Effect Alkaloid and aqueous extraction of *Convolvulus Scammonia* on microtubules of CHO cell line(china hamster)

Ibrahim hade , Zenia T.A

Department of Biology , College of Science, University of Diyala

Department of Biology, College of Eduction Ibn-AlHaitham, University of Baghdad

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Abstract

The study evaluated the effect of selected on crude Alkaloid and aqueous extraction from roots of *(Convolvulus Scammonia*) the microtubule network of CHO cell line (china hamster) GFP tubulin labeled and the recovery of its disruption. Model experiments were used for demonstration of the quantification of microtubule network changes induced by crude alkaloids using computer-assisted image analysis.

Cells were treated with Alkaloid and aqueous extraction from roots of *(Convolvulus Scammonia*) at various concentrations from 2 µg/l to 800 µg/l for 60 min, or with crude alkaloid at a concentration of 4615 µg/l and 9230 µg/l for 60 min. Microtubules were detected by means of indirect Immunofluorescence.

The damage was examined in a fluorescence microscope. Also, cells were treated for 60 min with alkaloid at concentrations of 20 µg/l or 800 µg/l and the recovery process was studied in time intervals of 6, 7, 8, 9, 10 hours, or 8 and 12 hours, respectively. Differences in the arrangement of microtubules were assessed by means of quantification of the cytoskeleton changes in cells treated with alkaloid at a concentration of 20 µg/l and in untreated control cells.

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Introduction

In eukaryotic cells the cytoskeleton is formed by three major structural elements microtubules ,microfilament , and intermediate filaments (1). The cytoskeleton plays aspecific role in cell division ,maintenance and changes of cell shape in intracellular contacts ,interaction with membranes , extracellular matrix, and in cell motion.

Microtubules are filaments with the diameter of cytoskeletal components .microtubular net work is important for the execution of many cell function ,The play an important role in cell division. The microtubular diameter measures about 25 nm. Microtubules are composed of 13 equally spaced profilaments(2).

Tubulin is the basic protein of the microtubules and molecules of tubulin are arranged in dimmers containing bith its forms α- tubulin and β-tubulin .microtubules are continuously changeable structures(3) and polmerisation and depolymerisation microtubules of is regulated extracellular and intracellular factors(4).

The presence of GTP at microtubule ends is necessary to maintain the stability of the polymer (5). Vinca alkaloids micritubular toxin of chemically similar nature(6)that disrupt microtubule function by binding to site on β tubulin and suppressing microtubule dynamics.Vincristin have been used widely to treat cancer (leukaemia ,Hodgkins disease) (7). *Convolvulus* is derived from the Latin, *convolere*, meaning "to entwine", and *arvensis*, meaning "of fields." The genus *Convolvulus* contains about 250 species. (8) but *arvensis* is understood to contain alkaloids that are toxic. However, extracts of the plant, largely comprised of proteoglycan molecules (9). appear nontoxic in animal studies, and have been shown to have potent anti-angiogenetic effects. In models of angiogenesis, mouse sarcoma, mouse Lewis lung carcinoma, PMGwas found to have potent anti-angiogenic and tumor-inhibitory effects. Inhibition of angiogenesis was 18 to73 percent; inhibition of tumor growth was 35 to 80 percent in the cancer models represented; and lymphocytes were increased 12 to 46 percent in respective models(10).

Convolvulues to mice were investigated. Bindweed contains several alkaloid , including pseudotropine, and lesser amounts of tropine, tropinone, (11). Mice fed bindweed exclusively died or were euthanized after 4-7 d and had severe hepatic necrosis. Mice fed low doses of bindweed along with standard laboratory mouse diet for 6 w had no clinical disease on

necropsy examination but did have histologic lesions of mild multifocal hepatitis and gastritis(12) .

Material and methods

cell line

In our experiments we used the stable heteroploid line of CHO cells obtained from the Department of Biology, Faculty of Medicine, wuhan University in china . The cells were grown on uncoated coverslips in a Dulbecco's Minimal Essential Medium (DMEM) (PAA Laboratories GmbH, Linz, Austria), supplemented with 10% foetal bovine serum (PAA), 2 mM glutamine (PAA), 100 Uml penicillin, and 100 μg \ml streptomycin (PAA Laboratories, Austria) in ahumidified atmosphere of 95 % air and 5 % CO_2 at 37 °C. The cells grew as monolayers.They were subcultured (three times) per week.

