doi 10.18699/bgrs2024-3.2-18

In vitro cytotoxic potential in human cancer cell line (C33a HPV-negative counterparts cervical cancer cell) of plant alkaloid, terpenoid and steroid derivatives

Hamad M.S.[1], Pokrovskii M.A.[1], Hamad S.S., Usenov K.[1], Pokrovskii A.G.[1]., Shinkarenko E.M.[1, 2], Finke A.O.[1, 2], Mironov M.E.[1, 2], Shults E.E.[2]

[1] *Novosibirsk State University, Novosibirsk, Russia*

[2] *Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia*

** mohammed_s1983@yahoo.com*

Key words: Medicinal plant; Anticancer; Antitumor; Cytotoxicity; Herbal medicine; apoptosis

Motivation and Aim: A tumor is a pathological process based on unregulated cell reproduction [1]. Cervical cancer is a malignant tumor of the mucous membrane of the vaginal portion and/or cervical canal. In 2023, malignant neoplasms claimed the lives of around 10 million individuals, with prevalent cancers encompassing breast, lung, colon and rectum, non-melanoma skin tumors, prostate, stomach, and cervical cancers. Cervical cancer, with approximately 16.5 thousand new cases annually in Russia, ranks fifth among cancers affecting women. Detection rates in early stages stand at 65% of patients, predominantly impacting middle-aged women. The historical use of herbal products in medicine, including anticancer drugs, remains significant [2]. Clinical trials have demonstrated the efficacy of various natural compounds like alkaloids, terpenes, phenols, and flavonoids [3]. Plant-derived substances form a substantial class of antitumor drugs, intervening in metabolic processes and enzymes crucial for their effects. Plant alkaloids, terpenoids, steroids and their synthetic derivatives, disrupt DNA integrity, transcription, repair, and mitosis, halting tumor cell proliferation [4, 5]. This report provided some data on the antitumor effects and mechanisms of alkaloids, terpenoids and steroids in the context of cervical cancer's hallmarks. A comparative analysis efficacy in cervical tumors cell line (C33a) in vitro. The paper provides an overview of the anticancer properties In vitro cytotoxic potential in human cell line (C33a HPV-negative counterparts), cervical cancer cell effects of derived plant alkaloids, terpenoids and steroids, emphasizing their pivotal role in the therapeutic landscape against cancer in future. The aim is to study the cytotoxic activity and the molecular mechanism of apoptosis-inducing effects of plant terpenoids, alkaloids and steroids in human tumor cells.

Materials: Human cell line cervical cancer (C33a HPV-negative counterparts), 148 compounds of derivatives of alkaloids, terpenoids and steroids, obtained in the laboratory of medical chemistry (No. 13-LMX). (Head of the laboratory, doctor of Chemical Sciences, Professor Shults Elvira Eduardovna). A mother (stock) solution of compounds at a concentration of 10 mM was obtained by dissolution in dimethyl sulfoxide and subsequently stored at -20 °C. FA, CHK, C -are alkaloids, LAN are derivatives of anthranilic acid (aromatic compounds), SV, Path - are sesquiterpenoids, HC – anthraquinone derivatives, KHAR, PD, PA, PA, – diterpenoids and MM –

