



Effect of Alkaloid-Free and Alkaloid-Rich preparations from *Uncaria tomentosa* bark on mitotic activity and chromosome morphology evaluated by Allium Test

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ABSTRACT

Ethnopharmacological relevance: *Uncaria tomentosa* (Willd.) DC. is the most popular Peruvian plant, used in folk medicine for different purposes. It contains thousands of active compounds with great content of alkaloids.

Aim of study: Two different fractions of Alkaloid-Rich and Alkaloid-Free were researched on chromosome morphology, mitotic activity and phases indexes.

Materials and methods: Cells of Allium Test (meristematic cells of root tips) were incubated up to 24 h in different concentrations of Alkaloid-Free and Alkaloid-Rich fraction obtained from *Uncaria tomentosa* bark followed by 48 h of postincubation in water. The chromosome morphology was analyzed and the content of mitotic and phase indexes were done. Individual compounds, oxindole alkaloids, phenolic compounds and sugars were determined.

Results: In Alkaloid-Rich and Alkaloid-Free fractions (different in chemical composition) we observed condensation and contraction of chromosomes (more in Alkaloid-Rich) with retardation and/or inhibition of mitoses and changed mitotic phases. Postincubation reversed results in the highest concentration which was lethal (in mostly Alkaloid-Rich fraction).

Conclusions: Our studies indicate that different action can depend on different groups of active compounds in a preparation either containing alkaloids or not. Other fraction analysis may be useful in the future.

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1. Introduction

Uncaria tomentosa (Willd.) DC. of the Rubiaceae is a lignified liana growing in Amazonian countries (Reinhard, 1997). It is commonly known as cat's claw, uña de gato or in Poland – Vilcacora as it is sold here by the Center of 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, tumors (Reinhard, 1997; Keplinger et al., 1999).

Among many pharmacologically active compounds, which were found in *Uncaria*, the oxindole alkaloids, including tetracyclic and pentacyclic ones, seem to be the most noteworthy (Keplinger et al.,

1989). The former act mainly on the nervous system, central and peripheral, while the secondly act mainly on the cells of immunological system, especially those responsible for non-specific and for direct and indirect cell immunities (Keplinger et al., 1989; Stuppner et al., 1993; Wurm et al., 1998; Lemaire et al., 1999). Tetracyclic alkaloids include rynchophylline, isorynchophylline, corynoxine and isocorynoxine; pentacyclic ones include isomitraphylline, isopteropodine (Uncarine E), mitraphylline, pteropodine (Uncarine C), speciophylline, and Uncarine F. According to the studies of Reinhard (1997, 1999) and Keplinger et al. (1999) 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 have been identified over 50 different compounds, including tannins, pentacyclic triterpenes with a variety of ursolic acid derivatives, quinovic acid glycosides, sterols and procyanidins (Wirth and Wagner, 1997; Heitzman et al., 2005). Although tannins, present in high concentration in cat's claw bark, acting as DNA protector and antioxidants,

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may have a beneficial effect, but in higher doses they may cause stomach upset, renal damage, hepatic necrosis, and increased risk of oesophageal and nasal cancer (Mukhtar, 1992; Kemper, 1999). Quinovic acid glycosides (3 β -O-(β -D-quinovopyranosyl)-(27-1)- β -D-glucopyranosyl ester and (28-1)- β -D-glucopyranosyl β -D-glucopyranosyl ester were found as anti-inflammatory and anti-viral agents (Aquino et al., 1989, 1990, 1991). Ursolic acid was proved to possess very strong anti-proliferative and proapoptotic properties (Sheng et al., 1998; Riva et al., 2001; Ya-Ling et al., 2004). β -Sitosterol, campesterol and stigmasterol were shown to have anti-inflammatory and antiarteriosclerotic properties (Falkiewicz and Łukasiak, 2001), whereas strong antioxidant potency was assigned to proanthocyanidins, the main phenolic phytochemicals identified in cat's claw (Desmarchelier et al., 1997; Gonçalves et al., 2005; Pilarski et al., 2006). 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 and therefore influence all biochemical processes in cells (DiSilvestro, 2005).

The main aim of these studies was to identify the antimutagenic activity of *Uncaria* preparations, differing principally in their chemical composition and the effect they have on chromosome morphology in the Allium cell Test. The test was initially used by Levan (1938) in the studies on the effect of plant extracts and various chemical composition on meristematic cells. Up to now, it has been widely used for detection of cytostatic, cytotoxic and mutagenic properties of different compounds, including anticancer drugs of plant origin (Kuraś and Malinowska, 1978; Majewska et al., 2000; Kuraś et al., 2006). The preparations used in these studies were obtained by fractionation of water bark extracts with various organic solvents.

