The effect of *Uncaria tomentosa* bark water extract on chemokinetic activity of spleen lymphocytes in mice

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Abstract

Uncaria tomentosa (Wild.) D.C. also known as "vilcacora", "una de gato", or "cat's claw", is a 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 local natural medicine for ages. This plant displays a large range of bioactive secondary metabolites including tetracyclic and pentacyclic oxindole alkaloids, glycosides, catechins, flavonoids, procyanidins and triterpenes. Organic solvent extracts of Uncaria were shown to have contraceptive, cytostatic, anti-inflammatory, anti-mutagenic, anti-bacterial and anti-viral activities.

The aim of this work was to study the in vivo effect of Uncaria bark water extract on the ex vivo chemokinetic activity of splenic lymphocytes in mice. Mice were fed Uncaria extracts in daily doses 200 or 2000 μg for 7 days. The chemokinetic activity of splenocytes was determined in 24-hour cell cultures in capillary tubes. Both doses of extracts stimulated splenocytes mobility but the lower dose of extract was most effective.

Key words: *Uncaria tomentosa, mice, splenocytes, chemokinesis.*

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Introduction

Uncaria tomentosa (Willd.) DC. of the Rubiaceae is a lignified liana growing in Amazonian countries [1]. It is commonly known as cat's claw, uña de gato or, in Poland, Vilcacora. *Uncaria tomentosa* is one of the most popular Peruvian therapeutic plants used for thousands of years in folk medicine, and preparations obtained from its bark, leaves or roots are basic natural medicines of these countries. Traditionally, these preparations have been used in therapy against virus infection, inflammations and tumors [1, 2].

Among many pharmacologically active compounds which were found in *Uncaria* the oxindole alkaloids, including tetracyclic and pentacyclic ones, seem to be the most noteworthy [3]. The former act mainly on the nervous system, central and peripheric, while the second act mainly on the cells of immunological system, especially those responsible for non-specific and for direct and indirect cell immunity [3-5]. Tetracyclic alkaloids include rynchophylline, isorynchophylline, corynoxeine and isocorynoxeine; pentacyclic ones include isomitraphylline, isopteropodine (Uncarine E), mitraphylline, pteropodine (Uncarine C), speciophylline, and Uncarine F. According to the studies of Reinhard [6] and Keplinger [2] the interaction of tetra- and pentacyclic alkaloids may be antagonistic. Hence, the determination of the content of these alkaloids in the bark of *Uncaria tomentosa* is crucial in defining its therapeutic value.

Besides the above-mentioned alkaloids, there are identified over fifty different compounds have been identified, including tannins, pentacyclic triterpenes with a variety of ursolic acid derivatives, quinovic acid glycosides, sterols and procyanidins [7]. Although tannins present in

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high concentration in cat's claw bark, acting as DNA protector and antioxidants, may have a beneficial effect, in higher doses they may cause stomach upset, renal damage, hepatic necrosis, and increased risk of oesophageal and nasal cancer [8]. Quinovic acid glycosides (3β-*O*-β-D-quinovopyranosyl)-(27-1)-β-D-glucopyranosyl ester and (28)-β-D-glucopyranosyl β-D-glucopyranosyl ester were found as anti-inflammatory, anti-bacterial [9] and anti-viral agents [10, 11]. Ursolic acid was proved to posses very strong anti-proliferative and proapoptotic properties [12-14]. β-sitosterol, campesterol and stigmasterol were shown to have anti-inflammatory and antiarteriosclerotic properties [15], whereas strong antioxidant potency was assigned to proanthocyanidins, the main phenolic phytochemicals identified in cat's claw [16, 17]. The most important thing is the anti-proliferative effect of these extracts. They induce delayed-type apoptosis and, depending on concentration, strongly inhibit proliferation of human cancer cells *in vitro*: HL-60 leukemia and lymphoma line (Raji) from B cells transformed with EBV virus [13] and breast cancer cells line [12]. 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 [18, 19]. Additional research on toxicity carried out on experimental animals showed that *U. tomentosa* extracts are not toxic [18, 20]. Additionally beneficial effects of cat's claw on human health may be associated with particular combinations of macro- and microelements contained in its bark. It is believed that the great majority of these elements act as key components of essential enzyme systems, therefore, influence all biochemical processes in cells [21].