Spirostanes, diosgenin derivatives (derivatives of steroid compounds). That is, all groups of substances are obtained by modification of natural compounds.
Method: *Cytotoxicity analysis of alkaloids, terpenoids and steroids using MTT test*
The cytotoxicity of alkaloids, terpenoids and steroids was evaluated using MTT test on potential in human cell line (C33a HPV-negative counterparts), cervical cancer carcinoma). According to stander protocol. Analysis of apoptosis by flow cytometry According to manufacturer's protocol.
Results: Study of the cytotoxic activity of plant derivatives, alkaloids, terpenes and steroids testing for cytotoxic activity in tumor cells humans was carried out in cell cultures C33a. Quantitative characteristics of cytotoxic activity, GI50, GI80 and GI90, as a result, it was discovered that some compounds are more active against 50% and 90% growth inhibition in cell lines C33a HPV-negative. C33a HPV-negative (MM-763, MM-765, MM-770, MM-771, MM-774, MM-775, MM-776, MM-784, MM-786, MM-787, MM-788, MM-789**,** MM-811, SHE-011**,** SHE052, SHE-022, SHE-027, SHE062, SHE078, FA-DM-13-1, FA-6921, FA-DM-648, FA-685, CK-24-11, CK-24-46, CK-28-84, MM-8092). Studying the ability of the most active compounds of terpenes, alkaloids, and steroids to induce apoptosis in C33a HPV-negative cells line in vitro.The cytotoxicity assay results obtained in the first phase of this project show high activity of a number of alkaloids, terpenes and steroids in suppressing cancer cell growth. We chose the most effective method for studying the type of cell death induction - cytometry, to search for the most active plant compounds alkaloids and steroids (**SHE052, FA-DM-13-1, CK-24-46, MM-763, MM-771, MM-811**) in two concentration **GI_{50}, µM**, **GI_{90}, µM.** Labeling cells with annexin-FITC markers makes it possible to detect early apoptosis (expression of phosphatidylserine in outer leaf of the cytoplasmic membrane and binding of annexin to phosphatidylserine) and propidium iodide – late cell death (fragmentation of nuclear DNA and penetration of propidium iodide through holes in the cytoplasmic and nuclear membranes and binding to the nuclear DNA).
Annexin V binds to phosphatidylserine. Usually, phosphatidylserine is found only in the inner layer of the cell membrane, but at the very beginning of apoptosis, PtdSer (phosphatidylserine) is transferred to the outer layer leaf. There it can bind the annexin V protein in a Ca2+-dependent manner. Thus, the early apoptosis phase is easily detected by cell labeling annexin V-FITC conjugate using flow cytometry (FACS).
Discussion: It has been reported that natural products steroids, terpenoids and alkaloids from plant sources have different biological activity such as antioxidant, anti-inflammatory or antiproliferative. Many of the reported anticancer effects natural compounds also target cellular proteins that play important role in signal transduction, apoptosis or cell cycle arrest. Our study reports on exploring the potential cytotoxic/antiproliferative activity of 148 secondary compounds metabolites found in plants such as alkaloids, steroids and terpenes, in the fight against tumor cells C33a HPV-negative. It has been shown that some of these plant-derived compounds have different mechanisms for the destruction of cancer cells, such as cell arrest cycle and intensification of the process of apoptotic cell death. These discoveries may pave the way for the development of new cancer treatments that exploit the therapeutic potential of natural compounds. Was found that C33a HPV-negative (Cervical cancer) cells exposed to effects of MM-763 and FA-DM-13-1, steroid derivatives, show high decreased metabolic activity (MTT test), externalization phosphatidylserine (Annexin-V). Of the 148 compounds studied from among derivatives of alkaloids, terpenoids and steroids, as shown in this work, 27 compounds showed high cytotoxic activity against

one or several cancer cell lines (C33a HPV-negative, 2 of these 27 compounds showed induction of apoptosis in a cell line (C33a HPV-negative counterparts), cervical cancer cell. Cells treated with compounds for (6) hours **GI_{50}, μM** MM-763,/ FA-DM-13-1 (at concentrations of 2.9 μM, 3.5 μM) contained 2.2%, 0.1%, 10.5% / 0.1%,0.1%, 0.5% cells in the stage of early apoptosis, respectively and **GI90, μM** MM-763, FA-DM-13-1 (at concentrations of 13 μM, 80 μM). contained 1.5%, 1.5%, 10.7% / 55.0% cells in the stage of early apoptosis, respectively, for 12 hours **GI_{50}, μM** MM-763, FA-DM-13-1 (at concentrations of 2.9 μM, 3.5 μM) contained 0.3%, 0.4%, 22.0% / 0.4%, 0.4% , 1.4% cells in the stage of early apoptosis, respectively and **GI90, μM** MM-763, FA-DM-13-1 (at concentrations of 13 μM, 80 μM), contained 2.0%,4.1%,67.1% / 0.6%, 6.7%, 78.5% cells in the stage of early apoptosis, respectively, for 24 hours **GI_{50}, μM** MM-763, FA-DM-13-1 (at concentrations of 2.9 μM, 3.5 μM), contained 0.4%, 1.0%,70.0% / 0.0%, 0.0% , 0.7% cells in the stage of early apoptosis, respectively and **GI90, μM** MM-763, FA-DM-13-1 (at concentrations of 13 μM, 80 μM), contained 0.8%, 0.8%, 69.2% / 0.0%, 0.2%, 48.3% cells in the stage of early apoptosis respectively.

References

1. American Cancer Society. Cancer Facts & Figures 2016. Atlanta, GA, USA: American Cancer Society, 2016
2. Ye H., Wang L., Ma L., Ionov M., Qiao G., Huang J., Cheng L., Zhang Y., Yang X., Cao S., Lin X. Protein kinases as therapeutic targets to develop anticancer drugs with natural alkaloids. *Front Biosci (Landmark Ed).* 2021;26(11):1349-1361
3. Hasanpourghadi M. et al. Phytometabolites targeting the warburg effect in cancer cells: A mechanistic review. *Curr Drug Targets*. 2017;18(9):1086-1094
4. Dey P., Kundu A., Chakraborty H.J., Kar B., Choi W.S., Lee B.M., Bhakta T., Atanasov A.G., Kim H.S. Therapeutic value of steroidal alkaloids in cancer: current trends and future perspectives. *Int J Cancer*. 2019;145(7):1731-1744
5. Huang Y., Li G., Hong C., Zheng X., Yu H., Zhang Y. Potential of steroidal alkaloids in cancer: Perspective insight into structure-activity relationships. *Front Oncol*. 2021;11:733369