2. Materials and methods

2.1. Raw material and preparation of extracts

The alkaloids standards: Uncarine F, Speciohylline, Mitraphylline, Isomitraphylline, Pteropodine, Isopteropodine were purchased from ChromaDex (USA). The bark of the *Uncaria tomentosa* used to obtain the extract was recommended by the Center of Vilcacora, Lomianki near Warsaw. Our preparations, obtained from this extract (Alkaloid-Rich and Alkaloid-Free), originated from Peru were supplied by the Center of Vilcacora, Poland. The voucher material was deposited at the Laboratory of Phytochemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland. Powdered bark (10 g) with 100 mL of distilled water was sonicated (2 \times 15 min) in a 320 W sonicator (Electronic Berlin, Germany). After centrifugation, the water extract was fractionated according to the method described in Patent Pending (2002). The steps for preparation of Alkaloid-Free (AFP) and Alkaloid-Rich (ARP) preparations are presented in Fig. 1. The dry mass of ARP was redissolved in DMSO (0.2 g/400 μ L) and adjusted with H₂O to 1.125 mg/mL (final concentration DMSO was 0.4%). The final concentration of DMSO (0.4%) did not cause structural changes in the cell (Rowinsky et al., 1988; Majewska et al., 2000). The residue from evaporation of the water layer (AFP) was dissolved in distilled water to a concentration of 65 mg/mL. Both solutions were used in biological tests as stock solutions.

2.2. Quantitative and qualitative determination of oxindole alkaloids in AFP and ARP by an HPLC method

The bark water extract of *Uncaria tomentosa* was used for AFP and ARP. Preparations were analyzed for oxindole alkaloid content

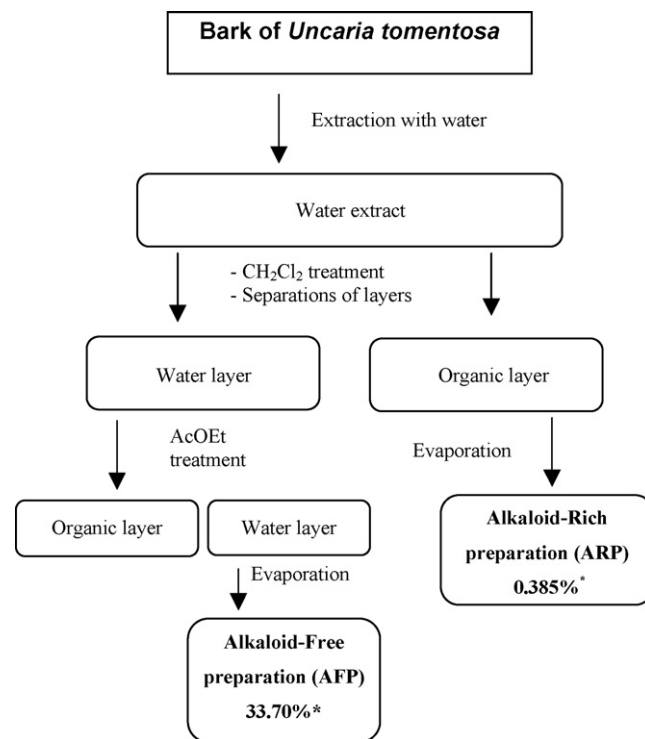


Fig. 1. The model scheme of Alkaloid-Rich preparation (ARP) and Alkaloid-Free preparation (AFP) (Patent Pending, 2002). *Yield of preparation according to the amount of start material (water extract).

according to the widely applied cat's claw standardization protocols (Stuppper et al., 1992; Sheng et al., 2000). Sulphuric acid (2% aqueous solution, 15 mL) was added to 100 mg of the analyzed material and this solution was sonicated for 15 min (Bandelin Sonorex RK 103H). Next, the mixture was centrifuged at 3000 rpm for 10 min and extracted three times with 10 mL portions of ethylacetate. The aqueous phase was separated and adjusted to pH 10 with 10% of NH₄OH then extracted three times with 10 mL of ethylacetate each. The organic extracts were combined, evaporated to dryness and the residue was dissolved in 1 mL of methanol. The alkaloid contents were determined by the HPLC fingerprint analysis [HPLC: L-7100 Intelligent Pump (Merck-Hitachi), L-7200 Autosampler (Merck-Hitachi), L-7450 Diode Array Detector (Merck-Hitachi), Software: D-7000 Chromatography Data Station Software ver. 4.0; Column: LiChrospher® 100 RP-18 (250 mm \times 4 mm, Merck); Precolumn: LiChrospher® 100 RP-18 (4 mm \times 4 mm, Merck); solvents: A, phosphate buffer solution (10 mM, pH 6.6); B, methanol:acetonitrile (1:1); gradient: (60% A and 40% B) to (30% A and 70% B); time: 30 min; washing: 20% solvent A and 80% solvent B; temp: 21 °C; flow rate: 1.0 mL/min; detection: 245 nm].

2.3. Determination of compounds in preparations by GC/MS method

Samples of AFP and ARP were subjected to GC/MS analyses on a Hewlett-Packard gas chromatograph Model 5890/II with a mass-selective detector Model 5971A (Hewlett-Packard Co., Palo Alto Ca, USA). The instrument was equipped with a DB5 fused silica capillary column (30 m \times 0.25 mm i.d.). The carrier gas was helium at a flow-rate of 1 mL/min. The column temperature was programmed from 180 °C (held for 2 min) it was increased to 300 °C at 5 °C/min, and maintained for 10 min. The injector temperature was 250 °C. Mass spectra were recorded in the range of 50–650 amu. Individual compounds of the preparations were identified by comparison

of retention times or mass spectra of standard compounds. Qualitative determination was done by HP ChemStation software. All analyses were done at least in duplicate.