Exposure to Crude Alkaloid and aqueous extraction

To investigate the action of Alkaloid and aqueous extraction from roots of *(Convolvulus Scammonia*) to the microtubule network, a solution containing 1 mg of alkaloids in 1 ml SDS was mixed final concentration of alkloids, or aqueous was 2, 10, 20, 30, 40, 80, 100, 200, 400, 800 μg/ml, or 4615 μg/ml and 9230 μg/ml Each concentration was in two dishes. The cells were exposed to the drugs for 60 min at 37 °C. the concurrently with control samples in phosphate-buffered saline (PBS,pH 6.9) and processed for immunofluorescence microscopy.In the second series of experiments the cells were cultivated for 2, 5, 10, 20, 30,and 60 minutes at 37 °C in media with a final alkaloids concentration of 20 μg/ml, or for 5 minutes in a medium containing alkaloids at a concentration of 800 μg/ml. The samples were washed three times for 4 minutes concurrently with control samples in PBS, pH 6.9, and processed for immunofluorescence microscopy. the performed with alkaloids at a final concentration of 20 μg/ml. The cells were exposed to the drug for 60 min. After the treatment, the drug-containing medium was poured off and monolayers were subjected to three washing procedures concurrently with control samples with phosphate-buffered saline (PBS) (pH 6.9).Two slips with the cells were then subjected immediately to fixation and detectionof the microtubular network as well as two slips which were cultivated for 60 min in -free growth

medium as a control. The other Petri dishes were refilled with fresh growth medium and incubated for another 6, 7, 8, 9, and 10 hours in order to evaluate the recovery processes. Recovery progressed at 37 °C in a humidified atmosphere of 95 % air and 5 % CO2. The other control monolayers treated with a growth medium only containing alkaloids-free DMEM were cultured in the same conditions as the alkaloids-treated cells recovered for 10 hours. After fixation, the microtubular components were visualised and viewed in a fluorescence microscope. A similar attempt was provided for a alkaloids concentration of 400 μg/ml. The cells were exposed to the drug for 60 min, and after the washing procedure, they were recovered for 8 or12 hours in the same condition as last mentioned. Microtubular components were visualised and examined in a fluorescence microscope.

Visualisation of microtubular network

Cells were washed three times for 4 min in the phosphate-buffered saline (PBS, pH 6.9) and fixed by 3 % paraformaldehyde in PBS. Thereafter, the cells were permeabilised by 0.2 % Triton X-100 solution in PBS. The microtubules were detected by means of the tubulin monoclonal antibody TU-01 (Institute of Molecular Genetics,Prague, Czech Republic), diluted 1:300 by PBS, and a secondary antibody SwAM/FITC (conjugated swine anti-mouse globulin/fluorescein isothiocyanate;Institute for Sera and Vaccines, Prague, Czech Republic), diluted 1:100 by PBS.The cells were washed in the phosphate buffer three times for 5 min between the application of individual agents. The samples were then closed in the Vectashield mounting medium (Vector Laboratories, Inc., Burlingham, CA, USA).

Results

Untreated control cells (*Fig. 1*) showed a microtubule network regularly distributed along the whole cell content. Cells exposed to Alkaloid and aqueous extraction from lroots of *(Convolvulus Scammonia*) drugs at concentrations of 2 µg/l for 60 min did not show considerable changes in the distribution of microtubules.Cells exposed to at concentrations of 10 (*Figs. 2*,), 20, 30,40, 80, 100, 200, 400, and 800 µg/l for 60 min showed changes in the arrangement of the microtubular network. The network of cytoplasmatic microtubules at concentrations of 10, 20 µg/l was thinned down, and individual fibres had a wavelike shape.

(*Figs. 3*), The network damage increased with increasing concentration of cytostatics. The microtubules were more thinned down and fragmentation of fibres occurred. At a higher concentration of 400 µg/l, (*Fig. 4*) sometimes blebs were formed (*Fig. 5*). Cells exposed to alkaloids at concentrations of 4615 µg/l and 9230 µg/l formed paracrystals (*Fig. 6*)and (*Fig. 7*) No significant difference was detected in alkaloid and aqueous treated cells.When cells were exposed to alkaloid at a concentration of 20 µg/l for 2, 5, or 10 minutes, no noticeable changes occurred in the microtubule network. The 20- min treatment at a concentration of 20 µg/l caused disruption of microtubules. The network was thinned down, and individual fibres had a wavelike shape. The cells exposed to alkaloid at a concentration of 800 µg/l for 5 minutes showed a severely defective microtubular network.

