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ORIGINAL ARTICLE

Antiproliferative properties of a new plant alkaloid against cellular colorectal cancer cultures

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ABSTRACT

Purpose of the study. To evaluate the antiproliferative properties of the novel alkaloid (P1) against CRC cell lines HT-29, Caco-2, and HCT116.
Materials and methods. CRC cell lines (HCT116, HT-29, Caco-2) were used in the experiments. The alkaloid (P1) was isolated from *Petasites hybridus* (L.) G. Gaertn., B. Mey. & Scherb and identified using high-performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy (NMR). Cells were incubated with various concentrations of the alkaloid, and cell viability was assessed. Berberine, a well-known anticancer alkaloid, served as the reference compound.
Results. The alkaloid (P1) demonstrated pronounced antiproliferative activity across all tested colorectal cancer cell lines – HCT116, HT-29, and Caco-2. The highest sensitivity was observed in HCT116 cells, with an IC_{50} value of 15.73 μmol/L after 72-hour incubation, indicating a substantial inhibitory effect on tumor cell proliferation. Comparative analysis showed that (P1) exhibited greater cytostatic efficacy than berberine in Caco-2 ($IC_{50}^{(P1)}$ = 54.489 ± 8.3 μmol/L vs $IC_{50}^{(berb)}$ = 193.154 ± 13.1 μmol/L) and HT-29 cultures ($IC_{50}^{(P1)}$ = 55.375 ± 7.1 μmol/L vs $IC_{50}^{(berb)}$ = 90.22 ± 8.2 μmol/L).
Conclusion. The findings indicate that the alkaloid (P1) possesses significant antiproliferative potential against colorectal cancer cell lines, underscoring its promise as a prospective anticancer agent. Notably, its superior efficacy compared with berberine highlights the relevance of further investigation. These results support continued development of (P1) as a basis for novel therapeutic agents. Future work should include detailed preclinical and clinical studies to elucidate its mechanism of action, evaluate safety and *in vivo* efficacy, and optimize pharmacological properties for potential clinical application.

Keywords: colorectal cancer, plant alkaloid, berberine, cytostatic properties, cell cultures

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Compliance with ethical standards: the work was carried out in compliance with the ethical principles set forth in the World Medical Association Declaration of Helsinki (1964, revised 2013). The study was approved by the Ethics Committee National Medical Research Centre for Oncology (protocol No. 5/223 dated 06.09.2024). Informed consent was obtained from all participants of the study.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

Антипролиферативные свойства нового растительного алкалоида в отношении клеточных культур колоректального рака

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РЕЗЮМЕ

Цель исследования. Оценить антипролиферативные свойства нового алкалоида (Р1) в отношении клеточных культур КРР HT-29, Caco-2 и HCT116.

Материалы и методы. В эксперименте использовались клеточные культуры КРР (HCT116, HT-29, Caco-2). Алкалоид (Р1) был выделен из *Petasites hybridus* (L.) G. Gaertn., B. Mey. & Scherb и идентифицирован с помощью методов ВЭЖХ и ядерного магнитного резонанса. Клетки инкубировали с различными концентрациями алкалоида и проводили анализ жизнеспособности клеток. Контрольным соединением являлся известный алкалоид берберин.

Результаты. В ходе эксперимента алкалоид (Р1) продемонстрировал выраженное антипрофелиферативное действие на все исследуемые клеточные линии колоректального рака – HCT116, HT-29 и Caco-2. Наиболее высокая чувствительность была выявлена у клеток линии HCT116, где значение IC_{50} составило 15,73 мкмоль/л при 72-часовой инкубации, что свидетельствует о значительной способности алкалоида подавлять пролиферацию этих опухолевых клеток. Кроме того, при сравнении активности алкалоида (Р1) с контрольным соединением – известным противоопухолевым алкалоидом берберином – было установлено, что (Р1) проявляет более высокую цитостатическую эффективность в культурах Caco-2 (IC_{50}^{P1} = 54,489 ± 8,3 мкмоль/л против IC_{50}^{berb} = 193,154 ± 13,1 мкмоль/л) и HT-29 (IC_{50}^{P1} = 55,375±7,1 мкмоль/л против IC_{50}^{berb} = 90,22 ± 8,2 мкмоль/л).

