tomentosa* bark water and ethanol extract on the metabolic activity of blood granulocytes in mice.

Materials and methods

Preparation of extracts

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.

One gram of the bark was extracted in 10 ml of distilled water or 96% ethanol for 8 h at 37°C. Then, the extracts were centrifuged (MLW K70D) for 15 min at 4000 rpm. Supernatants were evaporated on Speed-Vac and next exsiccated with P2O5. All these extracts were analyzed by HPLC. Content of total alkaloids in different cat's claw preparations expressed in mg/100 g: water extract 430 mg and ethanol extract 3480 mg [22]. Both dry extracts were dissolved in 10% ethyl alcohol before administration to the animals.

Animals

The study was performed on 8-10-weeks old female inbred Balb/c mice, 20–22 g of body mass, delivered from the Polish Academy of Sciences breeding colony. For all experiments animals were handled according to the Polish law on the protection of animals and NIH (National Institutes of Health) standards. All experiments were accepted by the local Ethical Committee (nr 347/2004).

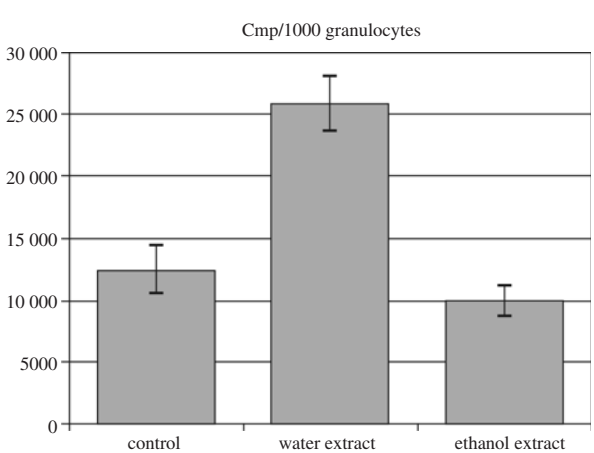

Fig. 1. The effect of feeding mice 200 µg/day *U. tomentosa* water and ethanol extracts on granulocyte chemiluminescence

In vivo experiment

Uncaria extracts were administered to Balb/c mice *per os* in 7 daily doses of 200 µg (each group consisted of 10 mice). These dose corresponded to 100 mg given to 70 kg person (applying the coefficient equal 7 for adjusting differences between mouse and human in relation of the surface to body mass). Mice received drugs by Eppendorff pipette, in 40 µl of 10% ethyl alcohol, for 7 days. Control mice (16 animals) were fed 40 µl of 10% ethyl alcohol. On the day 8th mice were bled in anaesthesia from retro-orbital plexus and sacrificed with Morbital.

Chemiluminescence test

Chemiluminescence test (CL) was measured using the method of Easmon and Cole with some modifications [9, 10] at room temperature, in scintillation counter (RackBeta 1218, KB, Sweden). Briefly: samples of 0.05 ml heparinised blood were diluted 1 : 4 with PBS (Biomed Lublin, Poland) supplemented with 0.1% BSA (Sigma-Aldrich, USA) and 0.1% glucose (Polfa, Poland). Next, 0.05 ml of this diluted blood was mixed with 0.2 ml of luminol (Sigma-Aldrich, USA) solution (10-5 M) in PBS and placed in a scintillation counter in the "out of coincidence" mode for background chemiluminescence measurement. Then, the cells were activated by addition of 0.02 ml solution of opsonised Zymosan (10 mg/ml) and chemiluminescence activity was measured for the next 15 min. Estimating the number of leukocytes and blood smears examination were performed by routine methods and the results were shown as the maximum value of chemiluminescence (cpm) obtained for 10^3 granulocytes.

Statistical analysis

Paired, normally distributed data were analyzed using the t-Student test.

Results

The results from the effect of *U. tomentosa* bark two extract feeding on chemiluminescence activity of mouse blood grnulocytes *in vitro* are presented on the Fig. 1 (granulocyte activity test) and Table 1 (statistical significance of number of blood leukocytes and chemiluminescent activity). As can be seen from the table, water extract highly stimulated granulocyte chemiluminescence but ethanol extract slightly decreased the O_2-production and number of leukocytes.

Table 1. The effect of *U. tomentosa* extracts on chemiluminescent activity and number of blood leukocytes

Kind of tested extract (200 µg/day)	Leukocytes/mm^3 $\bar{x}$ ± SE	Significance	Cmp/1000 granulocytes $\bar{x}$ ± SE	Significance
Control (n = 10)	7610 ±680	–	12 506 ±1916	–
Water extract (n = 10)	7870 ±730	NS	25 948 ±2225	< 0,001
Ethanol extract (n = 10)	5830 ±577	< 0.1	10 005 ±1222	NS

Discussion

Our experiments with chemiluminescence test showed *in vivo* immunostimulatory effect of *U. tomentosa* water extracts on mice granulocytes activity. Granulocytes provide the first line of defence against microbal pathogens and kill several of them. The essential part of killing process is the generation of reactive oxygen species during the oxidative burst. This process leads to the emission of light proportional to free radical quantity-chemiluminescence (CL) [23].

The results obtained show that the water extract highly stimulated granulocyte chemiluminescence whereas ethanol extract gave slightly inhibitory effect. Similar stimulatory properties were observed during research on the influence of extracts of *Rhodiola rosea* and *Rhodiola quadrifida* on mice splenocytes chemokinetic activity [24]. *Uncaria* water extract proved to be a strong immunostimulator of non-specific cellular defence, depending on the first line cells, granulocytes. Earlier experiments performed by us concerned evaluation of the effect of *U. tomentosa* water extract on the course of *Pseudomonas aeruginosa* infection in mice. Our results demonstrated that extract does not directly work on bacteria however it inhibits bacterial infection as an immunomodulating substance [17].

Content of total alkaloids measured by HPLC in both extracts showed that ethanol extract has tenfold more alkaloids then water extract. It was shown that high concentrated extract of these substances did not activate granulocytes. Moreover it has slightly decreased the total number of leukocytes. Besides the above-mentioned alkaloids, over fifty different compounds have been identified, including tannins, pentacyclic triterpenes with a variety of ursolic acid derivatives, quinovic acid glycosides, sterols and procyanidins many of which with strong immunostimulating properties [5].

It might been suggested that daily dose of 100 mg per day of *Uncaria* water extract in human would effectively stimulate granulocyte-dependent immunity. Use of dietary supplements containing *U. tomentosa* bark ethanol extract in the same daily doses might be dangerous because of the potential suppression of the first line defence against microbal pathogens infection.

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