The cytotoxic activity of the dry extract from dahlia herb of *Ken's flame* variety studied on an in vitro model of human liver cell culture

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Anthocyanins are phenolic substances of a group of flavonoids that color fruits, leaves and petals of plants in colors ranging from pink to black and purple and have a wide range of biological activity. The actual problem of modern pharmacy is the search for promising plants with a high content of anthocyanins to create herbal medicines based on them. Of scientific interest are plants of the dahlia variety from the family *Asteraceae*.

Considering the literature data on the presence of anthocyanins in the composition of dahlia herb, as well as information about the known pharmacological effects of this group of substances, it can be assumed that extracts obtained from herb of *Ken's Flame* dahlia variety containing a sum of anthocyanins may show both cytotoxic activity and increase cell proliferation in culture [4–7].

The purpose of this study was to determine the presence and degree of manifestation of the cytotoxic or pro-proliferative activity of aqueous solutions of an extract obtained from *Ken's Flame* variety dahlia herb on an *in vitro* model of human liver cell culture [2–3].

Materials and methods. The dry extract, provided for research, was received at the NuPh Botany Department under the supervision of prof. Gontova T.M.

An experimental study was performed using a culture of human liver cells by means of the Nitro Blue Tetrazolium (NBT) Reduction test, which is widely used as a screening method for measuring cell survival and is included in most molecular biology and medicine protocols [1].

Cells were cultured *in vitro* at 37°C in DMEM (BioWest, France), supplemented with fetal bovine serum (BioWest, France) at a concentration of 10%. Cell culture was maintained in culture vials with a volume of 25 cm^2 until a dense monolayer was formed at 37 ± 0.5 ° C, 5% CO2, and atmospheric humidity 95%. Cells were incubated with test aqueous solutions of dahlia extract for 48 and 72 hours. The 0.5; 0.25; 0.125; 0.063; 0.03; 0.015% concentrations of the extract were investigated. Simultaneously, control samples of cells were incubated with buffer solution instead of the test solutions.

Cell survival upon contact with the test solutions was determined spectrophotometrically and calculated using the formula: (optical density of the experimental well / optical density of the control well) x 100%.

The data obtained were statistically processed using the Statistica 11.0 program. Firstly, the significance of differences of optical densities between the experimental and the control wells was evaluated, then the percentage of surviving cells in each well of the plate was calculated. As a result, each concentration of the sample was characterized by a set of values of surviving cells. Differences were considered significant at $p<0.05$.

Results obtained and their discussion. The estimation of human liver cells viability by means of the NBT Reduction test showed that the ability of a cell culture to restore tetrazolium in the presence of aqueous extracts from the dahlia herb of *Ken's Flame* variety has a dose and time dependence. The study results of the dahlia herb extract influence on the human liver cells viability are presented in Figure 1.