Заключение. Результаты проведенного исследования демонстрируют, что алкалоид (Р1) обладает выраженными антипролиферативными свойствами в отношении клеточных линий колоректального рака, что свидетельствует о его значительном потенциале в качестве противоопухолевого агента. Особенно важно отметить его высокую эффективность в сравнении с известным алкалоидом берберином, что подчеркивает перспективность дальнейших исследований данного соединения. Эти данные открывают новые возможности для разработки инновационных лекарственных препаратов на основе природных соединений. В дальнейшем необходимы углубленные доклинические и клинические исследования, направленные на изучение механизмов действия алкалоида (Р1), его безопасности и эффективности *in vivo*, а также оптимизацию его фармакологических свойств для возможного применения в клинической практике.

Ключевые слова: колоректальный рак, растительный алкалоид, берберин, цитостатические свойства, клеточные культуры

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование было одобрено Этическим комитетом ФГБУ «Национальный медицинский исследовательский центр онкологии» Министерства здравоохранения Российской Федерации (протокол №5/223 от 06.09.2024). Информированное согласие получено от всех участников исследования.

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Конфликт интересов: автор статьи Кит О. И. является главным редактором журнала «Южно-Российский онкологический журнал»; авторы статьи Дженкова Е. А. и Златник Е.Ю. являются членами редакционной коллегии журнала «Южно-Российский онкологический журнал». Статья прошла принятую в журнале процедуру рецензирования. Об иных конфликтах интересов авторы не заявляли.

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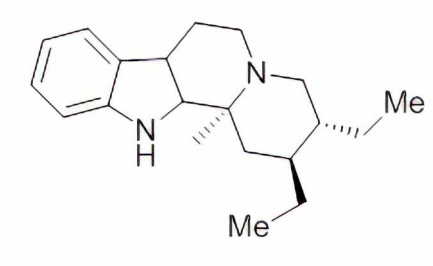
BACKGROUND

Cytostatic agents used in oncology represent an important class of drugs that play a key role in the treatment of various types of cancer, including colorectal cancer (CRC) [1]. According to the World Health Organization (WHO), the incidence of CRC continues to rise, underscoring the need to develop new cytostatic compounds with high antiproliferative activity and low toxicity [2].

In recent years, plant alkaloids have attracted increasing interest due to the established cytostatic properties demonstrated by many representatives of this group. One of the most extensively studied alkaloids, berberine, has shown the ability to inhibit cancer cell proliferation and induce apoptosis [3]. Berberine acts on key protein targets within signaling pathways that regulate cell growth, including phosphatidylinositol-3-kinase (PI3K), protein kinase B (Akt), and the mechanistic target of rapamycin (mTOR) in the PI3K/Akt pathway, as well as Raf kinase, mitogen-activated protein kinase (MEK), and extracellular signal-regulated kinase (ERK) in the MAPK pathway. These mechanisms allow berberine to be considered a promising antitumor agent [4].

However, despite encouraging results, berberine has several limitations, including low bioavailability and potential adverse effects, which may restrict its clinical applicability [5, 6]. In this context, the search for novel plant-derived alkaloids has become particularly relevant.

The compound (P1) investigated in our study belongs to the class of indole alkaloids and is structurally related to alkaloids isolated from plants of the genus *Corynanthe sp*., known for their analgesic and anti-inflammatory properties [7] (Fig. 1)



Chemical Formula: $C_{20}H_{30}N_2$
Molecular Weight: 298,4740

Fig. 1. Structural formula of compound (P1) isolated from *Petasites hybridus (L.)* G.Gaertn., B.Mey. & Scherb.

According to preliminary data, this compound demonstrates notable cytostatic effects against pancreatic cancer cell lines and non-small cell lung adenocarcinoma [8]. Comparing the activity of the novel alkaloid (P1) with berberine on CRC cultures will allow assessment of its efficacy and potential as a therapeutic candidate.

Purpose of the study: to evaluate the antiproliferative properties of the novel alkaloid (P1) against CRC cell lines HT-29, Caco-2, and HCT116.

MATERIALS AND METHODS

Dried and powdered rhizomes of *P. hybridus* (L.) were placed in a Soxhlet extractor; 250 mL of tetrachloroethylene (C_2Cl_4) was added to the extraction flask. Extraction was carried out under heating in the Soxhlet apparatus with a reflux condenser for 24 hours. After extraction, tetrachloroethylene was distilled off from the resulting 250 mL mixture, leaving 5 mL of extract in the distillation flask. The concentrated solution was applied to a chromatographic column packed with silica gel ($SiO_2 \cdot xH_2O$). The eluents used sequentially were C_2Cl_4, CH_2Cl_2, and a CH_2Cl_2/ EtOH mixture at a ratio of 10:1.

