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Uncaria tomentosa (Willd.) DC.—Ethnomedicinal use and new pharmacological, toxicological and botanical results

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Abstract

The medicinal system of the Asháninka Indians in Perú is portrayed. Three categories of medical disorders and healers are recognized. A human is viewed to consist of a physical and a spiritual being who communicate with each other by means of a regulating element. The significance of *Uncaria tomentosa* (Willd.) DC. (Rubiaceae), locally known as uña de gato, in traditional medicine is emphasized by its exclusive use by priests to influence this regulation. Pharmacological and toxicological results obtained with extracts or isolated compounds are summarized. Pentacyclic oxindole alkaloids stimulate endothelial cells in vitro to produce a lymphocyte-proliferation-regulating factor. Tetracyclic oxindole alkaloids act as antagonists. A significant normalization of lymphocyte percentage was observed in vivo although total leucocyte numbers did not change.

Keywords: *Uncaria tomentosa*; Uña de gato; Oxindole alkaloids; Lymphocyte proliferation; Asháninka; Perú

1. Introduction

1.1. General

In Perú approximately 60000 Asháninka Indians are living in the triangle between the rivers Pichis-Palcázu, Ucayáli and Perené-Tambo. In the course of our long-standing interest in their culture (Keplinger, 1993a,b) and their medicinal plants, we gathered information especially on a mighty climbing vine of the rain forest, *Uncaria tomentosa* (Willd.) DC. (Rubiaceae). Because of its curved hooks the Spanish call it 'uña de gato' which translates to English as 'tomcat's claw'. Rumours of miraculous healings evoked scientific

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and commercial interest which can be traced back at least a quarter of a century. By 1997 more than 50 dietary supplement manufacturers in the United States were offering cat's claw products. Therapeutic applications for abscesses, arthritis, asthma, cancer, chemotherapy side-effects, contraception, disease prevention, fevers, gastric ulcers, haemorrhages, inflammations, menstrual irregularity, recovery from child birth, rheumatism, skin impurities, urinary tract inflammation, weakness and wounds have been claimed. Peruvian scientists have pointed out that the two South American *Uncaria* species, *U. tomentosa* and *Uncaria guianensis* (Aubl.) Gmel., are often confused (Lock de Ugaz and Callo, 1991; Obregón-Vilches, 1994). Several books on this subject have been published (Cabieses, 1994; Jones, 1995; Schauss, 1996). Attempts have been made to identify the vine's potentially therapeutic compounds. We have found that there are actually two chemotypes of *U. tomentosa* (Laus et al., 1997), a fact that has been ignored by most manufacturers. Our latest findings have shown target cell-specific effects of the oxindole alkaloids of *U. tomentosa* root. Now we wish to report on the present state of affairs and also summarize here relevant results of other workgroups and two hitherto unpublished toxicological studies.

1.2. Alkaloidal and other constituents

In the first report on its constituents, the leaves and stems of *U. tomentosa* were found to contain rhynchophylline and isorhynchophylline as the major alkaloids, mitraphylline, isomitraphylline, dihydrocorynantheine, hirsutine and hirsuteine, together with their N-oxides. In addition, rotundifoline and isorotundifoline were found as minor alkaloids in one herbarium sample (Hemingway and Phillipson, 1974; Phillipson et al., 1978). The presence of the stereoisomeric alkaloids pteropodine, isopteropodine, speciophylline, uncarine F and isomitraphylline in the bark of 'uña de gato' (either *U. tomentosa* or *U. guianensis*) was reported (Montenegro de Matta et al., 1976). In recent years several analytical HPLC and CE methods were developed (Stuppner et al., 1992a,b and Laus and Keplinger, 1994) which confirmed the presence of six pentacyclic and in exceptional cases, two tetracyclic oxindole alkaloids as major alkaloids in the roots. Lately an allegedly first report on supercritical carbon dioxide extraction of *U. tomentosa* root was published, even though the stereoisomers were neither distinguished nor quantitatively determined by GC/MS and HPLC/MS (Lopez-Avila et al., 1997). Methods for quality control of the alkaloids and flavonoids in the bark by TLC were introduced (van Ginkel, 1996; Laus and Keplinger, 1997). By analysis of a collection of 16 individual plants we established the existence of two chemotypes of *U. tomentosa*, one containing pentacyclic, the other containing tetracyclic indole and oxindole alkaloids in various parts of the plant (Laus et al., 1997). These alkaloids are summarized in Table 1. Eight quinovic acid glycosides, four polyhydroxylated triterpenes, the precursor alkaloid 5α-carboxystrictosidine, oleanolic acid and ursolic acid have been isolated by an Italian research group from the root bark (Cerri et al., 1988; Aquino et al., 1989, 1990 and Aquino et al., 1991). β-Sitosterol, stigmasterol and campesterol were identified in the steroid fraction of an extract of the bark (Senatore et al., 1989).