2.4. Thin layer chromatography (TLC)

The analysis of phenolic compounds and sugars was done by thin layer chromatography. For TLC aluminium sheets 20 cm × 20 cm (Silica gel 60 F₂₅₄ Merck) were used. In the case of phenolic compounds, the following mobile phases were used: AcOEt:MeOH:H₂O:NH₃ (12:3:3:0.5) and isopropanol:MeOH:H₂O (5:2:3); while for sugar analysis: isopropanol:AcOEt:H₂O (5:2:3). For staining of phenolic compounds, Gibbs reagent was used, whereas sugars were detected by naphthoresorcine (Stahl and Kaltenbach, 1962; Svobodová et al., 1977).

2.5. *Allium cepa* Test

Adventitious root tips of onion *Allium cepa* L., var. Dawidowska were used for the investigation as previously described (Kuraś and Malinowska, 1978; Keightley et al., 1996; Majewska et al., 2000; Kuraś et al., 2006). The roots were grown in distilled water in 200 mL Erlenmeyer flasks under laboratory conditions (on light, 24 °C). After reaching a length of 3 cm (±0.5 cm), roots were incubated in the AFP at 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/mL concentrations. In the case of ARP, the roots were treated with 0.0175, 0.035, 0.07, 0.14, 0.28, and 0.56 mg/mL concentrations. During the 24 h incubation, the root tips were collected after 3, 6, 12 and 24 h. Then the root residues were rinsed several times, and kept in pure water for postincubations. The postincubation roots were collected after 3, 6, 12, 24 or 48 h. In order to study the effect of different preparations from bark of *Uncaria tomentosa* on the mitotic activity and phase index of cells *Allium* Test during the experiment, the 2–3 mm root tips were cut off, stained and macerated in 2% dilution of aceto-orceine with 1N HCl addition to prepare for squash preparations on a microscope slide. The mitotic and phase indexes were counted according to the Lopez-Saez and Fernandez-Gomez (1965) method. For each variant of the experiment, five roots of three analogous onions were taken. Average results and standard deviation were presented on charts. Chromosome morphology and their changes were photographed under a light microscope (NU Zeiss), using a photographic camera (Nikon). A parallel control treatment in distilled water was employed as previously described (Podbielkowska et al., 1994, 1995, 1996; Keightley et al., 1996).

2.6. Statistical analysis

The analyses of control roots were carried out at the same time, and the values at the beginning of our experiment were taken as 100%. The *n* values are the number of different onions studied. All values are expressed as the mean ± S.D. The statistical analyses were performed with the Student's *t*-test.

3. Results

3.1. The chemical composition of the studied preparations

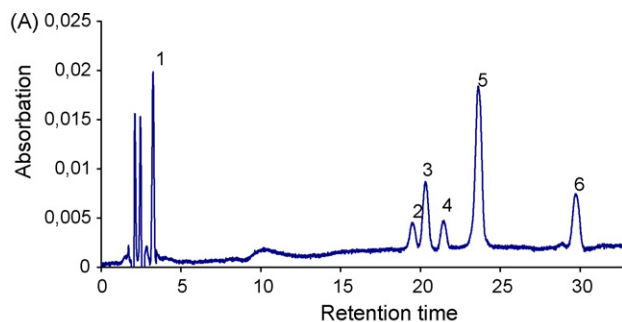
During treatment of water bark extract with organic solvents (Fig. 1) different preparations were obtained. These, an object of our studies (marked in Fig. 1 with thicken frame), differed distinctly in their chemical composition (Tables 1 and 2). As shown in Fig. 2 the bark of *Uncaria tomentosa* contained pentacyclic oxindole alkaloids. Among the analyzed alkaloids the highest percentage contribution was shown by isomitraphylline and pteropodine, the lowest one by mitraphylline and Uncarine F (9.24 and 9.26%, respectively).

Table 1
Compounds identified in ARP and AFP.

Preparation	Compounds identified/instrumental method applied
ARP	Compounds identified with HPLC/TLC: Alkaloids: Uncarine F, Speciohylline Mitraphylline, Isomitraphylline, Uncarine C, Uncarine E Compounds identified with GC/MS: Hydroxybenzoic acid, 4-hydroxy 3 methoxy-benzoic acid, di-methoxy- <i>p</i> -hydroxybenzoic acid, mono- and disugars, linoleic acid (di-unsaturated acid), oleic acid (unsaturated acid), stearic acid (saturated acid)
AFP	Compounds identified with HPLC/TLC: Tannins Compounds identified with GC/MS: Mono- and disugars, other proanthocyanidins

Table 2
Contents of particular alkaloids in ARP expressed in mg/100 g dry weight and their percentage contribution.

Alkaloids	ARP	
	mg/100 g	Percentage contribution
Uncarine F	2,712	5.38
Speciohylline	10,937	21.7
Mitraphylline	8,014	15.9
Pteropodine and isomitraphylline	19,303	38.3
Isopteropodine	9,435	18.72
Total	50,401	100



Peak no.	Compound	Bark	
		mg/100g	%
1	Caffeine*	-	-
2	Uncarine F	67.16	9.26
3	Speciohylline	115.25	15.89
4	Mitraphylline	67.01	9.24
5	Isomitraphylline/Pteropodine	371.39	51.21
6	Isopteropodine	104.44	14.40
2-6	Total	725.25	100

Fig. 2. The oxindole-alkaloid profile of the bark (A) HPLC-chromatograms (B) content of alkaloids expressed in mg/100 g dry weight and percentage contribution. *Internal standard.