The structure of the isolated alkaloid (P1) was confirmed by nuclear magnetic resonance spectroscopy (^1H and ^{13}C NMR) [3]. After purification, the alkaloid was dissolved in dimethyl sulfoxide (DMSO) (Biolot, Russian Federation) to prepare a stock solution at a concentration of 8.8 mmol/L. A stock solution of berberine (25 mmol/L) was similarly prepared in DMSO from dry berberine chloride (Sigma-Aldrich, USA).

The experiment utilized CRC cell lines HT-29, Caco-2, and HCT116 obtained from the Cell Culture Collection of the Institute of Cytology, Russian Academy of Sciences (St. Petersburg, Russian Federation), as well as peripheral blood mononuclear cells (PBMCs) collected from healthy donors. Cancer cell lines were seeded at 5,000 cells per well in 96-well plates using complete culture medium (CCM) DMEM (Servicebio, China) supplemented

with 10 % fetal bovine serum (Gibco, USA), 1 % glutamine (Biolot, Russia), 1 % non-essential amino acids (Biolot, Russia), and 1 % penicillin-streptomycin (Biolot, Russia). After cell adhesion, the medium was replaced with CCM containing the tested alkaloids in serial twofold dilutions: 125 μmol/L to 10.12 μmol/L for berberine, and 44 μmol/L to 0.34 μmol/L for the novel alkaloid (P1). Cells were incubated for 24 and 72 hours at 37 °C in an atmosphere of 5.0 % CO_2. Multiple dilutions of berberine were tested in preliminary experiments, allowing us to determine the optimal concentrations for this study [9, 10].

PBMCs from healthy donors were obtained from venous blood collected in EDTA tubes (MiniMed, Russian Federation). On the day of collection, the blood was diluted 1:1 with RPMI 1640 medium (Servicebio, China) and layered onto Ficoll (Biolot, Russia), followed by centrifugation for 30 minutes at 730 g. The PBMC ring at the phase interface was carefully collected and transferred into a separate tube. The isolated PBMCs were washed once with RPMI 1640, counted, and seeded at 5,000 cells per well in 96-well plates in RPMI 1640 supplemented with 10 % fetal bovine serum. The alkaloid (P1) was added in serial twofold dilutions (44 μmol/L to 0.34 μmol/L), and the cells were incubated for 72 hours at 37 °C in an atmosphere of 5.0 % CO_2.

After incubation, both adherent cancer cell lines and PBMCs were stained using a mixture of nuclear dyes Hoechst 33342 (1 mg/mL) (ThermoFisher, USA) and ethidium bromide (10 mg/mL) (Servicebio, China) for direct counting of live and dead cells. Visualization was performed using a LionheartFX imager (BioTek, USA), and nuclear quantification was carried out using Gen5 software (BioTek, USA). Cell viability was calculated as the percentage of live cells in treated wells relative to untreated controls. Each experimental condition was plated in 8 replicates, and each experiment was repeated three times. Results are expressed as mean ± SD.

Statistical significance between mean viability values was assessed using the Student's t-test with Bonferroni correction. Dose–response curves and half-maximal inhibitory concentration (IC_{50}) values were calculated using the online tool IC_{50} Calculator ("Quest Graph™ IC_{50} Calculator." AAT Bioquest, Inc., 13 Feb. 2025, https://www.aatbio.com/tools/IC50-calculator).

STUDY RESULTS

The study demonstrated that the alkaloid (P1) exhibits a selective antiproliferative effect against the tested malignant cell lines. Incubation with 44 μmol/L of (P1) for 72 hours resulted in a > 30-fold increase in the number of dead cells relative to untreated controls in the HCT116 culture, a 7.55-fold increase in Caco-2, and a 6.37-fold increase in HT-29, which was significantly higher than in PBMCs from healthy donors at the same concentration (2.5-fold) (Fig. 2A). A decrease in the concentration of the tested compound (P1) was accompanied by a reduction in the magnitude of differences between malignant and normal cell cultures. Thus, incubation with 22 μmol/L (P1) resulted in significantly higher levels of cell death in two cultures – HCT116 (7.59-fold) and Caco-2 (3.41-fold) – compared to PBMCs (2.03-fold), whereas no significant difference was observed in HT-29 (2.22-fold). Finally, at 11 μmol/L, a significant difference remained only between PBMCs (1.09-fold) and Caco-2 (1.72-fold).