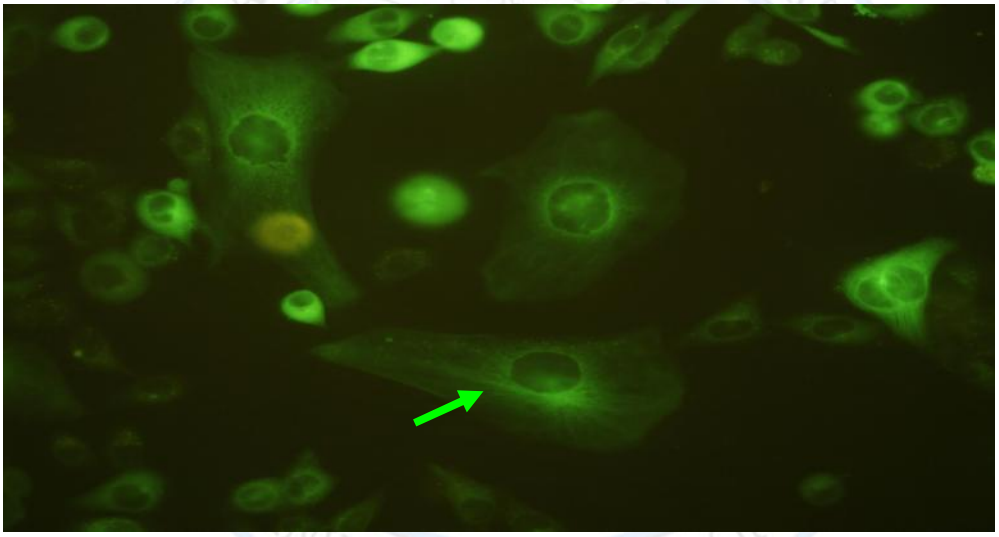

Figure 1

Microtubule Untreated control of GFP-tubulin labeled CHO cell .the network regularly distributed along the whole content.

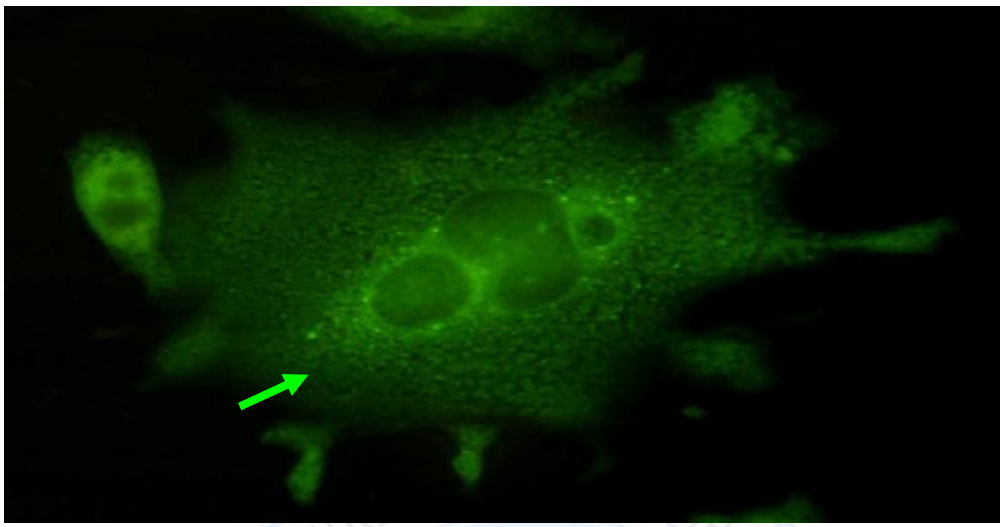

Figure 2

Microtubules of CHO line cells treated with alkaloids at a concentration of 10 µ/l for 60 min. The network of cytoplasmatic microtubules is thinned down, and individual fibres have a wavelike shape.

