Method of Bioindication for Evaluation of the Effects of Plant Extracts on Cell Monolayer in Acute and Chronic Experiments

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Acute and chronic toxicities of plant extracts for external use were studied on a model of cell monolayer. This method detects the toxic dose of the agent during the first stage of the study; the activity of this dose and severity of damaging effect can be further studied on experimental animals. An advantage of the method is high informative value of preliminary investigation in the development of new low-toxic agents.

Key Words: *cytotoxicity; bioindication; plant extracts; cell monolayer*

Extracts from medicinal plants for external application were developed. These plant extracts were selected with consideration for cell vital activity and differ by the quantity and composition of essential oils and method of their application. According to current requirements to toxicity studies of new drug, the method of application in the study should be the same as recommended for treatment [1], but traditional methods of investigation will hardly provide objective results, as these preparations contain hundreds of organic compounds.

Here we studied the effects of plant extracts in different concentrations on cell monolayer and evaluated their cytotoxicity.

MATERIALS AND METHODS

Aqueous and oil extracts from medicinal herbs (spearmint, wormwood, St. John wort, thyme, yarrow, calencula, celandine, camomile, pine buds, licorice root, dog rose fruit, fennel, and caraway) with essential oils were studied.

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Acute cytotoxicity was evaluated by the method of bioindication on L-41 (human leukocytes) and RH (renal human cells) cells and blood neutrophils. The criteria of evaluation were the density of cell monolayer, percentage of viable and dead cells, the state of cell monolayer (according to macroscopic examination), changes in cell morphology, and mitotic activity (MA).

The cells were grown on coverslips in penicillin flasks (8×10^4 cells/ml medium 199 supplemented with 10% bovine serum) at 37°C for 24 h. After attaining confluence the medium was replaced with fresh portion of serum-free medium 199.

The preparations were dissolved in medium 199 to a concentration of 0.08-2% (oil extract) and 0.1-10% (aqueous extract); native preparations were also used. After addition of the preparations the flasks with culture medium were incubated for 72 h in a thermostat, preparations for morphological examination were routinely prepared every 24 h (fixation in Carnoy fluid and staining with hematoxylin and eosin). Cell culture without extracts served as the control.

Chronic toxicity was studied by the method of bioindication on L-41 cells grown in 175-ml culture flasks to confluence. The monolayer was treated with Versene and the cells were suspended in medium 199 to a concentration of 10^5 cell/ml. The suspension was used in four variants of the experiment.

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In variant 1, cell suspension was incubated in 25-ml Carrel dishes (5 ml per dish) with 5 ml medium 199 supplemented with 10% bovine serum with or without 0.16% test drug at 37°C. The medium was not replaced util complete degeneration of the monolayer. The duration of incubation was 16 days for both control and test monolayers.

In variant 2, 5 ml cell suspension in 25-ml Carrel dishes was incubated with 5 ml medium 199 with or without 0.16% test preparation and 5 ml bovine serum. Culture medium was replaced every 2 days. The monolayer survived 21 days in both control and experimental dishes. The state of the monolayer was evaluated daily.

In variant 3, the cells were grown in 175-ml culture flasks in 10 ml medium 199 containing 0.16% test agent and 10% bovine serum at 37°C for 72 h. After attaining confluence, the cells was transferred onto coverslips every 2 days for morphological examination (10-min fixation in ethanol and staining with hematoxylin and eosin). A total of 10 passages were carried out (16 flasks per passage).

The remaining cell suspension was incubated for 72 h to confluence (variant 4). The cells were then treated with Versene and transferred into penicillin flasks and cultured in medium 199 with 10% serum in the absence (control, 16 flasks) and presence of test agent (0.08%, 16 flasks). Preparations for morphological analysis were prepared every 24 h. A total of 5 series were carried out.

RESULTS

Oil extract in a concentration below 0.16% exhibited no acute toxicity. The morphology of the cell monolayer did not differ from that in the control, cell cytoplasm was well spread, the nuclei were large and round