1.3. Isomerization of oxindole alkaloids

Spiro oxindole alkaloids isomerize in aqueous solutions to give pH-dependent mixtures of iso-

Table 1
Alkaloids of *Uncaria tomentosa* (Wild.) DC.

	Pentacyclic alkaloids	Tetracyclic alkaloids
Oxindole alkaloids	Pteropodine Isopteropodine Speciophylline Uncarine F Mitraphylline Isomitraphylline	Rhynchophylline Isorhynchophylline Corynoxeine Isocorynoxeine
Indole alkaloids	Akuammigine Tetrahydroalstonine Isoajmalicine	Hirsutine Dihydrocorynantheine Hirsuteine Corynantheine

mers. The proposed mechanism involves a retro-Mannich ring opening, rotation and Mannich ring closure. The isomerization is slowed by protonation and decreasing polarity of the solvent. A highly satisfactory logarithmic correlation between the rate coefficients and the Dimroth–Reichardt solvent polarity parameter was obtained. These results support the existence of a zwitterionic intermediate (Laus et al., 1996, Laus, 1998). Of course, this behaviour seriously impedes the evaluation of pharmacological properties of single isomers. For example, speciophylline cannot be used in a week-long test because its concentration drops to 5% of the initial value after only 2 h at 37°C and the other isomers appear instead.

1.4. Enhancement of phagocytosis

Extracts and pure alkaloids were tested using a granulocyte-smear test, a chemoluminescence model and an in vivo carbon-clearance test to evaluate the stimulating effect on the phagocytic activity of granulocytes. Phagocytosis was enhanced by pteropodine, isomitraphylline and isorhynchophylline. The strongest stimulation was observed with isopteropodine whereas mitraphylline and rhynchophylline had no effect. In the in vivo carbon-clearance test, activity was observed only after the admixture of catechin to the otherwise inactive alkaloids (Kreutzkamp, 1984; Wagner et al., 1984 and Wagner et al., 1985).

1.5. Antiviral activity of quinovic acid glycosides

Antiviral activity of six quinovic acid glycosides from *U. tomentosa* was tested against two RNA virus infections (vesicular stomatitis virus and rhinovirus 1B) in CER and HeLa cells, respectively. An inhibitory effect against VSV infection was observed for all six glycosides at MIC_{50} values of 20–60 mg/l. In contrast, only one compound, quinovic acid-β-D-glucopyranosyl-(28 → 1)-β-D-glucopyranosyl ester with an undefined position of the glycosidic linkage, reduced the cytopathic effect of rhinovirus 1B by 50% at 30 mg/l (Aquino et al., 1989).

1.6. Anti-inflammatory activity of a quinovic acid glycoside

Several extracts of *U. tomentosa* root bark and fractions thereof were tested for anti-inflammatory activity using the carrageenan-induced rat paw oedema. A new quinovic acid glycoside was isolated as one of the active principles. Quinovic acid-3-β-O-(β-D-quinovopyranosyl)-(27 → 1)-β-D-glucopyranosyl ester reduced the inflammatory response by 33% at 20 mg/kg p.o. It could not be ruled out that the strong anti-inflammatory effect of the extracts could be due to a combination of compounds (Aquino et al., 1991).

1.7. Mutagenic and antimutagenic activity

The Ames test (*Salmonella*/mammalian microsome test) with and without metabolic activation was used to evaluate the mutagenic potential of extracts of *U. tomentosa*. Antimutagenic activity was studied on photomutagenesis induced by 8-methoxypsoralen and UV-A irradiation in *Salmonella typhimurium*. Extracts and fractions of *U. tomentosa* bark showed no mutagenic effect in several strains of *S. typhimurium* but rather a protective antimutagenic activity in vitro against photomutagenesis. A decoction of *U. tomentosa* ingested daily for 15 days by a smoker decreased the mutagenicity of the subject's urine (Rizzi et al., 1993).