The main compounds of ARP (Table 1) were pteropodine and isomitrphylline (pentacyclic oxindole alkaloids) whose percentage contribution was ca. 40% (Table 2). Two other compounds identified beside the undefined ones found in this preparation belonged to the derivatives of benzoic mono- and disugars, linoleic acid, oleic acid, stearic acid (Table 1). The AFP was very poor in relation to its chemical composition. It did not contain alkaloids but its main components were tannins, proanthocyanidins and benzoic mono- and disugars (Table 1). No saponins or coumarins were looked for.

3.2. Changes of morphology and chromosome structure in cells of the *Allium Test* after incubation with ARP and AFP followed by postincubation in water

During mitotic division in the control (Fig. 3A–E), particular phases may be distinguished by taking into account the condensation grade of chromatin. The first one was prophase, in which gradual condensation of chromatin strands proceeds. Chromosomes, primarily those maximally despiralized, appeared in the form of a tangled mass inside a nucleus, and they next began to

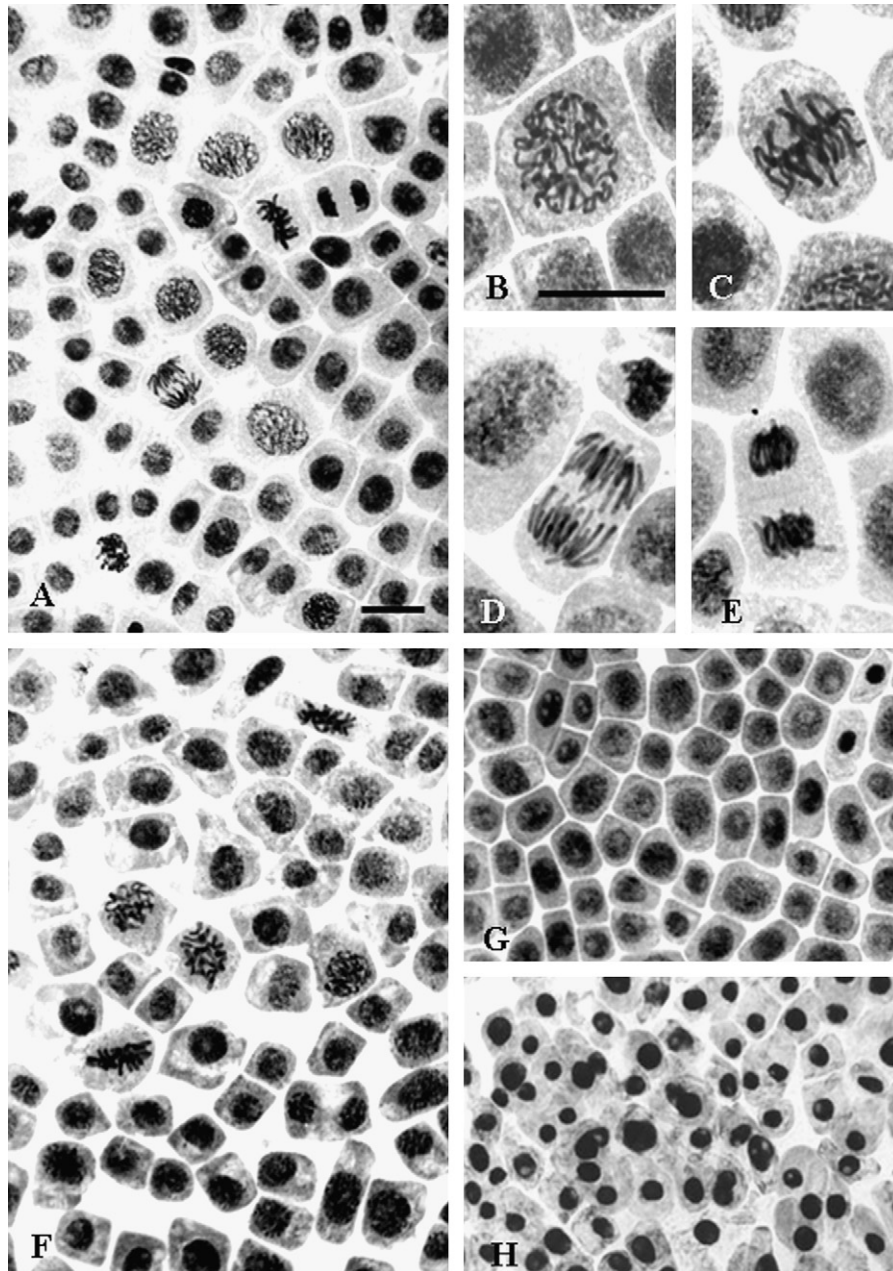


Fig. 3. Microphotographs of control meristematic cells of *Allium Test* (A–E) and after incubation in solutions of ARP (F–H). Squeezed preparations, stained in 2% acetoorcein and observed in light microscope; bar = 10 μ m. All preparations and photographs were made in the same way (Figs. 3 and 4). (A) A typical view of different shape and size, basophilicity of interphase nuclei and mitotic phases; (B) cell in the typical prophase; (C) typical control metaphase (chromosomes arrayed in equatorial plate); (D) typical anaphase (regular alignment of anaphase chromosomes); (E) telophase cell; (F) low mitotic activity and changed divisions (cc prophases and metaphases) following 6 h of incubation in ARP at the concentration of 0.14 mg/mL; (G) different size, shape and basophilicity of nuclei in different phase of interphase after 6-h postincubation following a 24-h incubation in the ARP at the concentration 0.14 mg/mL; (H) no mitosis cell—the marked contraction of cell nuclei after 24-h postincubation following a 24-h incubation in the ARP at the concentration 0.28 mg/mL.

