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

JULITA NOWAKOWSKA1, EWA SOMMER2, ANDRZEJ CZUBAJ1, MIECZYSŁAW KURAŚ1, EWA SKOPIŃSKA-RÓŻEWSKA2

1Laboratory of Electron and Confocal Microscopy, Warsaw University, Warsaw, Poland
2Department of Pathology Biostructure Center, Medical University, Warsaw, Poland
3Department of Molecular Plant Physiology, Warsaw University, Warsaw, Poland

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 O₂-production and number of leukocytes.

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

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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).

**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 10⁵ 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 granulocytes *in vitro* are presented on the Fig. 1 (granulocyte activity test) and Table I (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 O₂-production and number of leukocytes.

![Fig. 1. The effect of feeding mice 200 μg/day *U. tomentosa* water and ethanol extracts on granulocyte chemiluminescence](image-url)
The in vitro effect of Uncaria tomentosa water and ethanol extract on the metabolic activity of blood granulocytes in mice

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

<table>
<thead>
<tr>
<th>Kind of tested extract (200 μg/day)</th>
<th>Leukocytes/mm³</th>
<th>Significance</th>
<th>Cmp/1000 granulocytes</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>7610 ±680</td>
<td>–</td>
<td>12 506 ±1916</td>
<td>–</td>
</tr>
<tr>
<td>Water extract (n = 10)</td>
<td>7870 ±730</td>
<td>NS</td>
<td>25 948 ±2225</td>
<td>&lt; 0,001</td>
</tr>
<tr>
<td>Ethanol extract (n = 10)</td>
<td>5830 ±577</td>
<td>&lt; 0.1</td>
<td>10 005 ±1222</td>
<td>NS</td>
</tr>
</tbody>
</table>

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 microbial 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 chemokinetick 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 be 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 microbial pathogens infection.

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References