1.8. Antileukaemic activity

Leukaemic HL60 and U-937 cells were incubated with different concentrations of alkaloids for 7 days. The antiproliferative effect was measured by colorimetric and clonogenic assays. The pentacyclic oxindole alkaloids of *U. tomentosa* inhibited the growth of HL60 and U-937 leukemic cells. IC_{50} values were in the range 10^{-5} to 10^{-4} mol/l. The most pronounced effect was found for uncarine F. Selectivity between leukaemic and normal cells was observed (Stuppner et al., 1993).

2. Methods

2.1. The medicinal system of the Asháninka Indians

Women and men from the villages Kiviníki, Shintoriáto, Churingavéni and Pachacutéc in the region of Chanchamáyo and Neváti near Puerto Bermudez, Perú, were independently interviewed about their knowledge on health, illness and healing plants. The Spanish language was used and, in cases of doubt, two Asháninka-Castellano dictionaries (Payne, 1980; Kindberg, 1980) were consulted. Taking notes during an interview was considered by the natives as disturbing. On the other hand, tape recording was accepted only in exchange for payment and thus became viewed as commercially exploitable. It was felt that due to this situation no trustworthy information could be expected. However, an amicable relationship of confidence developed when the interviewer abstained from taking notes. Later records were made from memory. Healers were willing to give away their knowledge, whereas a priest only occasionally came out with information. The correctness of the information gathered was substantiated on nine study trips in the course of 25 years.

2.2. Acute oral toxicity of U. tomentosa extract to mice

The study was conducted at the Huntingdon Research Centre (England). Ten mice of the CFLP strain, five male and five female, in the weight range 20–24 g, were starved overnight before treatment with a freeze dried aqueous root extract (containing 35 mg total pentacyclic oxindole alkaloids per g; 6% yield from crude drug). The extract was prepared as a 40% suspension in aqueous gum tragacanth (0.5%) and administered by oral intubation at a maximum dosage volume of 40 ml/kg bodyweight. Mice treated with aqueous gum tragacanth alone served as controls. During the observation period of 14 days, a record was kept of all mortalities and signs of toxicity. All mice that died were examined macroscopically in an attempt to identify the target organs and those animals surviving terminally were similarly examined to detect possible residual damage (Kynoch and Lloyd, 1975).

2.3. Four-week oral rat toxicity

The study was conducted according to the method recommended in the OECD Guideline 'Repeated dose oral toxicity. Rodent: 28 days study', no. 407 (1981), by Scantox Biological Laboratory (Denmark). An aqueous hydrochloric acid extract of *U. tomentosa* root (containing 7.5 mg total oxindole alkaloids per g and 300 mg sodium chloride per g as remnant from neutralization; 10% yield from crude drug) was given orally to five male and five female SPF Wistar rats in a daily dose of 1000 mg/kg for 28 days. Another five male and five female rats served as controls and were orally given the vehicle, distilled water, 10 ml/kg per day. All signs of ill health and any behavioural changes were recorded daily. A complete haematological examination was carried out before cessation of the treatment. Body weight, food consumption and food conversion ratio were monitored. All rats were subject to complete postmortem examination. The kidneys, liver, adrenals and testes were dissected and weighed. Tissue samples from these organs, from heart and spleen were prepared for microscopical examination (Svendsen and Skydsgaard, 1986).

2.4. Regulation of lymphocyte proliferation by alkaloid-stimulated endothelial cells

The alkaloids were extracted from the root bark by supercritical carbon dioxide and isolated by conventional acid–base work-up and column chromatography. Due to the inevitable isomerization, equilibrated mixtures of alkaloids were employed (aqueous solution, 37°C, 24 h) in order to guarantee a stable and defined composition. These mixtures are comparable to the traditionally used ones because extensive isomerization also occurs in the course of preparing a decoction. Human EA.hy926 endothelial cells (Edgell et al., 1983) were incubated with 10^{-6} mol/l pentacyclic oxindole alkaloids (POA: 28% pteropodine, 57% isopteropodine, 4% speciophylline, 6% uncarine

