Effect of plant-origin compounds on changes in the cytoplasmic skeleton in meristematic cells

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Abstract

The cell cycle is fundamental for the function of cells. It is defined as a series of events occurring in a eukaryotic cell and leading to its division. The events can be divided into several phases. The cell cycle is a vital process that is inextricably linked with cell divisions. Many factors have a direct effect on the course of the cell cycle as well as divisions of genetic material and cellular structures. Factors that exert an inhibitory effect on cell divisions and induce cell death comprise antimitotic compounds, i.e. cytostatics. The paper presents the results of an experiment conducted to determine the effect of the Rhiodiola extract on the cytoskeleton during mitotic divisions in meristematic cells. A correlation between the presence of the cytostatic compound and inhibition of cell divisions was shown.

Key words:

cytoplasmic skeleton, mitosis, extract, cell cycle

Introduction

The ability of cell to divide during the cell cycle is the most fundamental trait of each living organism [1]. The cell cycle in living organisms is a period between formation of the cell through mitosis and the end of the subsequent divisions. During this period, parts of the cell protoplast undergo many biochemical processes and structural transformations, which result in emergence of two separate somatic cells [2].

The most important stage of the cell cycle is phase M (mitosis), during which the genetic material is divided between daughter cells [3]. The final stage of the process consists in cytoplasm division (cytokinesis) followed by an interphase (a period between consecutive divisions) consisting of three phases: G1, S, and G2 [4].

Many phases are part of a full cell cycle and the most important ones include initiation of DNA replication (transition from phase G1 to phase S) and initiation of nuclear division (transition from phase G2 to mitosis), in which genetic material is replicated and divided between two cells [5].

The most crucial stage for normal progression of cell division is correct doubling of the number of chromosomes and separation of sister chromatids to the nuclei of the daughter cells [6].

The entry of the cell into the cell cycle and its consequent divisions or absence of divisions depends on multiple intracellular and extracellular factors to which the cell responds, e.g. the role of cyclin-dependent Cdk kinases, which influence the correct course of the mechanism of progression of cells through the cell cycle [7].

Progression of the cell through the three phases, i.e. phase G1, characterised by an increase in the cytoplasm volume and duplication of all cellular structures, phase S with the synthesis of histone proteins, duplication of the genetic material, and an increase in the cell volume [1], and phase G2 with intensive synthesis of the structural proteins of the karyo- and cytokinetic spindle and substances required for restoration of the nuclear envelope, enables the cell to enter the division stage [6].

The mitosis stage in the cell cycle is characterised by a number of structural changes in the nucleus, primarily aimed at separation of replicated genetic material to daughter cells [8]. It consists of four phases: prophase, metaphase, anaphase, and telophase, but sometimes a fifth element, i.e. prometaphase, is included in mitosis [2].

Prophase begins with chromosome condensation, in which a vital role is played by phosphorylation of histone H3. Equally important is condensin as well as non-histone proteins forming complexes with e.g. cohesin [9]. This phase is characterised by disappearance of nucleoli, which leads to degradation of the nucleus [10].

The next phase of mitosis consists in disintegration of the nuclear envelope (prometaphase) followed by metaphase beginning with arrangement of the chromosomes in the equatorial plane of the karyokinetic spindle (metaphase plate). Chromosome condensation and attachment to the spindle take place in this phase.

The progression into anaphase is determined by separation of sister chromatids as a result of cohesin unbinding. This mechanism relies on a number of enzymes, e.g. separase, which represents the protease group. The essence of anaphase is the movement of chromatids (daughter chromosomes) towards the cell poles driven by the mitotic spindle [6].

The final step of mitosis is telophase, which consists in restoration of nuclei, chromosome decondensation [2], and restoration of the nuclear envelope. Additionally, nucleoli and nuclear matrix are formed in this phase and the cell cytoplasm prepares for cytokinesis [6].

The basis of cytokinesis is the formation of a cellular septum in the division plane, which leads to separation of the cytoplasm and organelles contained therein [11]. The process is possible due to the involvement of the phragmoplast composed of microtubules and actin microfilaments [2].