The cytostatic activity of alkaloid (P1) against CRC cultures varied within a relatively narrow range. After 24 hours of exposure, the half-maximal inhibitory concentration (IC_{50}) was lowest for HCT116 cells (IC_{50} = 51.98 ± 4.8 μmol/L) and highest for HT-29 (IC_{50} = 55.375 ± 7.1 μmol/L). After 72 hours, HCT116 again showed the lowest IC_{50} value (15.73 ± 3.2 μmol/L), whereas the highest value was observed in Caco-2 cells (IC_{50} = 32.505 ± 9.2 μmol/L), with HT-29 showing a similar response (IC_{50} = 29.075 ± 7.4 μmol/L). Across all cell lines, the dose–response curves demonstrated a hormetic effect – an increase in cell viability at low concentrations of alkaloid P1. At 24 hours, hormesis was observed in HCT116 and HT-29 cultures (Figs. 2C, 2D), whereas in Caco-2 cells the effect was more pronounced at 72 hours (Fig. 2B).

The sensitivity of the CRC cell lines to the antiproliferative effects of berberine varied across

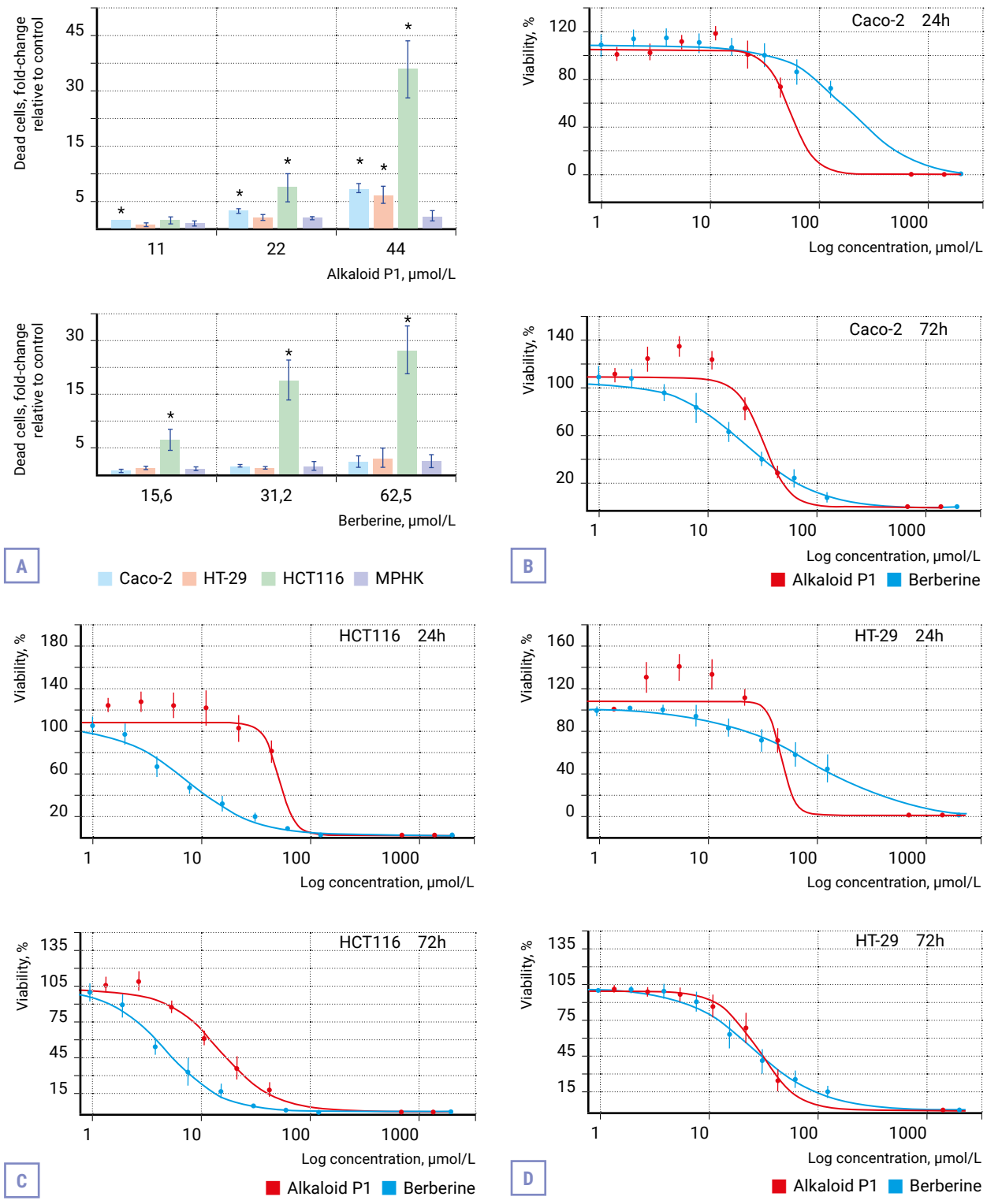


Fig. 2. Antiproliferative and cytostatic effects of alkaloid (P1). A – comparison of the antiproliferative activity of berberine and alkaloid (P1) against CRC cell cultures and PBMCs from healthy donors, 72 h exposure; B – dose–response curves for Caco-2; C – dose–response curves for HCT116; D – dose–response curves for HT-29.
* – the difference between CRC and PBMC values at the corresponding (P1) concentration is statistically significant, $p < 0.05$.
PBMC – peripheral blood mononuclear cells.

a broader range than in the assays with (P1). At 24-hour exposure, the IC_{50} for berberine was lowest in HCT116 (IC_{50} = 7.43 ± 2.4 μmol/L) and highest in Caco-2 (IC_{50} = 193.154 ± 13.1 μmol/L), with intermediate values in HT-29 (IC_{50} = 90.22 ± 8.2 μmol/L). At 72 hours, the lowest IC_{50} remained in HCT116 (IC_{50} = 4.94 ± 1.2 μmol/L), while the highest IC_{50} was observed in HT-29 (IC_{50} = 26.269 ± 4.5 μmol/L), with values in Caco-2 being similar (IC_{50} = 23 ± 3.1 μmol/L).

A direct comparison of the antiproliferative properties of the two alkaloids showed that at 24 h exposure, (P1) exhibited stronger cytostatic activity than berberine in Caco-2