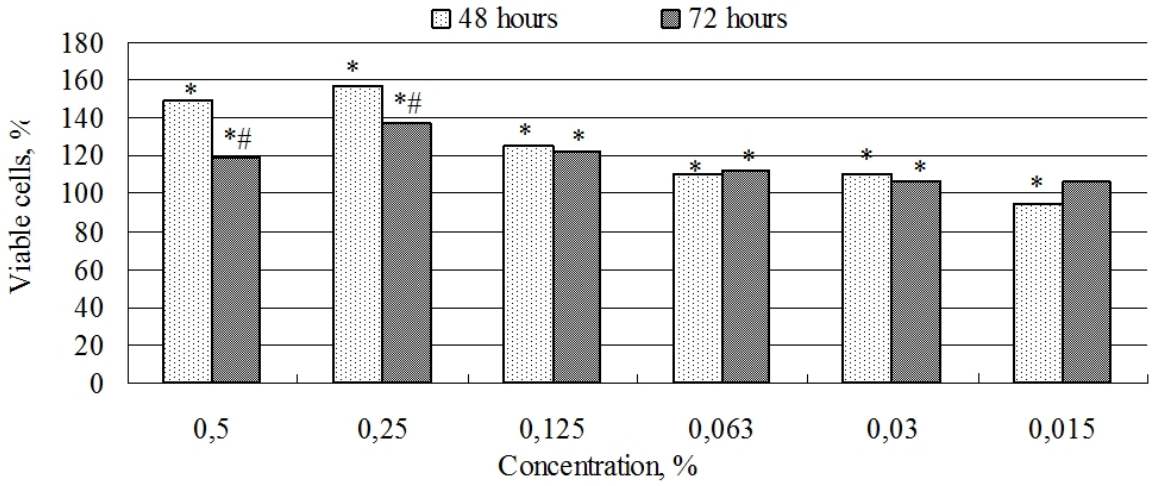

Fig. 1. The effect of the aqueous extract from the dahlia herb of *Ken's Flame* variety on the human liver cells viability determined in the NBT Reduction test. Note: * - the differences are significant relative to the control, $p<0.05$; # - differences significant between the 48 and 72 hours experimental options (depending on the incubation time), $p<0.05$; $n = 3$.

Solutions of dry dahlia herb extract at concentrations of 0.03 and 0.063%, when they contacted with cells for 48 hours, stimulated cell proliferation in culture, increasing the viability index to 110,69 and 110.07% ($p<0.05$), respectively. Concentration of 0.125%, during 48 hours exposure, increased the viability index to 125,07% ($p<0.05$). The most pronounced pro-proliferative activity had concentrations 0.03 and 0.063%, increasing the viability index to 156.95 and 149.25% ($p<0.05$), respectively. An aqueous solution of the extract at a concentration of 0.015% did not have a significant effect on the viability of human liver cells at an exposure of 48 hours.

An increase of the contact time of dahlia herb extract solutions with cells up to 72 hours resulted in a significant decrease in the number of viable cells in concentrations of 0.25 and 0.5% to 136.82% and 119.80 ($p<0.05$), respectively, compared with 48-hour exposure, which is probably

due to inactivation of the components of the studied substance. There was no significant difference in the *in vitro* vitality of human liver cells after incubation with an aqueous solution of dahlia extract in concentrations of 0.015 - 0.125% depending on the exposure.

Conclusions.

1. The application of a cell model of human liver cells makes it possible to establish and evaluate *in vitro* the existence of pro-proliferative activity of solutions made from dahlia herb extract which can be used in the future to analyze tolerability and increase the effectiveness of treatment with drugs based on the studied extracts.

2. The existence of pro-proliferative activity of the studied solutions from dahlia herb of *Ken's flame* variety has a dose and time dependence.

3. Aqueous solutions of dahlia herb extract have a pro-proliferative effect in concentrations of 0.03 – 0.5% and do not affect the passage of human liver cells through the cell cycle at a concentration of 0.015% in all the studied exposures.

Literature

1. Cancer cell culture: methods and protocols / Ser. Methods in Molecular Medicine. Vol. 88. (Ed. S.P. Langdon). Totowa, NJ: Humana Press, 2003. 165-169 p.
2. *Donato M. T.*, *Jover R.*, *Gomez-Lechon M. J.* Hepatic cell lines for drug hepatotoxicity testing: limitations and strategies to upgrade their metabolic competence by gene engineering // Curr. Drug Metab. *2013*. V. 14. p. 946–968.
3. Gómez-Lechón M. J., Tolosa L., Donato M. T. Cell-based models to predict human hepatotoxicity of drugs. Rev. Toxicol. 2014. V. 31. p.149—156.
4. Razina T. G. Antitumor effects of Sorbus aucuparia L. Extract highly saturated with anthocyans and their mechanisms // Bulletin of experimental biology and medicine. 2016. T. 162. №. 1. C. 93-97.
5. Reque P. M., Steffens R. S., Jablonski A., Flores S. H. et al. Cold storage of blueberry (Vaccinium spp.) fruits and juice: Anthocyanin stability and antioxidant activity. Journal of food composition and analysis. 2014. V. 33. №. 1. p. 111–116. doi: 10.1016/j.jfca.2013.11.007
6. Rugină D., Sconţa Z., Leopol, L., Pintea A. et al. Antioxidant activities of chokeberry extracts and the cytotoxic action of their anthocyanin fraction on HeLa human cervical tumor cells // Journal of medicinal food. 2012. V. 15. №. 8. p. 700-706.
7. Sagdic O., Ekici L., Ozturk I., Tekinay T., Polat et al. Cytotoxic and bioactive properties of different color tulip flowers and degradation kinetic of tulip flower anthocyanins // Food and chemical toxicology. 2013. V. *58*. p. 432-439.