The cytoskeleton, which is present in each living cell, is formed of microtubules, microfilaments, and intermediate filaments. It plays an important role in determination of the cell division plane, chromosome movement in anaphase, the direction of cell expansion, organelle movement, and diffusive and apical expansion; it also exerts an effect on signal transduction [8].

Disturbances and disorders of the cell cycle may be associated with several groups of substances, for instance compounds with antimitotic activity, i.e. the so-called cytostatics, which inhibit cell divisions or exert a lethal effect on cells.

An example of cytostatics is 5-fluorodeoxyuridine, an alkylating agent inhibiting synthesis of thymidine acid. A similar effect is also produced by cyanides, azides, and phenols, which inhibit vital cell processes, e.g. glycolysis or respiration, and induce changes in the physical and chemical properties of DNA under the effect of maleic hydrazide [12].

Cytostatics can induce disorders in the structure of the cell cytoskeleton and disturbances in normal function of the karyokinetic spindle (inhibition of separation of sister chromosomes, formation of polyploid cells); they also affect formation of the phragmoplast of the karyokinetic spindle [6].

An important group of plant-origin compounds are secondary metabolites. This large group of chemical compounds is not involved in plant metabolism and serves a crucial function in the response to stress conditions. Due to their antitumour and antimitotic activity, many secondary metabolites are important for medicine and the pharmaceutical industry.

Colchicine isolated from the autumn crocus (*Colchicum autumnale*) is a secondary metabolite. It disrupts the separation of sister chromosomes into daughter cells and induces embryogenesis in *in vitro* cultures of microspores and anthers, which is reflected in polyploidisation [13].

Caffeine, i.e. a purine alkaloid isolated from *Coffea* L. leaves, is a biostimulator of the central nervous system and an antioxidant agent [14]. It exerts an impact on normal phragmoplast formation by inducing disturbances in the progress of cytokinesis and meiotic divisions [15].

The Rhodiola extract derived from roots and rhizomes of *Rhodiola rosea*, a representative of *Crassulaceae*, is a mixture of many active compounds. It exhibits antibacterial and antioxidant activity and psychostimulant and antimitotic properties. Additionally, it has a protective effect on neurons and erythrocytes in oxidative stress conditions. The major compounds contained in the extract include rosavins (responsible for the cytotoxic, adaptogenic, and cardioprotective properties of the extract) and salidroside (involved in the antioxidant and anticancer activity) [16]. The extract inhibits mitotic divisions and causes changes in the mitochondrial ultrastructure through reduction of the number or size of prophasic and metaphasic chromosomes [17].

The extract from *Uncaria tomentosa* is another chemical agent with valuable properties used both in the pharmaceutical industry and in medicine. Thanks to the presence of bioactive compounds, e.g. indole and oxindole alkaloids, it regulates cell proliferation and stimulates the immune and nervous systems [18]. Additionally, its activity depends on its concentration: a low concentration leads to reduced mitotic activity and strong chromosome condensation, whereas higher concentrations induce cell apoptosis [19].

Taxol, a plant-origin cytostatic extracted from the bark, needles, and roots of various species of yew, e.g. *Taxus baccata*, *T. brevifolia*, and *T. media*, is a polycyclic diterpene with antileukemic and antimalarial activity [13]. The compound enhances polymerisation of tubulin and inhibits depolymerisation of microtubules; therefore, it is applied in treatment of neoplastic diseases (ovarian, mammary, and lung cancer). It arrests mitotic divisions at metaphase, exerts a cytotoxic effect, and at higher concentrations leads to cell death [20].

The extract from *Rosmarinus officinalis* L. leaves contains biologically active compounds, e.g. antiseptic tannins, antioxidant flavonoids, and antibacterial essential oils [21].

Aim

The aim of this study was to determine the effect of the *Rhodiola rosea* extract on the cytoskeleton during mitotic divisions in meristematic cells of *Allium cepa* roots.