F, 2% mitraphylline and 3% isomitraphylline) in RPMI-1640 medium (Bio-Whittaker, USA) containing 10% foetal calf serum (Bio-Whittaker), 0.002 mol/l glutamine (PAA-Laboratories, Austria), 50 units/ml penicillin G and 50 μg/ml streptomycin (PAA-Laboratories) for 7 days to produce active supernatants (SN-POA). For comparison tetracyclic oxindole alkaloids (TOA: 67% rhynchophylline and 33% isorhynchophylline) were also used. Supernatants (SN) were filtered and aliquots were stored at -20°C. Normal human umbilical vein endothelial cells (HUVEC, ATCC CRL-1730) were cultivated in HAM F12 medium, completed with 10% foetal calf serum, 60 μg/ml endothelial cell growth supplement (Becton Dickinson) and 100 μg/ml heparin (Calbiochem, USA). Normal B and T lymphocytes (isolated according to Freundlich and Avdalovic, 1983; Indiveri et al., 1980) were cultured at a density of 2×10^6 cells/ml in RPMI-1640, 0.1 ml were used per well in triplicate wells. They were stimulated with different concentrations of the endothelial cell supernatant for 5 days, then pulsed with 1 μCi [^{3}H]thymidine (NEN-Du Pont, Germany) and harvested 18 h later. Epstein-Barr virus-transformed human lymphoblastoid Raji cells (ATCC CCL86), leukaemic Jurkat cells (ATCC E6.1) and normal human B and T lymphoblasts (from peripheral blood or tonsils) were cultured at 10^5 cells/ml, incubated with the supernatant for 2 days, pulsed with 0.5 μCi and harvested 5 h later. [^{3}H]Thymidine uptake was measured using a 2200CA β-scintillation counter (Tri-Carb, Packard, Canberra). Supernatant of untreated endothelial cells (SN-medium) was used as control. Alkaloids alone in medium were also tested to detect any direct effect on proliferation, in this case medium was used as control. Endothelial cells were grown without alkaloids and the alkaloids were added finally to the SN in order to prove that stimulation of the cells by the alkaloids was necessary for the production of activity. In order to exclude the possibility of a cytotoxic effect on lymphoblastoid cells, the viability of the cells was monitored by trypan blue exclusion (Wurm, 1997).

2.5. Increase of lymphocytes in humans

Thirteen individuals with HIV-infection, who refused to receive other therapies, voluntarily took 20 mg per day of a hydrochloric acid extract of *U. tomentosa* root (containing 12 mg total pentacyclic oxindole alkaloids per g and 300 mg sodium chloride per g as remnant from neutralization; 10% yield from crude drug). The test persons were 24–38 years-old (mean 33, S.D. 4), 11 male/2 female, classified according to CDC as follows: 2 A1, 3 A2, 1 A3, 2 B2, 1 C2, 1 C3 and 3 unknown. Results of blood tests were made available to us at the beginning and after 2.2–5.0 months of intake.

3. Results

3.1. The medicinal system of the Asháninka Indians

In the Asháninkas' view a human consists of a physical (ivátsa = his flesh) and a spiritual (isancáne = his deepest) being which come into existence at the time of birth. Both communicate by means of a regulating element (ineatátsiri = he talks to him). Medical disorders can arise from each of these three components and are classified accordingly into obvious physical diseases (catsiaréntsi = acting in the non-dark), hidden psychological complaints (mantsiaréntsi = acting in the dark) and deterioration of the regulation (aparéntsi = degeneration). Interestingly, there are also three levels of healers. For the treatment of simple ailments people called 'anteaviári' (having strong medicine) are consulted. Intellectual leaders and healers at the same time, people called 'seripeári' (taking tobacco) work in the socio-religious and socio-medicinal fields. They include the family or even village of the patient in the cure which can be a behaviour therapy or a taboo. Selected men who keep to a vegetarian diet and celibacy are appointed to 'sancóshi' (knowing the signs) after years of education by their mentors in complete seclusion in the forests. As priests they protect the harmony in the world and in individual humans as advised by the god Pavá. Indeed

