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Analysis of apoptotic activity of *Helichrysum arenarium* extract *in vitro*

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Motivation and Aim: Sandy immortelle (*Helichrysum arenarium* (L.) Moench) is a perennial herbaceous plant of the Asteraceae family, growing in Europe and Central Asia. An infusion of immortelle flowers is used as a choleretic agent. It is known that immortelle flowers contain a significant amount of flavonoids (at least 20 compounds) belonging to various groups. Today, flavonoids are one of the most promising substances of plant origin for the creation of drugs, including those for the fight against cancer. A previous *in vivo* study of immortelle sandy extract on rats with transplanted sarcoma 45 established the positive effect of the extract in the treatment of malignant neoplasms: a decrease in tumor volume, as well as necrotic and dystrophic processes in it. The purpose of the work was to study the possible apoptotic activity of a flavonoid-containing extract of immortelle sandy on a culture of human kidney cancer cells A498.
Methods and Algorithms: The material for preparing the extracts was the flowers of the sandy immortelle. Immortelle extract was obtained according to the method described in Patent for invention No. 2482863: "Method of obtaining a dry extract from plant materials with biological activity". A study of the antitumor activity of immortelle extract was carried out on human kidney cancer A498 tumor cells obtained from the bank of tumor cultures of the Russian Cancer Research Center named after. N.N. Blokhina. Cell cultivation was carried out in plastic bottles in RPMI 4. Cells were cultured in a CO_2 incubator at 37 °C for 24 hours, after which they were stained. A Nikon microscope was used to visualize the cells. ImageJ software was used for cell counting. To detect morphofunctional changes in cells, staining with two dyes was used: acridine orange, which stains living cells, and propidium iodide, which penetrates non-viable cells.
Results: We studied the following concentrations of immortelle extract (mg/ml): 0.9; 1.8; 3.6; 7.2. The tests were carried out after 24 and 48 hours. A comparison was made in the control and experimental groups according to the following indicators: the number and ratio of cells with sickles to the number of living cells, the number and ratio of cells with pyknosis to the number of living cells, the number and ratio of cells in apoptosis to the number of living cells cells. The data were then statistically analyzed using SPSS 17.0 software. The normality of the distribution of characteristics in groups was determined using the Shapiro–Wilk test. The distribution of characteristics did not correspond to normal, therefore, to assess the presence of statistically significant differences, the nonparametric Mann–Whitney test (U-test) was used. Quantitative data were described using median, minimum and maximum values, 1st and 3rd quartiles. A result was considered statistically significant if the probability of rejecting the null hypothesis of no differences did not exceed 5 % ($p < 0.05$). Such indicators of apoptotic

activity as the number of cells with sickle-shaped nuclei, cells with pyknosis of the nucleus, as well as the ratio of the number of such cells to the number of living cells did not reveal differences between cells under the influence of immortelle extract and control. However, the number of cells that disintegrated into apoptotic bodies under the influence of immortelle extract at concentrations from 0.9 to 3.6 mg/mg after 24 hours was slightly higher than in the control, which indicates the presence of weak apoptotic activity of the extract as a whole. Weak apoptotic activity was also detected after 48 hours on the second day: at an extract concentration of 0.9 mg/ml, after 48 hours there is an increase in cells that disintegrated into apoptotic bodies, compared with 24 hours.
Conclusion: Thus, the extract of immortelle sandy has a weak apoptotic effect on the culture of human kidney cancer cells A498.
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