Material and methods

The investigations were based on application of the Rhodiola extract to meristematic cells of *Allium cepa*

roots. The extract used in the experiment was provided by the Department of Biology and Pharmaceutical Botany, Faculty of Pharmacy, Medical University of Warsaw, after dilution in 1 ml of methanol and 0,65 ml of DMSO.

The *Allium cepa* roots were incubated with the Rhodiola extract in experimental variants of 2, 4, 8, 16, and 24 h and fixed in a mixture of 4% paraformaldehyde in MSB. Next, the samples were rinsed in MSB buffer, dehydrated, and supersaturated in Stedman's wax polymer. The research material was cut into 3-µm thick slices and subjected to immunological reaction with the use of anti-β-tubulin antibodies in 0,1% BSA in 0,1 M PBS and FITC in 0,1% BSA in 0,1 M PBS. Next, the material was treated with the DAPI fluorochrome. The samples were analysed under a fluorescence microscope with excitation light at a wavelength of λ- 510 nm (for FITC) and λ-300 nm (for DAPI). The experiment results were recorded as black-and-white images at a magnification of 420x and 990x.

Observations

The experiment revealed normal progression of the individual stages of the mitotic division (control sample in H_2O) in the meristematic cells analysed under the fluorescence microscope. There were cortical microtubules located at the cell walls in the mitotic prophase (Tab. I, Fig. 1), metaphase with the karyokinetic spindle produced by the microtubules extending from the plate to the cell poles (Tab. I, Fig. 2), and DAPI fluorochrome-stained chromosomes (Tab. I, Fig. 3). We observed normal anaphase with a visible karyokinetic spindle and polar and chromosomal microtubules (Tab. I, Fig. 4) as well as chromosomes moving to the cell poles after staining with the DAPI fluorochrome (Tab. I, Fig. 5). The final phase of mitosis (telophase) in the control sample was characterised by visible phragmoplast microtubules between the cell poles and the central plane, which resulted in formation of a fluorescent fold, a primary septum, and a cell wall (Tab. I, Fig. 6 and 7).

Cells with microtubule immunofluorescence incubated with the Rhodiola extract for 2 hours exhibited a low number of microtubules in the area occupied by the phragmoplast in late telophase (Tab. I, Fig. 8) and various shapes of the nuclei of adjacent cells after staining with the DAPI fluorochrome (Tab. I, Fig. 9).

Root cells incubated with the extract for 4 hours exhibited a karyokinetic spindle composed of an evidently low number of microtubules in metaphase (Tab. I, Fig. 10). At this incubation, we observed a very weak structure of the spindle skeleton with an impaired polar part in anaphase (Tab. I, Fig. 11) and a weak phragmoplast-devoid cytoskeleton in telophase (Tab. I, Fig. 12).

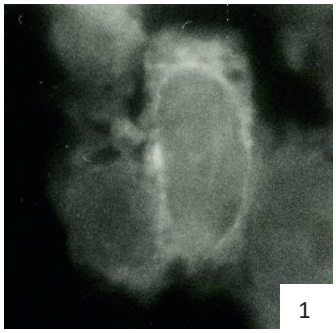
In cells with microtubule immunofluorescence incubated with the Rhodiola extract for 8 hours, we observed late prophase with short tufts of microtubules located at the chromosomes (Tab. II, Fig. 1), DAPI fluorochrome-stained chromosomes (Tab. II, Fig. 2), and telophase with small clusters of polar microtubules along chromatids (Tab. II, Fig. 3). The incubation carried out for the specified time caused distinct differentiation of the shape of the DAPI-stained cell nuclei (Tab. II, Fig. 4) and revealed cortical microtubules that did not occupy the polar part of the cell (Tab. II, Fig. 5).

Cells subjected to the 16-hour incubation with the Rhodiola extract exhibited an alternate arrangement of round and strongly flattened DAPI-stained nuclei (Tab. II, Fig. 6) and a low density of microtubules surrounding the karyokinetic spindle and phragmoplast (Tab. II, Fig. 7).