($IC_{50}^{(P1)}$ = 54.489 ± 8.3 μmol/L vs
$IC_{50}^{(berb)}$ = 193.154 ± 13.1 μmol/L) (Fig. 2B)
and HT-29 ($IC_{50}^{(P1)}$ = 55.375 ± 7.1 μmol/L vs
$IC_{50}^{(berb)}$ = 90.22 ± 8.2 μmol/L) (Fig. 2D).
In HCT116, the opposite pattern was observed: berberine demonstrated markedly higher potency, with an IC_{50} nearly one order of magnitude lower
($IC_{50}^{(P1)}$ = 51.98 ± 4.8 μmol/L vs
$IC_{50}^{(berb)}$ = 7.43 ± 2.4 μmol/L).
With prolonged exposure (72 h), IC_{50} values for both alkaloids converged in Caco-2 and HT-29: Caco-2:
$IC_{50}^{(P1)}$ = 32.505 ± 9.2 μmol/L vs
$IC_{50}^{(berb)}$ = 23 ± 3.1 μmol/L.
HT-29: $IC_{50}^{(P1)}$ = 29.075 ± 7.4 μmol/L vs
$IC_{50}^{(berb)}$ = 26.269 ± 4.5 μmol/L.
In HCT116, the higher sensitivity to berberine remained evident
($IC_{50}^{(P1)}$ = 15.73 ± 3.2 μmol/L vs
$IC_{50}^{(berb)}$ = 4.94 ± 1.2 μmol/L).

DISCUSSION

The findings of this study indicate that the alkaloid (P1), isolated from *Petasites hybridus* (L.) G. Gaertn., B. Mey. & Scherb., induces dose-dependent cell death in several colorectal adenocarcinoma cell lines. Its cytostatic effect is influenced not only by concentration but also by exposure time, with the strongest effect occurring after 72 hours of incubation.

Among the tested lines, HCT116 demonstrated higher sensitivity to both (P1) and berberine compared with Caco-2 and HT-29. This highlights the importance of selecting appropriate cell lines for assessing the activity of novel anticancer agents, given the notable inter-line variability in drug response.

The CRC lines studied have distinct molecular and metabolic features that likely underlie their differing sensitivities to (P1) and berberine. For example, HCT116 is more sensitive than HT-29 to FOLFOX chemotherapy and hypoxia, possibly due to the absence of p53 loss in HCT116, whereas HT-29 carries a p53 deficiency and displays microsatellite instability [11]. Caco-2 cells lack activating mutations in KRAS, NRAS, BRAF, and PIK3CA and are resistant to cetuximab [12]. Their capacity for enterocyte-like differentiation is strongest among the three lines and often used in drug absorption studies rather than tumor modeling [13, 14].