shorten (Fig. 3B). During metaphase, chromosomes formed the equatorial plate (Fig. 3C) and during anaphase—each of the sister chromosomes shifted to the opposite cell pole (Fig. 3D). Telophase began when chromosomes reached the poles (Fig. 3E). After the cycle was finished, the chromosomes elongated and returned to the interphase state. During our experiment, the mitotic activity of control cells was at the level of about 9% (Fig. 3A).

During the incubation with ARP and AFP, we observed changes in mitoses and decrease of mitotic activity proportional to the concentrations used. In particular, the prophase chromosomes had thickened (Fig. 4A); such thickened chromosome-containing cells increased with the time of incubation and the concentration used. Abnormal metaphases were visible with condensed and contracted chromosomes (c.c. – chromosomes) in an abnormally skewed position in the metaphasal plate (Fig. 4A and B) or in the form of C-metaphases scattered over the whole area of a cell (Fig. 4C). Fig. 3G shows that interphase nuclei after incubation at the lowest concentrations ARP did not differ from the structures of the nuclei of control cells (Fig. 3A).

Differences in the effects of both preparations were observed after a longer time in the interphase cells. Incubation for 24 h in high concentrations of ARP (0.28 and 0.56 mg/mL) caused the appearance of the nuclei with strongly condensed chromatin (probably apoptotic cells) (Fig. 3H). Incubation at the highest concentration of AFP (4.0 mg/mL) caused vacuolization of nuclei and autolysis in the cytoplasm (Fig. 4E). These changes were irreversible in the postincubation stage. During the postincubation the mitoses restarted, and reduction of changed division was also observed (especially in AFP) (Fig. 4D). Inhibition of mitoses during incubation in ARP (0.28–0.56 mg/mL) was not reversed even during long postincubation (48 h).

3.3. Analysis of mitotic activity in control cells under incubation with ARP and AFP, as well as in postincubation in water

At all concentrations used in the incubation of roots in both preparations caused retardation and/or inhibition of mitotic activity proportional to the time of incubation (Tables 3 and 4). Already after 3 h the highest concentrations of AFP (2.0 and 4.0 mg/mL) and ARP (0.28 and 0.56 mg/mL) decreased the mitotic index to ca. 40% in comparison to the control. After 12 h of incubation in ARP, its value was close to zero, and complete inhibition of mitotic activity appeared after 24 h.

At the highest concentration (4.0 mg/mL) of AFP used and the highest two concentrations of ARP (0.28 and 0.56 mg/mL), the restoration of cell divisions was not observed during the postincubation. These concentrations showed lethal action (MLC – minimal lethal concentration) on cells of *Allium Test*. The restoration

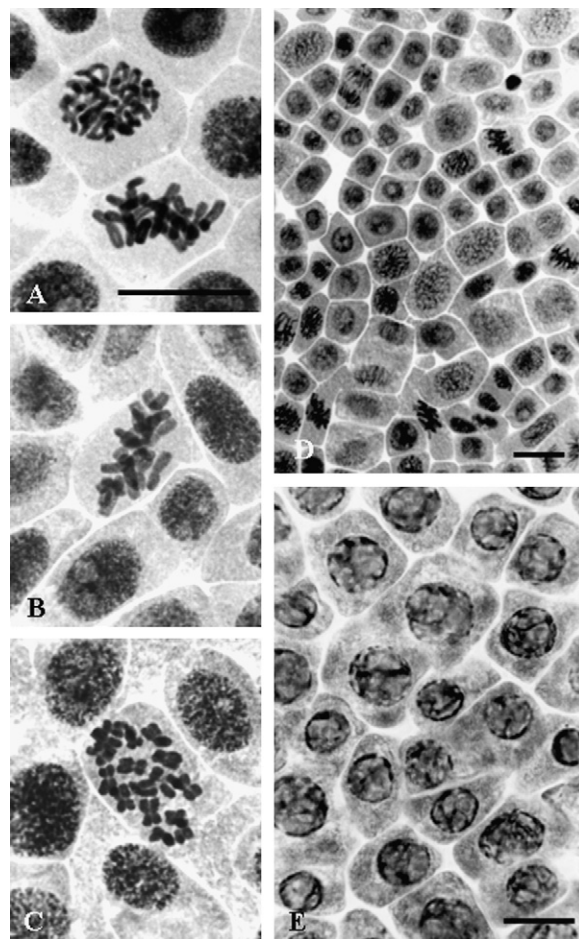


Fig. 4. Microphotographs of cell structure and forms of changed mitoses following different variation of incubation in AFP (A–C and E) and during postincubation in water (D). (A) cc prophase and cc metaphase with condensed and contracted chromosomes skewed positioned in the metaphasal plate, after 12 h of incubation in the AFP at the concentration of 0.5 mg/mL; (B) cc metaphase after 12 h of incubation in the AFP at the concentration of 1.0 mg/mL; (C) typical C-metaphase with “sky” chromosomes (with the set of 16 chromosomes, typical for *Allium*), following 24 h of incubation in the AFP at the concentration of 0.5 mg/mL; (D) resumed normal cell divisions after 12-h postincubation following a 24-h incubation in the AFP at the concentration 1.0 mg/mL – sublethal concentration; (E) the strong vacuolization of cell nuclei and autolysis in the cytoplasm after 24 h of incubation in the AFP at the concentration 4.0 mg/mL – lethal concentration.