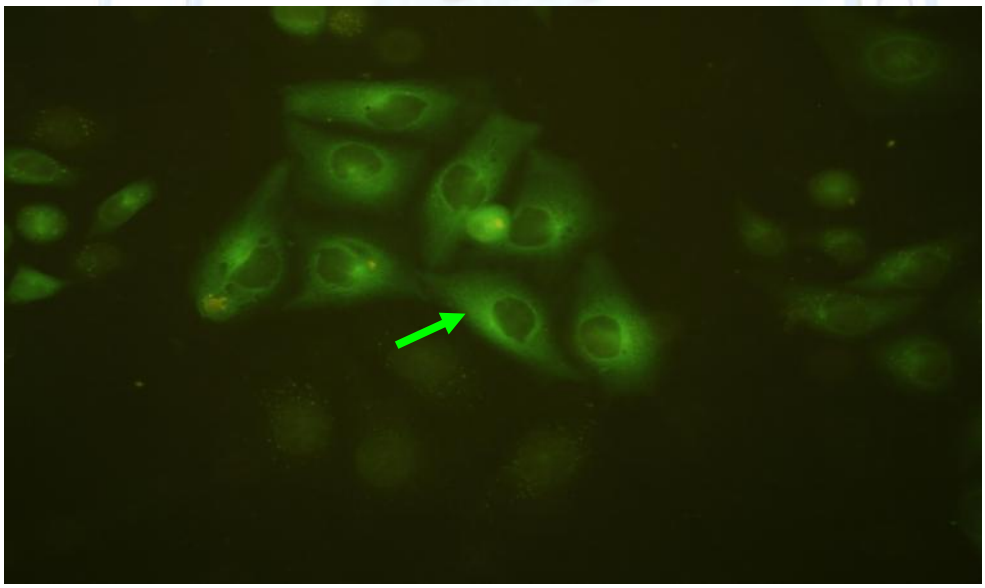

Figure 3

Microtubules of CHO line cells treated with aqueous at a concentration of 10 µ/l for 60 min. The network of cytoplasmatic microtubules is thinned down, and individual fibres have a wavelike shape.

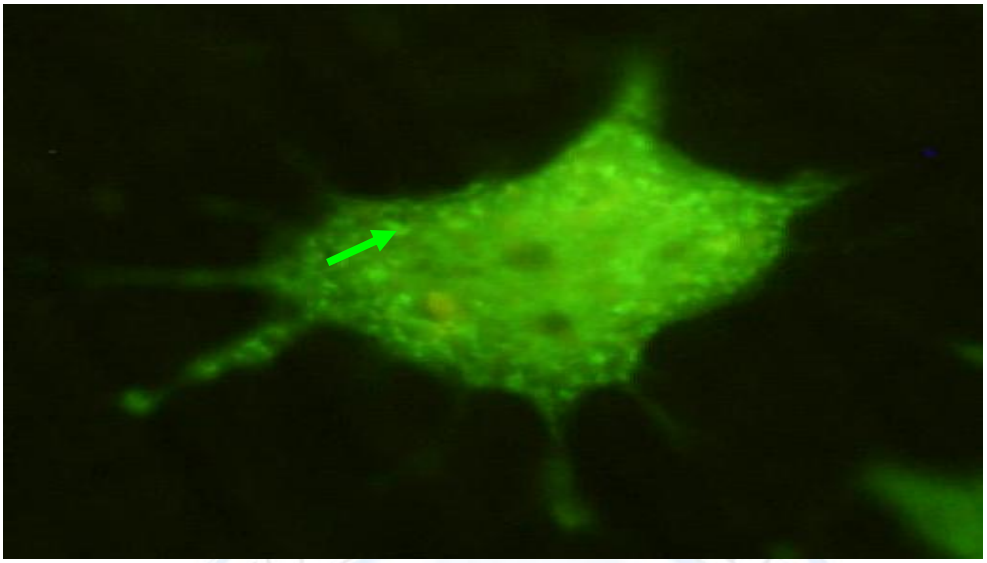

Figure 4

Cells of CHO line treated with aquoues at a concentration of 400 µg/l for 60 min. There are blebs formed on the periphery of the cells. The microtubules perished and free tubulin was detected along the whole cell content