HCT116 is highly aggressive, poorly differentiated, and enriched in cancer stem cell-like subpopulations [15]. It exhibits MDR1 overexpression and associated chemoresistance linked to NOX and Nrf2 gene activity [16]. Molecularly: HCT116 harbors KRAS codon 13 mutations; HT-29 expresses KRAS, APC, and BRAF V600E [12, 17].

Thus, differences in response to (P1) and berberine may reflect variations in KRAS, TP53, and MLH gene status, which regulate RAS/RAF/MAPK and PI3K/Akt/mTOR signaling, cell cycle progression, apoptosis, and DNA repair [18, 19].

Berberine, widely recognized for its antitumor activity, also demonstrated substantial cytostatic effects against CRC cells. Its mechanism of action includes inhibition of enzymes involved in glucose and lipid metabolism, as well as modulation of signaling pathways such as the AMPK pathway [20]. Studies have shown that berberine can induce apoptosis through caspase activation and suppression of protein kinases [21].

We compared our findings on berberine with results reported by other international research groups, as the cytostatic activity values (IC_{50}) obtained in our study varied within a broad range. In several studies, depending on the exposure time, IC_{50} values for berberine in HT-29 cells ranged from 34.6 to 52.37 ± 3.45 μM [22, 23], while in HCT116

cells they ranged from 31 to 55.27 μM [24–26]. At these concentrations, berberine reduced the expression of aquaporins 1, 3, and 5 and increased PTEN expression in HT-29, SW-480, and HCT116 cultures. Upregulation of PTEN contributed to suppression of the PI3K, AKT, and mTOR signaling pathways, leading to enhanced apoptosis in CRC cells and reduced migratory capacity – findings consistent with those reported by other researchers [23].

An important aspect relevant to our study is the previously documented sensitivity of HCT116 cells to several plant-derived compounds. For example, their in vitro growth is inhibited by flavopiridol – a compound developed from a natural molecule through substitution of a flavonoid moiety with a nitrogen-containing heterocyclic alkaloid. Flavopiridol has been shown to inhibit CDK9 kinase and exhibit antitumor activity in lymphoproliferative disorders. In recent years, HCT116 cells have also demonstrated sensitivity to a number of additional plant-derived and synthetic compounds [26–28]. In the study by Parry R. A. et al., various fractions obtained from extracts of Alcea rosea were tested, and HCT116 cells exhibited greater sensitivity to these fractions in an MTT assay compared with HT-29 cells [29].

Our findings support the hypothesis that the novel plant alkaloid (P1) exerts markedly greater efficacy against CRC cells compared with berberine.

CONCLUSION

The novel alkaloid (P1), isolated from *Petasites hybridus* (L.) G.Gaertn., B.Mey. & Scherb, demonstrates clear dose-dependent cytostatic activity against colorectal cancer (CRC) cell cultures, while exerting minimal effects on peripheral blood mononuclear cells (PBMCs). Among the tested cell lines, HCT116 exhibited the highest sensitivity to P1. The results of this study indicate that the new plant-derived alkaloid (P1) is markedly more effective and possesses strong antiproliferative potential against CRC cells.

Thus, the findings highlight the relevance of P1 as a promising candidate for the development of new therapeutic strategies against colorectal cancer. However, further research is required to draw definitive conclusions, including comprehensive evaluation of its toxicity, pharmacokinetics, and mechanism of action.