Table 3

Mean mitotic index in 24 h incubation followed by 48 h postincubation in the different concentration of ARP (counted as percentage of control time (0 + 0)).

Time (h)	Concentration (mg/mL)					
	0.0175	0.035	0.07	0.14	0.28	0.56
Incubation						
0+0	100 ± 5.1	100 ± 4.1	100 ± 3.3	100 ± 4.5	100 ± 4.5	100 ± 5.1
3+0	72.2 ± 4.3	66.5 ± 5.3	65.3 ± 4.5	43.5 ± 5.3	40.2 ± 5.1	35.2 ± 4.3
6+0	57.2 ± 6.1	54.3 ± 5.5	50.2 ± 2.1	20.1 ± 5.4	15.2 ± 3.3	11.3 ± 2.3
12+0	60.2 ± 3.3	45.6 ± 4.1	35.2 ± 5.3	18.3 ± 4.1	0	0
24+0	68.2 ± 6.3	40.6 ± 6.3	30.6 ± 3.2	0	0	0
Postincubation						
24+3	75.3 ± 7.5	61.3 ± 5.2	59.6 ± 4.3	15.2 ± 4.2	0	0
24+6	87.6 ± 5.2	69.5 ± 7.3	62.3 ± 5.1	20.3 ± 5.3	0	0
24+12	92.5 ± 4.1	78.5 ± 5.2	72.6 ± 4.3	85.6 ± 7.6	0	0
24+24	95.6 ± 5.3	83.6 ± 4.4	80.1 ± 4.3	45.3 ± 5.3	0	0
24+48	98.3 ± 6.3	94.6 ± 6.3	82.3 ± 5.2	63.4 ± 6.3	0	0

Table 4

Mean mitotic index in 24 h incubation followed by 48 h postincubation in the different concentration of AFP (counted as percentage of control time (0+0)).

Time (h)	Concentration (mg/mL)					
	0.125	0.25	0.5	1.0	2.0	4.0
Incubation						
0+0	100 ± 3.1	100 ± 4.3	100 ± 5.4	100 ± 4.3	100 ± 5.3	100 ± 3.3
3+0	102.1 ± 4.2	108.3 ± 5.3	97 ± 4.5	60.2 ± 5.3	42.6 ± 4.2	40.6 ± 4.5
6+0	67.2 ± 2.3	70.2 ± 5.6	65.2 ± 6.2	38.2 ± 5.5	27.2 ± 6.3	25.3 ± 2.2
12+0	46.5 ± 5.2	52.4 ± 4.3	33.3 ± 3.3	18.1 ± 3.2	7.6 ± 2.2	0
24+0	32.4 ± 3.1	23.6 ± 6.1	20.5 ± 6.2	0	0	0
Postincubation						
24+3	64.2 ± 4.1	59.8 ± 5.2	43.1 ± 7.1	0	0	0
24+6	70.6 ± 5.2	93.4 ± 7.0	110.8 ± 5.2	10.3 ± 5.2	0	0
24+12	81.1 ± 4.2	135.4 ± 5.1	84.3 ± 4.2	31.5 ± 7.1	4.3 ± 0.5	0
24+24	92.4 ± 4.3	111.2 ± 4.1	70.6 ± 5.6	38.1 ± 5.2	39.5 ± 5.0	0
24+48	75.2 ± 5.3	80.6 ± 6.3	78.1 ± 6.2	60.3 ± 6.3	58.6 ± 6.3	0

of divisions during postincubation after their inhibition was observed at the 1.0 and 2.0 mg/mL concentrations of AFP and 0.14 mg/mL of ARP. After 48 h of postincubation, the value of mitotic index of these concentrations reached ca. 60% of the initial value. These concentrations showed sublethal action (MSC – minimal sublethal concentration). Other lower concentrations of AFP (0.125–0.5 mg/mL) and (0.0175–0.07 mg/mL) of ARP did not cause complete inhibition of cell division. In the case of AFP, an increase of mitotic activity in the first hours of incubation was observed (Fig. 4). This process was not observed for ARP (Fig. 3).

During the postincubation (Table 4) the increase of mitotic activity, particularly fast for the AFP at concentrations 0.5 mg/mL after 6 h and 0.25 mg/mL after 12 and 24 h, reached values exceeding the mitotic activity of the control. After 48 h, the mitotic index for these concentrations decreased to 80% of the initial value. In the case of lower concentrations of ARP, a gradual increase of the quantity of mitotic divisions during the postincubation was observed, but this did not reach the control values.