The 24-h incubation caused spiralisation of chromosomes stained with the DAPI fluorochrome in mitotic prophase (Tab. II, Fig. 8) and revealed overlaps of microtubules on the nucleus surface in the upper and lower side of the cell (Tab. II, Fig. 9). At the same incubation time, we observed very few longitudinally arranged microtubules forming a karyokinetic spindle in prometaphase and metaphase (Tab. II, Fig. 10).

Discussion and conclusions

The observations of the cytoplasmic tubulin skeleton in cells exposed to the activity of the Rhodiola extract revealed a number of differences from the control samples.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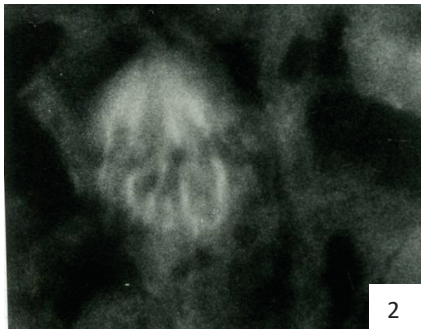
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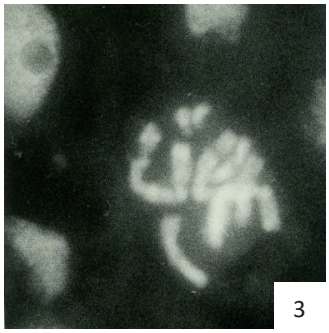
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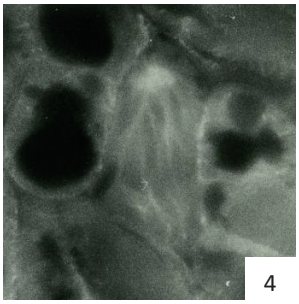