References

1. Shi C, Yang EJ, Tao S, Ren G, Mou PK, Shim JS. Natural products targeting cancer cell dependency. J Antibiot (Tokyo). 2021 Oct;74(10):677–686. https://doi.org/10.1038/s41429-021-00438-x
2. Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag CJ, Laversanne M, et al. Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. Gut. 2023;72(2):338-344. https://doi.org/10.1136/gutjnl-2022-327736
3. Zlatnik EYu, Enin YaS, Burov ON, Bondarenko ES, Sagakyants AB, Kutilin DS, et al. Molecular and cellular aspects of the impact of secondary metabolites from common barberry and hybrid goat's rue on the HeLa cell line. Research and Practical Medicine Journal. 2023;10(4):31–47. (In Russ.). https://doi.org/10.17709/2410-1893-2023-10-4-3
4. Filippova SYu, Timofeeva SV, Sitkovskaya AO, Mezhevova IV, Enin YaS, Burov ON, et al. Influence of berberine on energy phenotype of breast cancer cell cultures. International Journal of Applied and Fundamental Research. 2021;10:42–46. (In Russ.). EDN: CQWAKN
5. Timofeeva SV, Kit OI, Filippova SYu, Sitkovskaya AO, Mezhevova IV, Gnennaya NV, et al. Some Plant Metabolites from Petasítes sp. and Their Effect on Cancer Cells Motility in vitro. Journal of Clinical Oncology. 2022;40(16):15077. https://doi.org/10.1200/jco.2022.40.16_suppl.e15077
6. Timofeeva SV, Zlatnik EY, Vaschenko LN, Enin YS, Nepomnyashchaya EM. Molecular mechanisms of berberine action on tumor cells. Kazan Medical Journal. 2025;106(2):267–276. (In Russ.). https://doi.org/10.17816/kmj643366
7. Li J, Li JX, Jiang H, Li M, Chen L, Wang YY, et al. Phytochemistry and biological activities of corynanthe alkaloids. Phytochemistry. 2023 Sep; 213:113786. https://doi.org/10.1016/j.phytochem.2023.113786

8. Mezhevova IV, Filippova SYu, Chembarova TV, Gnennaya NV, Zlatnik EYu, Novikova IA, et al. Some plant secondary metabolites as promising candidates for the treatment of lung cancer and pancreatic cancer. Problems of Biological, Medical and Pharmaceutical Chemistry. 2024;27(9):32–40. (In Russ.). https://doi.org/10.29296/25877313-2024-09-05
9. Mezhevova IV, Filippova SYu, Timofeeva SV, Sitkovskaya AO, Shamova TV, Enin YaS, et al. Antimigratory effect of berberine in T98G, U87MG and primary glioma cell culture. Journal of Clinical Oncology. 2021;39(S15): e15045.
10. Filippova SY, Shamova TV, Timofeeva SV, Sitkovskaya AO, Mezhevova IV, Gnennaya NV, et al. Influence of some metabolites from plants of the genus Petasítes sp. on cancer cells motility in vitro. Genes & Cells. 2022;17(2):60–63. (In Russ). https://doi.org/10.23868/202209009
11. Ieranò C, Righelli D, D'Alterio C, Napolitano M, Portella L, Rea G, et al. In PD-1+ human colon cancer cells NIVOLUMAB promotes survival and could protect tumor cells from conventional therapies. J Immunother Cancer. 2022 Mar;10(3):e004032. https://doi.org/10.1136/jitc-2021-004032
12. Bovio F, Epistolio S, Mozzi A, Monti E, Fusi P, Forcella M, Frattini M. Role of NEU3 Overexpression in the Prediction of Efficacy of EGFR-Targeted Therapies in Colon Cancer Cell Lines. Int J Mol Sci. 2020 Nov 20;21(22):8805. https://doi.org/10.3390/ijms21228805
13. Shchulkin AV, Tranova YuS, Abalenikhina YuV, Esenina AS, Slepnev AA, Yakusheva EN. Cells of the Caco-2 line as a model for studying the absorption of medicinal substances. Experimental and Clinical Gastroenterology. 2022;(10):63–69. (In Russ.). https://doi.org/10.31146/1682-8658-ecg-206-10-63-69
14. Hoffmann P, Burmester M, Langeheine M, Brehm R, Empl MT, Seeger B, Breves G. Caco-2/HT29-MTX co-cultured cells as a model for studying physiological properties and toxin-induced effects on intestinal cells. PLoS One. 2021 Oct 7;16(10):e0257824. https://doi.org/10.1371/journal.pone.0257824
15. Yeung TM, Gandhi SC, Wilding JL, Muschel R, Bodmer WF. Cancer stem cells from colorectal cancer-derived cell lines. Proc Natl Acad Sci U S A. 2010 Feb 23;107(8):3722–3727. https://doi.org/10.1073/pnas.0915135107
16. Waghela BN, Vaidya FU, Pathak C. Upregulation of NOX-2 and Nrf-2 Promotes 5-Fluorouracil Resistance of Human Colon Carcinoma (HCT-116) Cells. Biochemistry (Mosc). 2021 Mar;86(3):262–274. https://doi.org/10.1134/s0006297921030044
17. Ghodousi-Dehnavi E, Hosseini RH, Arjmand M, Nasri S, Zamani Z. A Metabolomic Investigation of Eugenol on Colorectal Cancer Cell Line HT-29 by Modifying the Expression of APC, p53, and KRAS Genes. Evid Based Complement Alternat Med. 2021 Nov 18;2021:1448206. https://doi.org/10.1155/2021/1448206
18. Tatar M, Varedi M, Naghibalhossaini F. Epigenetic Effects of Blackberry Extract on Human Colorectal Cancer Cells. Nutr Cancer. 2022;74(4):1446–1456. https://doi.org/10.1080/01635581.2021.1952454
19. Mikhalenko AP, Shchayuk AN, Kilchevsky AV. Signaling pathways: a mechanism for regulating the proliferation and survival of tumor cells. Molekulyarnaya i prikladnaya genetika. 2019;26:145–157. (In Russ.).
20. Filippova SY, Sitkovskaya AO, Timofeeva SV, Shamova TV, Mezhevova IV, Gnennaya NV, Novikova IA. Application of silicone coating to optimize the process of obtaining cellular spheroids by the hanging drop method. South Russian Journal of Cancer. 2022;3(3):15–23. https://doi.org/10.37748/2686-9039-2022-3-3-2
21. Timofeeva SV, Filippova SYu, Sitkovskaya AO, Gnennaya NV, Mezhevova IV, Shamova TV, et al. Bioresource collection of cell lines and primary tumors of the National Medical Research Center of Oncology of the Ministry of Health of Russia. Cardiovascular Therapy and Prevention. 2022;21(11):3397. (In Russ.). https://doi.org/10.15829/1728-8800-2022-3397
22. Li Q, Zhao H, Chen W, Huang P. Berberine induces apoptosis and arrests the cell cycle in multiple cancer cell lines. Arch Med Sci. 2021;19(5):1530–1537. https://doi.org/10.5114/aoms/132969
23. Tarawneh N, Hamadneh L, Abu-Irmaileh B, Shraideh Z, Bustanji Y, Abdalla S. Berberine Inhibited Growth and Migration of Human Colon Cancer Cell Lines by Increasing Phosphatase and Tensin and Inhibiting Aquaporins 1, 3 and 5 Expressions. Molecules. 2023 Apr 29;28(9):3823. https://doi.org/10.3390/molecules28093823
24. Samad MA, Saiman MZ, Abdul Majid N, Karsani SA, Yaacob JS. Berberine Inhibits Telomerase Activity and Induces Cell Cycle Arrest and Telomere Erosion in Colorectal Cancer Cell Line, HCT 116. Molecules. 2021 Jan 13;26(2):376. https://doi.org/10.3390/molecules26020376
25. Li P, Hao Z, Liu H, Zhu B, Dang L, Ma C, et al. Quantitative Proteomics Analysis of Berberine-Treated Colon Cancer Cells Reveals Potential Therapy Targets. Biology (Basel). 2021 Mar 23;10(3):250. https://doi.org/10.3390/biology10030250