the Asháninka expression for 'I am healthy' (nocarátanáje) literally means 'I carry harmony'. During the rainy season illness is often attributed to the intruding of 'irampavanto' (cloud woman) into the villages. In order to undermine the 'mother of the disease' the symptoms are treated with herbal preparations (avintaróntsi = medicine), diet cures, irritation therapy (*Urera baccifera*, Urticaceae; Spanish name: chalanga morada), taboos and rituals. In serious cases the 'mother of the disease' (ina-, e.g. inaporóqui = mother in the face; cause of smallpox) is driven out by medicines poisonous to her (quepearivénqui = poisonous healing plant), e.g. a decoction of *Lonchocarpus* sp. (Fabaceae) bark as a pyretogen. An antibiotic from the dispensary would also be assigned to this category. Psychologically caused disorders are believed to be religious matters. Treatments include socio-psychological measures as well as herbal remedies, e.g. decoctions of *Erythroxylon coca* (Erythroxylaceae) leaves (nocanéshi = my haze-bringer) or the anxiolytic drug *Banisteriopsis caapi* (Malpighiaceae). Anxiety is considered the major disruptive factor in the communication between body and spirit. Preparations of 'powerful plants' (savéntaro) are used to eliminate this disturbance in order to restore health. These plants are believed to be inhabited by good spirits (manincaaríte = living hidden in water). It remains unclear to how many species, if more than one, this title is awarded, but *U. tomentosa* is one of them. It is not regarded as a healing plant because it is attributed to the sphere of religion. Only the 'sancóshi' priests are able to perceive the presence of the good spirits in individual plants of this species. As a consequence it is not found in any register of Peruvian healing plants, but one of the dictionaries (Kindberg, 1980) relates 'savéntaro' to 'uña de gato, espezie de planta espinoza' which clearly is a reference to the *Uncariae*. These results reveal an integrated medicinal system with a strong link between religion and medicine.

3.2. Traditional use

In Perú, a 'sancóshi' was observed boiling approximately 20 g of sliced root bark in 1 l of water for 45 min. The liquid was decanted and losses due to evaporization were replenished. This bitter decoction was said to be a 10-days ration. From HPLC analyses using a previously published method (Laus and Keplinger, 1994) of similarly prepared decoctions the daily dose was estimated at 4 mg oxindole alkaloids. However, the dose is probably adapted to the circumstances.

3.3. Botanical classification and description

The genus *Uncaria* Schreb. belongs to the family *Rubiaceae*, subfamily *Cinchonoideae*. According to the rearrangement of the traditional tribe *Cinchoneae* by Andersson and Persson (1991) which was accepted by Robbrecht (1993), *Uncaria* is grouped within the tribe *Coptosapelteae* and forms together with the closely related Old World tree genus *Mitragyna* Korth. the subtribe *Mitragyninae*. *Uncaria* contains 34 species concentrated in SE Asia, and only three of them occur in Africa and two in tropical America (Ridsdale, 1978). All *Uncaria* species are lianas with monopodial main shoots and more or less horizontally patent, plagiotropic lateral shoots of limited growth; therefore the lianas climb by their divergent spreading shoots. Additionally the gliding back of the lateral shoots from the supporting branches is hindered by thorns (hooks) which are homologous to the hypopodium of an axillary shoot (Troll, 1937, 854–855) and which can be sensitive (hook tendrils) or not. The partial inflorescences are globose pseudo-heads arranged in thyrsic or racemic order on the plagiotropic shoots. Secondary pollen presentation on the styles takes place (Puff et al., 1996). The two American species are listed in group VI in Phillipson et al. (1978) and treated one after another in Ridsdale (1978), suggesting quite a close relationship. But many important differences between the two species are evident in Teppner et al., 1984.

U. guianensis possesses loose buds. The stipules, completely glabrous on the upper side, lie just touching each other at the margins and then separate early in the bud development. The precocious leaf tips (salient tips, drip tips) then take over the bud-covering function (Ellenberg, 1985). The thorns are sickle-shaped to spirally twisted and sensitive. The first order lateral branches of

the inflorescence are simple, not ramified. The flowers have very short pedicels, the fruits on the other hand have long (0.5–1.7 cm) ones. The relatively large sepals (3.5–4 mm long) are coalesced to $^{2}/_{3}$–$^{3}/_{4}$ and the calyx falls off as a whole some time before the fruits are ripe. The 4–5 mm long narrow corolla tube is largely glabrous on the outer side; only the uppermost part together with the conic part and the lobes are very apparently bearded with 1–2 mm long whitish hairs. Hairs and outermost layers of ripe fruits and their stalks are shed off successively. The use of *U. guianensis* in traditional medicine was first mentioned by Ostendorf (1962).