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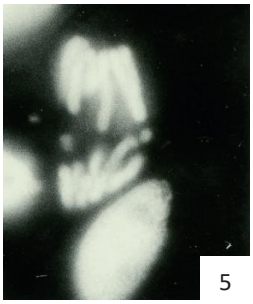
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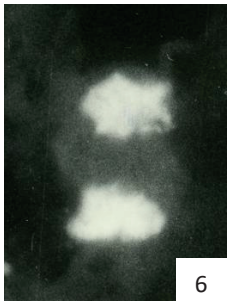
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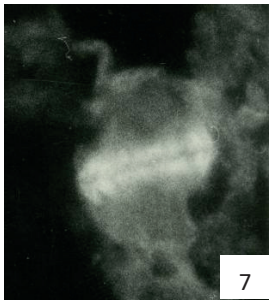
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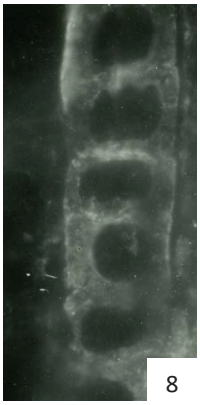
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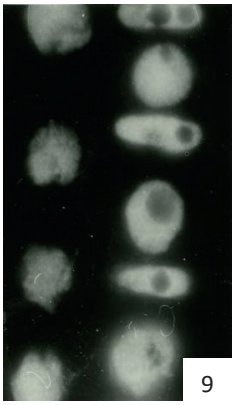
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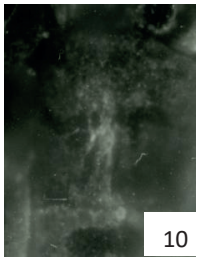
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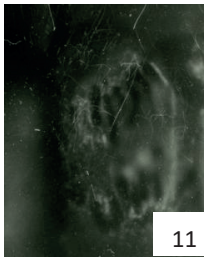
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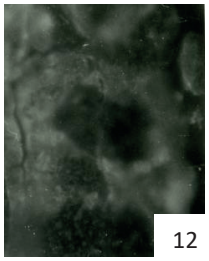
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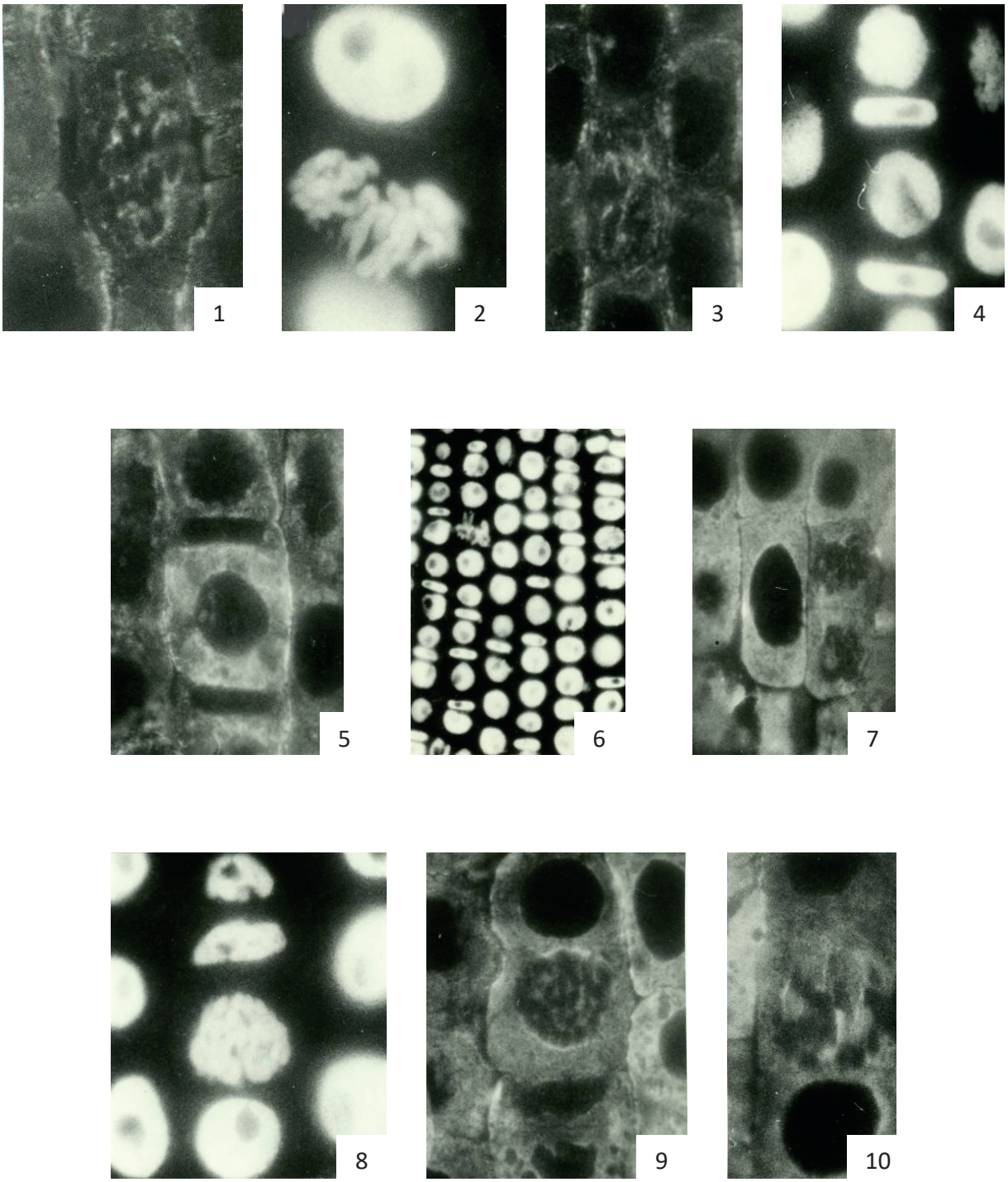
Tab. I