3.4. Analysis of changes in the phase index of *Allium Test* during the experiment

The phase index of control cells of *Allium Test* was characterized by the occurrence of all phases of correct mitosis in characteristic proportions: 44% of prophase, 21% of metaphase, 10% of anaphase, and 25% of telophase. All concentrations of both preparations used in the experiment caused changes in the percentage of particular phases' distribution in comparison to the control. The characteristic effect caused by both tested preparations was a gradual increase of prophase index during the incubation and simultaneous decrease of telophase index. Whereas during the postincubation, except at the highest (lethal) concentrations, a gradual decrease of prophase index and an increased quantity of telophase were observed, the phase index regained the control value (Fig. 5).

Furthermore, the incubation in the preparations changed the mitoses, and their quantity depended on the type of preparation, its concentration, and time of action on the cells of *Allium Test* (Fig. 5A–E). Significantly more changed divisions (mainly of prophases and metaphases) occurred after the incubation in the ARP (even up to 80% of all prophases at concentration 0.14 mg/mL after 12 h of incubation). During the postincubation, total decay of changed division was not observed even at the lowest concentration, although their number significantly decreased during the experiment (Fig. 5D–E). The incubation in AFP resulted in a reduced number of changed divisions (maximally up to 30% of all prophases at concentrations 1.0 and 4.0 mg/mL), which decayed totally during the incubation, much faster at lower concentrations (Fig. 5A–C). A complete lack of changed mitoses was noted after 24 h of postincubation for both lowest concentrations (0.125 and 0.25 mg/mL), and after 48 h—also for higher concentrations (0.5 and 1.0 mg/mL). However, several changed divisions (2.5%) were noted afterwards for the cells initially treated at 2.0 mg/mL.

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4. Discussion

For many years, laboratories from all over the world have been working on finding effective remedy for tumors, up to now the most severe disease of our civilization. Among many therapeutic plants that are objects of interest, the extracts from *Uncaria tomentosa* seemed particularly important. Water extracts from this plant have a very rich chemical composition and a range of biological properties, including immunostimulating, antioxidant or antimetabolic (Heitzman et al., 2005). In order to identify the compounds of *Uncaria* responsible for the biological activity, we created two preparations of considerably different chemical composition. Alkaloids-Rich preparation (ARP) contained pentacyclic oxindole alkaloids ca. 50% of dry mass, derivatives of benzoic mono- and disugars, and derivatives of linoleic acid, oleic acid and stearic acid. This preparation was poorly soluble in water. Alkaloid-Free preparation (AFP) was soluble in water and contained at most only negligible traces of any alkaloids, and its main compounds were proanthocyanidins, tannins and benzoic-mono and disugars. We found the relation between chemical composition of *Uncaria* preparations and their antimetabolic properties. The lethal concentration of the AFP was 4.0 mg/mL, while the ARP was 0.28 mg/mL. Thus AFP could be potentially less toxic while still inhibiting cell divisions. Irreversible changes in cells resulted in their degradation, which precluded the restoration of divisions during postincubation. Visible autolysis of cytoplasm and strong vacuolization of nuclei proved initiation of an irreversible process of cell death. Minimal sublethal concentration values were recognized as 1.0 mg/mL for AFP and 0.14 mg/mL for ARP. These concentrations caused inhibition of mitotic division during the incubation, but during postincubation the restoration of division activity was possible. These concentrations may be considered therapeutic because they did not show visible toxic effects.

The effects of mitotic inhibition of the AFP and ARP preparations may be compared with the results obtained for tumor cells (Sheng et al., 1998). These studies showed an antiproliferative effect of the *Uncaria tomentosa* extract on human line tumor cells and confirmed correlation between concentration of preparation and inhibition of proliferation. The same dependence was found in our other studies by the *Allium Test*. According to the obtained results, one can conclude that both alkaloids in ARP and tannins among other yet unidentified water-soluble compounds in AFP play a role in the antiproliferative effect, although the activity of the Alkaloid-Rich fraction is twice as high as that of the tannin-rich fraction. Other