26. Gong C, Hu X, Xu Y, Yang J, Zong L, Wang C, et al. Berberine inhibits proliferation and migration of colorectal cancer cells by downregulation of GRP78. Anticancer Drugs. 2020 Feb;31(2):141–149. https://doi.org/10.1097/cad.0000000000000835
27. Duda-Madej A, Viscardi S, Szewczyk W, Topola E. Natural Alkaloids in Cancer Therapy: Berberine, Sanguinarine and Chelerythrine against Colorectal and Gastric Cancer. Int J Mol Sci. 2024;25(15):8375. https://doi.org/10.3390/ijms25158375
28. Och A, Lemieszek MK, Cieśla M, Jedrejek D, Kozłowska A, Pawelec S, Nowak R. Berberis vulgaris L. Root Extract as a Multi-Target Chemopreventive Agent against Colon Cancer Causing Apoptosis in Human Colon Adenocarcinoma Cell Lines. Int J Mol Sci. 2024 Apr 27;25(9):4786https://doi.org/10.3390/ijms25094786
29. Parry RA, Mir IA, Bhat BA, Hussain MU, Ashraf S, Zaman GS, et al. Exploring the cytotoxic effects of bioactive compounds from Alcea rosea against stem cell driven colon carcinogenesis. Sci Rep. 2025 Feb 18;15(1):5892. https://doi.org/10.1038/s41598-025-89714-6

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