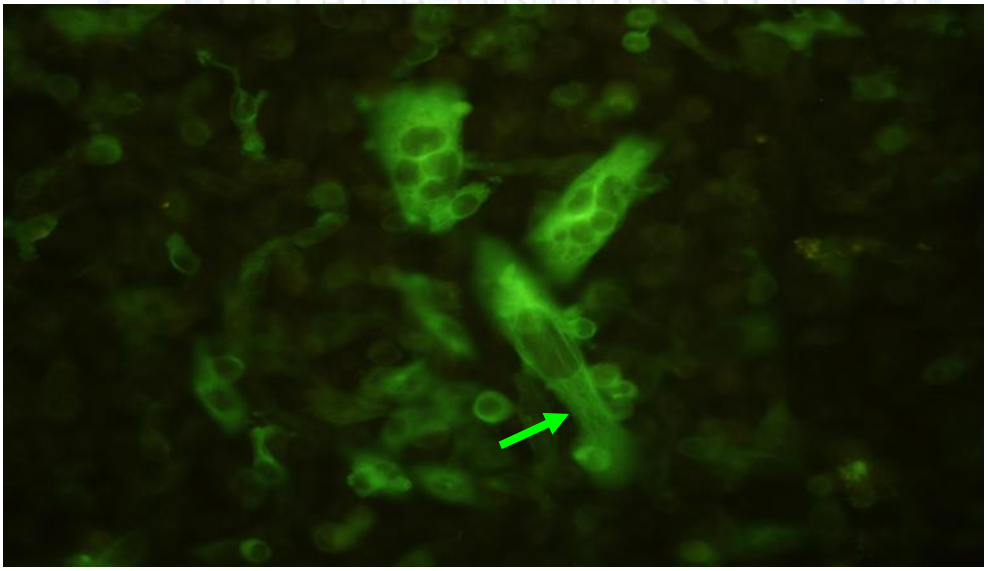

Figure 5

Microtubules of CHO line cells treated with alkaloids at a concentration of 400 µ/l for 60 min.Microtubules recovered for 8 hours. The network is partially restored

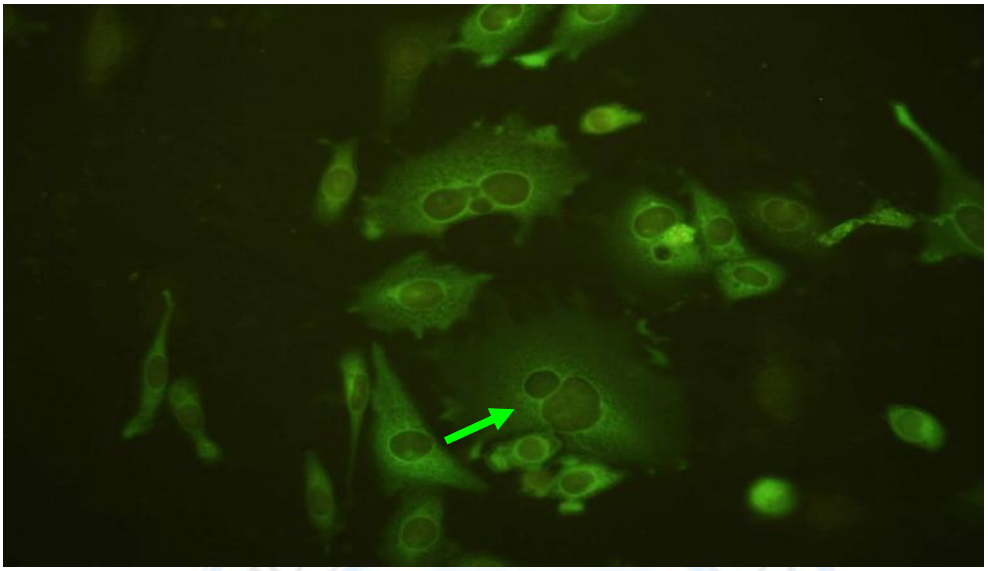

Fig. 6

HeLa cells treated with alkaloids at a concentration of 4615 µg/l for 60 min. Paracrystals are formed.