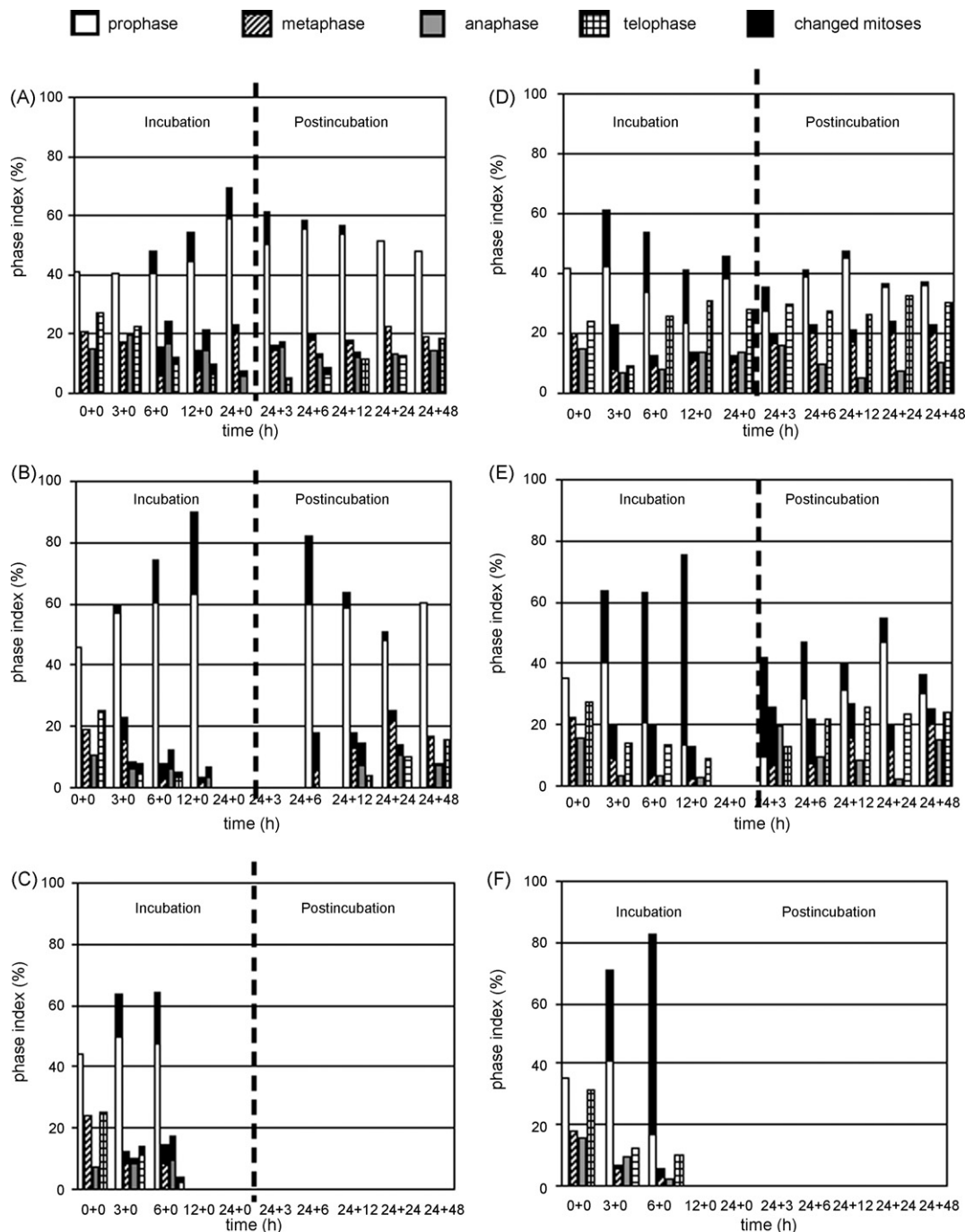


Fig. 5. Mean phase index of cells of *Allium Test* during incubation in the AFP at the concentration of 0.125 mg/mL (A); 1.0 mg/mL (B); 4.0 mg/mL (C) and in the ARP at the concentration of 0.0175 mg/mL (D); 0.14 mg/mL (E); 0.56 mg/mL (F) and postincubation in water.

compounds, existing in both preparations, may act synergistically and this effect has to be taken into consideration in future experiments. Our results obtained for the ARP confirmed earlier studies of Reinhard (1999) which, surprisingly, showed that the preparations considered totally free of alkaloids but containing tannins showed similar biological activity. Tannins-rich extracts of several *Sambucus* species and epicatechins were found to have antimetabolic properties (Zobel, 1999). Proanthocyanidins (Harborne, 1977) show ability to precipitate protein, which is their most important biological property (Horvath, 1981). As showed by Mukhtar (1992) and Kemper (1999), proanthocyanidins acted as astringents and antioxidants and have beneficial effects, and were used as poten-

tial antimetotics in folk medicine (Zobel, 1996). According to the present-day studies, at the concentrations used, proanthocyanidins have an antiproliferative effect similar to that of the epicatechins found in *Sambucus* (Zobel, 1977).

In contrast to the majority of cytostatic alkaloids of *Uncaria*, the Alkaloid-Free fraction showed a distinct but gentler cytostatic effect on cells, which is confirmed by the results obtained during the postincubation. The restored divisions were without aberration and no mutations causing irreversible changes were observed, quite in contrast to these of Taxol[®] or Taxotere[®] tested under the same conditions (Zobel, 1999; Majewska et al., 2000) when irreversible changes appeared in the form of chromosome fragmentations

resulting in creation of micronuclei. Such deformation of genetic material, together with thickening of chromosomes, inhibited the normal progress of mitotic division even long after removal of this factor. These mutations were not observed in the case of either ARP or AFP of the *Uncaria* preparations.

Besides quantitative differences, the action of both preparations as revealed by the Allium Test was qualitatively different in the cell. The changes were in the mitotic index and in a number of changed divisions in particular. Less pronounced action was showed by the AFP because there were considerably fewer changed divisions in comparison to the ARP. It is worth emphasizing that, at the concentrations which caused a similar effect on the mitotic index (1.0 mg/mL for AFP and 0.14 mg/mL for ARP), the number of changed divisions was considerably higher when the ARP was used. Furthermore, in the case of ARP, there was no total elimination of the changed divisions during postincubation. Interesting further research of different applications of both preparations should follow.

Further studies on different applications of both preparations should be of interest. Studies will also be carried out on direct standardization of preparation for obtaining highly active therapeutic remedies that are non-toxic. For that purpose, we are planning experiments on animal cells in test *in vitro* cultures, and on mice and rats *in vivo*.

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