The upper sides of the stipules in the buds of *U. tomentosa* are densely tomentose; the meshing of these hairs (often with curved tips) and the circumgrasping of the longer hairs on the leaf margins help the stipules to be strongly connected to each other along the margins for a long time; they split open just before the next inner pair of stipules has reached nearly the same size. Therefore the leaves exhibit hardly any bud-covering function. The thorns are straight to sickle-shaped, very pungent, and not sensitive. The lateral branches of the inflorescence are ramified. Flowers and fruits are nearly sessile. The minute 0.6–0.8 mm long calyx (with sepals coalesced little more than half their length) persists on the top of the fruit. The narrow part of the corolla tube measures 3.0–3.5 mm, the whole corolla is densely covered with short hairs on the outer side. The hairs on the fruits are evenly dense and persisting. In the fully ripe, dense fruiting heads, the maceration of head axis and stalk makes the opening of the capsules possible.

For general descriptions of the species see e.g. Andersson and Taylor (1994), Steyermark (1974), Dwyer (1980) and Standley and Williams (1975). The distribution of *U. tomentosa* ranges from Belize and Guatemala to Perú, Venezuela, Trinidad and Suriname. *U. tomentosa* is a giant liana of the rainforest canopy and possesses main stems with diameters of up to 20 cm or more. In young secondary forests and on the edges of the forest the plant forms nearly impenetrable thickets. We have observed some infraspecific variability, especially in the leaf form and pubescence, in the size and shape of the thorns, and in the colour of the inner bark in our Peruvian material.

Progeny has been grown from seeds of one *U. tomentosa* plant (Teppner et al., 1984) from 'Jardin el Botanico', La Merced, Perú, for some time in the greenhouse of the Botanical Garden in Graz. In four individuals the chromosome number of $2n = 44$ has been confirmed. The indications of alkaloid content (Phillipson et al., 1978; Lavault et al., 1983) seemed to support affinity of the two species. But our latest results are contradictory in that a number of samples of *U. guianensis* from Perú exhibited alkaloid patterns quite different from *U. tomentosa*.

Thus, we are of the opinion that *U. tomentosa* and *U. guianensis* are not closely related species. It rather seems that two members out of different groups within the genus *Uncaria* have reached or have survived in tropical America. One candidate of the Old World for a closer affinity to *U. tomentosa* can be *U. rhynchophylla* (Miq.) Havil. (Laus and Teppner, 1996) which apparently has the same complex and specialized opening mechanism of the fruits.

3.4. Acute oral toxicity to mice

Signs of reaction to treatment, observed shortly after dosing, consisted of lethargy and piloerection. Death of two out of ten mice occurred within 4 h of treatment. Autopsy revealed haemorrhage of the stomach and intestines, and pallor of the liver and spleen. Recovery of survivors, as judged by external appearance and behaviour, was apparently complete within five days of treatment. This observation was substantiated by normal bodyweight gains, compared with controls (Table 2) and normal autopsy findings. The acute median lethal dose (LD_{50}) to mice of the aqueous extract was found to be greater than 16 g/kg bodyweight (Kynoch and Lloyd, 1975).

3.5. Four week oral rat toxicity

The study has shown that the aqueous acid extract in a daily dose of 1000 mg/kg per day for 28 days caused a slight but statistically significant increase in percentage of lymphocytes and de-

Table 2
Mortality ratio and group mean bodyweight of mice dosed orally with an aqueous extract of *U. tomentosa* root

Sex	Dose (g/kg)	Bodyweight (g) at			Mortality ratio (number of eaths/number dosed)	Time of death after dosing (h)
		Dosing	1 week	2 weeks		
Male	0	23	30	34	0/5	—
Male	16	22	30	33	1/5	<4
Female	0	22	26	29	0/5	—
Female	16	21	23	27	1/5	<4

crease in percentage of neutrophil granulocytes and, in addition, an increase of the relative weight of the kidneys in rats of both sexes (Table 3). Since the histology of the kidneys was normal, there is no explanation for this finding. There were no differences between the test group and the control group with all other respects. There were no mortalities during the study. Thus, a no-effect dose level was not demonstrated by the study. However, from the modesty of the findings, a no-effect level is expected to be close to the dose used (Svendsen and Skydsgaard, 1986).