Fig. 1. Prophase with visible microtubules located at the cell walls. Presence of microtubules located at the pre-prophase ring site.
Fig. 2. Karyokinetic spindle formed by microtubules extending from the cell plate to the poles (metaphase).
Fig. 3. Metaphase with chromosomes located in the equatorial plate of the cell (DAPI fluorochrome staining).
Fig. 4. Anaphase. Visible karyokinetic spindle with chromosomal and polar microtubules
Fig. 5. Movement of chromosomes to the cell poles in anaphase visualised by DAPI fluorochrome staining.
Fig. 6. Nucleus restoration after mitotic division. DAPI fluorochrome staining
Fig. 7. Telophase. Visible phragmoplast microtubules connecting the cell poles and the central plane and forming a fluorescent fold and next a primary septum and cell wall.
Fig. 8. Late telophasic cell with few microtubules at the site occupied by the phragmoplast.
Fig. 9. Differentiated shape of cell nuclei in adjacent cells after DAPI fluorochrome staining.
Fig. 10. Metaphase. Karyokinetic spindle with few microtubules.
Fig. 11. Poorly developed spindle skeleton with an impaired polar part in anaphase. Presence of polar microtubules in the inter-chromatid space.
Fig. 12. Telophase. Weak phragmoplast-devoid cytoskeleton. Presence of few microtubules at the margins of the nuclei.



Tab. II

Fig. 1, 2. Late prophase with short tufts of microtubules located at the chromosomes. DAPI fluorochrome staining (2).
Fig. 3. Few polar microtubules along the chromatids in telophase.
Fig. 4. Differentiation of the shape of the cell nuclei after DAPI staining.
Fig. 5. Location of microtubules in the cytoplasm and around the nucleus and cortical microtubules that do not occupy the polar part of the cell.
Fig. 6. Alternate arrangement of round and strongly flattened DAPI-stained nuclei.
Fig. 7. Low density of microtubules surrounding the karyokinetic spindle and phragmoplast. Visible well-preserved cortical microtubules at different stages of formation of the pre-prophase ring.
Fig. 8. Strong spiralisation of DAPI-stained chromosomes.
Fig. 9. Prophase. Location of microtubules on the nucleus surface forming overlaps on the upper and lower side of the cell.
Fig. 10. Few longitudinally arranged microtubules forming a karyokinetic spindle in prometaphase and metaphase.

After the 2-h incubation, the shape of the interphase nuclei changed from oval to undulating. Additionally, the nucleoli were enlarged and had hardly regular shapes in comparison with the control cells. This was also observed during the other longer incubations.

The investigations have shown deviations in the phragmoplast structure in the 2-h incubation. Microtubules were observed in the cytoplasm of telophase cells, but no typical phragmoplast with microtubules was formed. In late telophasic cells, there were surprisingly few microtubules in the area occupied by the phragmoplast. The location of the microtubules was disorderly and did not resemble the longitudinal arrangement of the phragmoplast microtubules. The 4-h incubation revealed formation of a weak karyokinetic spindle composed of only few microtubules, likewise in the weak cytoskeleton of telophasic cells, which contained no typical phragmoplast and there were only a few microtubules at the cell margins.

At the other incubation times, the cortical microtubules at the different stages of formation of the preprophase ring were better preserved than the microtubules of the karyokinetic spindle and phragmoplast.

The *Rhodiola rosea* extract restrains cell divisions, triggers apoptosis thereby leading to cell death, and impairs normal formation of the cytoplasmic skeleton.

In normal conditions, the cell can enter mitosis only after completion of DNA replication, i.e. phase S of the cell cycle. This sequence can be altered by structural DNA damage or a slowdown or inhibition of DNA biosynthesis [22].

Besides mutations, chemical factors disrupt and block the internal checkpoints of phase S. This may result in impaired functioning of the mechanisms of control of normal DNA biosynthesis progression, which consequently leads to premature initiation of mitosis in cells that have not completed replication or post-replication repair of DNA. These factors include inhibitors of protein phosphatases, e.g. okadaic acid, and an inducer of premature mitosis, i.e. caffeine [23].

The experiment has demonstrated inhibition of cell divisions, which is in agreement with investigations conducted by other authors [24] reporting a reduced number of mitotic divisions under the impact of the *Rhodiola rosea* extract.

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