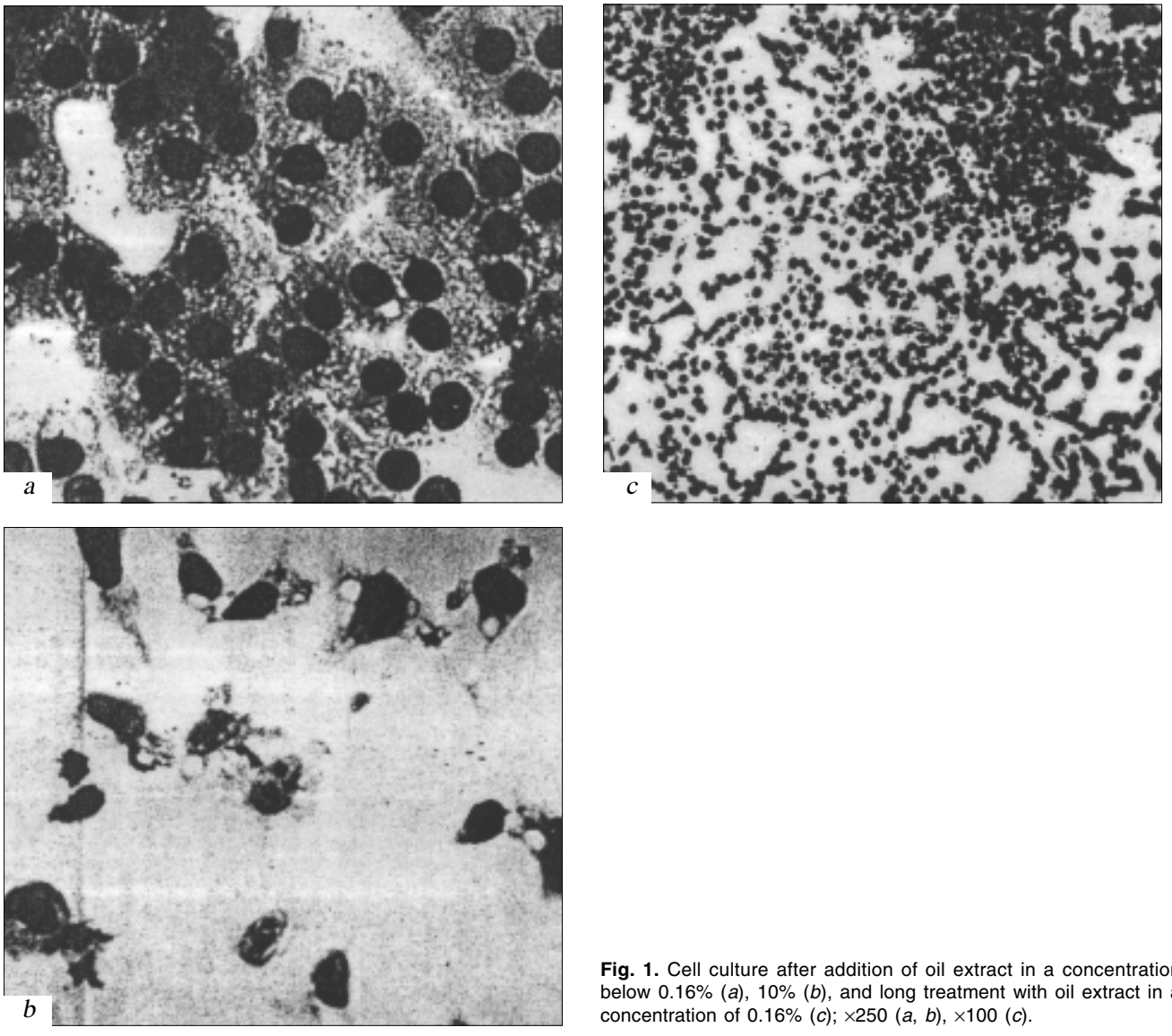

Fig. 1. Cell culture after addition of oil extract in a concentration below 0.16% (*a*), 10% (*b*), and long treatment with oil extract in a concentration of 0.16% (*c*); ×250 (*a*, *b*), ×100 (*c*).

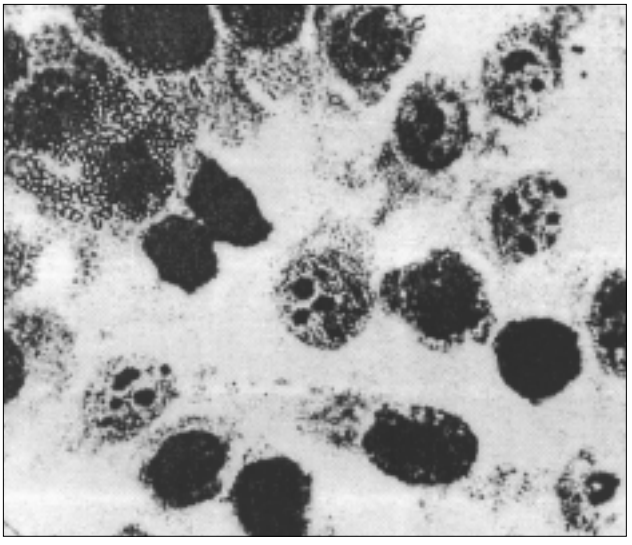

Fig. 2. Cell culture after addition of water extract in a concentration of 0.5%, ×400.

and contained 1-2 nucleoli. There were many young cells (Fig 1, *a*).

Oil extract in concentrations of 1-3% produced a weak cytotoxic effect: the monolayer was rarefied, cell cytoplasm vacuolized, and some cells were pyknotic.

Oil extract in concentrations of 3-10% was highly toxic for cells (50-70% dead cells, Fig. 1, *b*).

Water extract in concentrations of 0.1-0.5% was not toxic for cell monolayer (Fig. 2). The cells were well spread on the glass, the cytoplasm was finely granulated, the nuclei were round and contained 1-3 nucleoli, there were many young cells.

In studies of chronic toxicity we used oil extract in concentrations of 0.08 and 0.16% and water extract in a concentration of 0.5%. No morphological changes were found in cell monolayer after treatment with 0.16% oil extract: pathological mitoses were absent, the number of multinuclear and giant cells did not differ from normal for this cell strain, no nonspecific degenerative changes were seen (Fig. 1, *c*).

Treatment with 0.5% water extract produced no morphological changes: pathological mitoses were absent, the number of multinuclear cells (3.3 per 100 cells) and cells with giant nuclei (5.5 per 100) did not surpass the normal for this cells strain, no nonspecific degenerative changes were seen.

Hence, the cytotoxic effect of oil extract manifests at its concentrations >0.16%; the preparation is not toxic for cell monolayer in a dose of 0.04 ml/1.6×10^5 cells (*i.e.* about 1 g/kg human body weight). Water extract produced no toxic effects in concentrations below 0.5%, which corresponds to 3 g/kg.

According to the WHO International classification, these extracts belong to class 4 nontoxic substances and can be recommended for further studies in the above-mentioned concentrations.

The method of bioindication on cells can be used as express test system in development of new drugs, particularly for external application, because it clearly and accurately reflects the effects of test substances on cell viability.

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