3.6. Regulation of lymphocyte proliferation by alkaloid-stimulated endothelial cells

Supernatants of EA.hy926 endothelial cell cultures incubated with 10^{-6} mol/l pentacyclic oxindole alkaloids (POA) increase the proliferation of normal resting or weakly activated human B and T lymphocytes. In contrast, the proliferation of B and T lymphoblasts and Raji and Jurkat cell lines is significantly inhibited (Table 4), whereas the viability of the Raji and Jurkat cells was not impaired ($>90\%$ in all cases). The proliferation of the myeloid cell line U-937 was not affected by supernatants of POA-stimulated endothelial cell cultures. Activities produced by POA-stimulated (2×10^{-6} mol/l) HUVEC culture supernatants were somewhat weaker but significant, too. It was found that neither the alkaloids alone nor in combination with a supernatant of untreated endothelial cells exert an effect on the proliferation of lymphocytes. Thus it was shown that the pentacyclic isomers do not affect directly the proliferation but rather induce endothelial cells to release a yet to be identified factor which influences the proliferation of lymphocytes. The secretion of the factor was effected by the pentacyclic alkaloids but not by the tetracyclic alkaloids. Rather it was shown that the tetracyclic alkaloids act antagonistically on the release of the factor. Admixture of 0.01, 0.1 and 1 μM TOA to 1 μM POA (pteropodine isomers as well as mitraphylline isomers) as stimulant reduced the effect of the supernatants on Raji and Jurkat cells in a dose-dependent manner (Table 5) (Wurm, 1997). In the literature, endothelial cells were shown to produce interleukines (Salmi et al., 1995) that activate T cells and act as B cell-differentiation factors. However, these cytokines are not known to regulate the proliferation of lymphoid cells in such an unexpected way. IL-6 was detected in the SNs by ELISA, but its concentration in SN-POA and SN-medium was equal (85 pg/ml, 8 SNs tested). Thus, the endothelial cell-derived lymphocyte-proliferation-regulating factor seems to be novel. The identification of this factor will be the object of future research.

3.7. Increase of lymphocytes in humans

Although the total leucocyte number remained unchanged within the whole collective, it was found that low values (<4000 per μl; 2 of 13) were raised and high values (>9000 per μl; 2 of 13) were lowered. The relative and absolute lymphocyte count increased significantly in the 13 test persons (Table 6). The four cases who were below normal ($<20\%$) were raised above this level. However, no significant changes of T4/T8 cell ratios were observed.

Table 3
Leucocyte values and relative kidney weight in rats (group mean values ± S.D.)

Sex	Dose (g/kg per day)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes(%)	Relative kidney weight (%)
Male	0	12.2 ± 2.5	86.8 ± 2.5	0.6 ± 0.5	0.4 ± 0.5	0.63 ± 0.04
Male	1	8.6 ± 2.1*	90.4 ± 1.7*	0.8 ± 0.4	0.2 ± 0.4	0.70 ± 0.03*
Female	0	15.2 ± 2.5	83.4 ± 2.9	0.8 ± 0.8	0.6 ± 0.5	0.63 ± 0.02
Female	1	10.2 ± 2.4*	88.4 ± 1.1*	1.2 ± 1.6	0.2 ± 0.4	0.65 ± 0.01*

* $P<0.05$ ($n=5$).

4. Discussion

In the light of the frequent blames on phytomedicine, *U. tomentosa* puts up some especially challenging problems. The standardization of extracts and products thereof must take into account the fact that at least two chemotypes of this plant exist. They are distinguished by the Asháninka priests as demonstrated by a 'sancóshi'-guided harvest which yielded exclusively pentacyclic alkaloid-type plants. It still remains a secret how this selectivity was achieved. Chemotypes have been recognized within a number of species, e.g. of *Senecio*, *Papaver* and even *Valeriana officinalis*. The exact conditions of cultivation have to be a subject of further research, or else plants must be selected individually which is impossible for small plants and at least costly in the case of large plants. Moisture and pH of the soil, trace elements, as well as bacteria or fungi living in symbiosis with the respective plant could be points of scrutiny. The question of chemotype is especially important when antagonistic effects are to be expected from the wrong type, as in the case of *U. tomentosa*. This fact has been widely ignored by today's manufacturers who have never asked a native authority. We analyzed some fifty 'uña de gato' or cat's claw products from the Unites States, Central America and Perú and found varying mixtures of pentacyclic and tetracyclic (up to 80% of total) alkaloids. In addition, seasonal changes in oxindole alkaloid content of greenhouse-cultivated *U. tomentosa* (Reinhard, 1997) and related *Mitragyna* species (Shellard and Houghton, 1971 and Shellard and Houghton, 1972) have been observed. Deforestation and terrorism in Perú have added to the problems we encountered during our research activities on *U. tomentosa*. Although the oxindole alkaloids of the root bark surely are not the only active compounds in *U. tomentosa*, our research has focused on them and yielded some very interesting results.