In the present paper we evaluate the *in vivo* influence of *Uncaria tomentosa* bark water extract on chemokinetic activity of mice splenic lymphocytes *in vitro*.

Material and Methods

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. 10 g of powdered bark with 100 mL of distilled water was sonicated (2 × 15 min) in a 320W sonicator (Elecronic Berlin, Germany). 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.

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. *Uncaria* extracts were administered to Balb/c mice *per os* in 7 daily doses of 200 or 2000 μg (each group consisted of 8 mice). These doses corresponded to 100 or 1000 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. Splenocytes were isolated from spleens under sterile conditions by straining through stainless sieve and cotton gauze and centrifugation on Lymphoprep in order to remove erythrocytes.

Spleen cells chemokinesis (spontaneous migration) assay *in vitro* was performed according to the Sandberg method [22] in own modification [23, 24]. Briefly, isolated splenocytes were resuspended in Parker culture medium with 5% inactivated FCS, at the final concentration of 30 × 10^6 cells/ml. Afterwards, siliconized capillary tubes were filled with cell suspension, sealed with plasticine, centrifuged (5 min 450 g) and fixed on the glass plates. Cells levels were marked. After 24 h incubation (37°C, 5% CO_2 humidified atmosphere) the distances of migration were measured in milimeters (mm) at a magnification of 6.5 × and presented as migration units (1 MU = 0.18 mm). Stimulatory indices were calculated by dividing the results obtained for individual splenocytes cultures derived from *Uncaria* fed animals by the mean of the results of accompanying control cultures.

Statistical evaluation of the results was done by Student *t* test.

Table 1. Stimulatory *in vivo* effect of *Uncaria tomentosa* bark water extract on the *in vitro* chemokinetic activity of mouse splenic lymphocytes

	Chemokinetic activity	
***Uncaria* extract dose (μg/mouse)**	**Number of tests**	**Mean migration distance ±SE**
0	**25**	**12.03** ±0.21
200	**25**	**15.27** ±0.34 ***
2000	**25**	**14.11** ±0.44 ***

*** $p < 0.001$.

Results

The results from the effect of *Uncaria tomentosa* bark water extract feeding on chemokinetic activity of mouse spleen lymphocytes *in vitro* are presented on the Table 1.

As can be seen from the table, highly significant stimulation of splenocyte migration in *in vitro* cultures was obtained. Higher dose (2000 μg) was less effective than lower dose (200 μg).

Discussion

Among many therapeutic plants being the object of interest of many scientists the extracts from *Uncaria tomentosa* occupy particular place. The water extracts from this plant have very rich chemical composition and show various biological activity including immunostimulating, antioxidant or antimitotic properties. For example water extract from *Uncaria* bark stimulates alveolar macrophages in lungs to produce interleukin-6 and interleukin-1, which indicates its strong immunostimulating properties [25].

The aim of this study was to evaluate, for the first time, the *in vivo* effect of water extracts obtained from the bark of *Uncaria tomentosa* on chemokinetic activity of mice splenocytes in 24 h tissue cultures *in vitro*. The results obtained show higher *in vitro* migratory activity of splenocytes collected from mice fed lower dose of *Uncaria* extracts. Dose of 2000 μg was not inhibitory, but gave lower stimulatory effect. Similar properties were observed during research on water extracts of *Rhiodiola rosea* [26] and on different plant extracts [23, 27].

Earlier experiments performed by us were evaluation of the effect of water extract of *Uncaria tomentosa* on the course of *Pseudomonas aeruginosa* infection in mice. Our results demonstrated that extract does not directly works on bacteria, however it inhibits bacterial infection as a immunomodulating substance. Moreover the results showed that lower concentration of the exstract has most effective inhibitory effect on bacterial developement than higher [9].

It has been shown that in human, daily dose 100 mg per day of *Uncaria* extract would effectively stimulate lymphocyte-cependent immunity. Use of dietary supplements containing *U. tomentosa* bark water extract in daily doses higher than 1 g, might be dangerous because of the possibility of suppression of some lymphocyte activities.

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