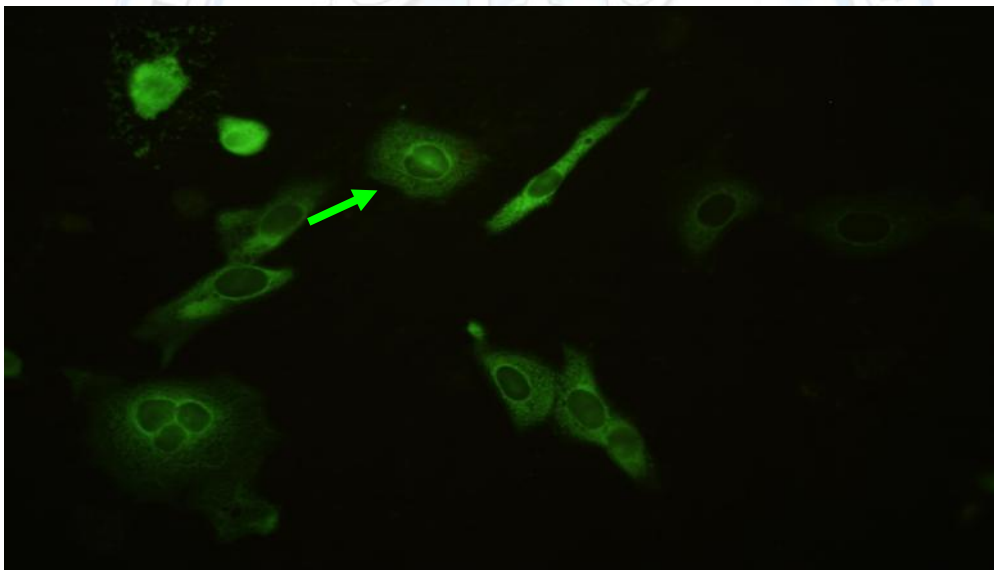

Figure 7

CHO cells treated with alkaloids at a concentration of 9230 µg/l for 60 min. Paracrystals are formed.

All the cells with the recovering period of 6 hours in a drug-free growth medium following vincristine treatment showed damage of microtubules. The cells after a7-hour recovery period had their microtubular network either fully restored or stilldamaged. After recovery for an 8-

hour period, some cells showed a partly defective(thinned-down) network, but the majority of the cells showed restored microtubules.When the cells were allowed to recover for 9 hours, the microtubules werespread out comparably to those observed in untreated control cells. The controlcells showed their microtubule network regularly distributed along the whole cell volume. When cells were recovered after treatment with alkaloids at a concentrationof 400 µg/l for 8 hours, the cytoskeleton was partially restored afterwards (*Fig. 5*). The microtubules were thinned down, and individual fibres had a wavelike shape. After a recovery period of 12 hours, the network was also damaged,only several cells showed nearly restored microtubules.

Discusion

The interaction of antitumour agents with compounds of the cytoskeleton is a theme studied in many papers (*5–12*). *Meng* (*10*) studied the interaction of alkaloids with tubulin, and compared alkaloid and aquoues leaves of *(Convolvulus arvensis*) . he studied e.g. the affinity of the drug for tubulin heterodimers. alkaloid exhibited a higher overall affinity for porcine brain tubulin than aquoues, but the affinity of the drug for tubulin heterodimers was identical for the two drugs. Under our experimental conditions we did not mark any differences between the two drugs. Some authors also studied the recovering processes of the cytoskeleton after treatment of cell cultures with physical factors or agents interfering with cytoskeleton compounds. alkaloid caused a sequence of morphological changes insensitive cells from three pleiotropic resistant MCF-7 human breast carcinoma cell lines mixed with vaginal adenocarcinoma cells. The cells were selected in serially increasing drug concentrations. These changes included precipitation of tubulin and disappearance of tubular structure. The changes occurred initially within 3 hours of incubation, but were expressed in all cells after 6 hours. If, after 3 hours of drug exposure, the cells were subcultured in drug-free media, the cytoskeletal structure reformed within 10 hours. The maximal recovery of the cytoskeletal structure occurred 22 hours after drug removal and was sustained up to 36 hours (*13*).

Treatment with alkaloid (*14*) eliminated the microtubule bundles, leaving only tubulin paracrystals. Within 24 hours after washing out the alkaloid, the microtubule bundles repolymerised in cultured hippocampal neurons**.**

They presented a decrease of the assembled tubulin after treatment with nocodazole and a less delicate structure of the remaining microtubules. This was indicated by a reduction of the parameters used. They also showed significant differences between the high and low metastatic cell lines.

Conclusions

The cells showed changes in the arrangement of microtubules even at the 10 µg/l concentration of cytostatics after 60-min exposition. Its damage increased with increasing concentration of cytostatics. No differences were recorded between the two cytostatics. At concentrations of 4615 µg/l (alkaloid) and 9230 µg/l (alkaloid) tubulin paracrystals appeared. Disruption of the microtubules was also time-dependent. It was more serious with prolonged cell treatment.

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