Table 4
Effect on proliferation of different human cells and cell lines by treatment with pentacyclic oxindole alkaloids (POA)-incubated EA.hy936 endothelial cell culture supernatants (SN) (% of control ± S.D.)

Treatment	Dilution	T lymphocytes[a]	B lymphocytes[a]	T blasts[b]	B blasts[b]	Raji CCL86[a]	Jurkat E6.1[a]	U-937 CRL-1593.2[b]
POA	1 μM	98 ± 11	89 ± 17	108 ± 7	99 ± 7	100 ± 4	100 ± 2	101 ± 5
SN-POA	1:2					15 ± 8***	23 ± 16**	95 ± 13
SN-POA	1:4	210 ± 59**	182 ± 87	55 ± 6		28 ± 14***	24 ± 17**	92 ± 7
SN-POA	1:8	237 ± 84***	153 ± 44**	57 ± 30		57 ± 33	22 ± 12**	90 ± 11
SN-POA	1:16	151 ± 47*	143 ± 34	62 ± 39	28 ± 20*			
SN-POA	1:32				45 ± 23			

Significantly different from control (Student t-test for paired samples): * $P<0.01$, ** $P<0.005$, *** $P<0.001$; [a] $n=7$ [b] $n=3$.

Table 5
Proliferation of lymphoblastoid cell lines after treatment with culture supernatants (SN) of EA.hy936 endothelial cells incubated with pentacyclic oxindole alkaloids (POA), tetracyclic oxindole alkaloids (TOA) and mixtures thereof (% of control ± S.D.)

Treatment	Raji CCL86 (POA = pteropodine isomers)	Raji CCL86 (POA = mitraphylline isomers)	Jurkat E6.1 (POA = pteropodine isomers)	Jurkat E6.1 (POA = mitraphylline isomers)
SN-POA(1 μM)	32 ± 3***	36 ± 6***	50 ± 2*	52 ± 2**
SN-[POA(1 μM)+TOA(0.01 μM)]	53 ± 4***	57 ± 1***	78 ± 24	74 ± 4**
SN-[POA(1 μM)+TOA(0.1 μM)]	67 ± 6**	73 ± 3**	83 ± 16	88 ± 3*
SN-[POA(1 μM)+TOA(1 μM)]	82 ± 16	85 ± 16	87 ± 23	89 ± 19
SN-TOA(1 μM)	100 ± 5	99 ± 5	113 ± 11	104 ± 1

Significantly different from control (Student t-test): * $P<0.01$, ** $P<0.005$, *** $P<0.001$; $n=6$.

The finding that extracts showed weaker effects in vivo (rats and humans) than the isolated alkaloids in vitro need not be seen as a disadvantage. In contrary, it can be judged as enabling the development of a mild phytomedicinal preparation which is close to the traditional way of use and can be safely used in long-term therapy whenever a normalization of lymphocyte counts is desired. In summary, the selection of 'manincaaríte'-inhabited *U. tomentosa* by the Asháninka priests has a scientifically sound background: the pentacyclic oxindole alkaloids.

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Table 6
Total leucocytes and percentage of lymphocytes in humans

Patient	Month	Leucocytes per μl	Lymphocytes (%)	Months	Leucocytes per μl	Lymphocytes (%)
1	0	3400	13.0	4.6	4000	29.0
2	0	3800	22.9	2.2	5780	35.8
3	0	4100	28.0	3.6	4800	24.3
4	0	4400	25.9	4.8	4100	37.6
5	0	5500	14.0	4.9	6700	25.1
6	0	5870	19.6	4.8	5690	22.7
7	0	6600	23.1	5.0	6700	47.5
8	0	6800	43.0	3.0	7700	43.0
9	0	7500	22.0	3.0	7650	24.2
10	0	7500	19.1	3.9	6900	39.0
11	0	8300	21.7	4.6	7900	40.0
12	0	9690	28.3	4.5	5700	31.9
13	0	16800	31.9	4.8	13700	37.5
Mean values ± S.D.		6943 ± 3506	24.0 ± 7.8	4.1 ± 0.9	6717 ± 2465	33.7 ± 8.1**

Significantly different from month 0 (t-test for paired samples): ** $P=0.002$ ($n=13$).

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