Research in Pharmaceutical Sciences, June 2025; 20(3): 408-415
Received: 15-03-2024
Peer Reviewed: 14-08-2024
Revised: 23-10-2024
Accepted: 09-11-2024
Published: 17-06-2025

School of Pharmacy & Pharmaceutical Sciences
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Original Article

Genistein potentiated the cytotoxic effect of entinostat in colorectal cancer cell lines

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Abstract

Background and purpose: Colorectal cancer (CRC) is the second leading cause of cancer death. While surgery and medicines offer complete treatment, recurrence and medication resistance pose challenges. This study assessed the cytotoxic impact of entinostat, a histone deacetylase (HDAC) inhibitor, and genistein, a soybean isoflavone, combination on CRC cells.
Experimental approach: The cytotoxic effect of genistein, combined with entinostat, was tested in HCT-116 and HT-29 cell lines, along with their impact on migration and colony formation. Gene expression of the cell cycle regulatory protein CDC25A was assessed using qPCR.
Findings/Results: The IC_{50} values of genistein, entinostat, and their combination in HCT-116 cells were 24.48 µM, 13.65 µM, and 14.55 µM, respectively. In HT-29, the IC_{50} values were 30.41 µM, 20.25 µM, and 19.98 µM, respectively. In the HT-29 cell line, a 1:1 ratio of entinostat and genistein resulted in a combination index of 0.6 using a concentration of 1.56 µM of each compound, indicating a synergistic effect. In contrast, no synergistic effect was produced between the two drugs in the HCT-116 cell line. In HCT-116 cells, genistein, entinostat, and their combination significantly reduced wound closure compared to the control. In contrast, in HT-29 cells, only the combination treatment was effective, while genistein and entinostat alone showed no notable impact. In HCT-116, entinostat, genistein, and their combination reduced the number of colonies significantly compared to the control, while in HT-29, only entinostat and the combination reduced the number of colonies significantly compared to the control. Furthermore, the combination of genistein with entinostat was more effective in reducing CDC25A expression in the HT-29 cells compared to entinostat treatment alone.
Conclusions and implications: Combining genistein with entinostat could potentiate the entinostat cytotoxic effect in CRC.

Keywords: CDC25A; Colorectal cancer; Entinostat; Genistein; Good health and well-being; Scratch assay.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer type worldwide in males and the second in females, with 1.9 million cases diagnosed in 2020 (1-3). On a global scale, 60% of CRC cases are typically diagnosed among individuals aged 50-74 years old, while nearly 10% manifest in adults under the age of 50 (4). In nations characterized by a very high human development index, the incidence rates were four times greater than in countries with a lower index (4). Several risk factors for CRC that can be modified have been recognized. These include an unhealthy diet characterized by high consumption of red and processed meat, and low intake of fruits, vegetables, and dietary fiber. Other modifiable risk factors encompass high alcohol consumption, physical inactivity, and excess body weight (5).

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Access this article online	
	Website: http://rps.mui.ac.ir
	DOI: 10.4103/RPS.RPS_59_24

Metastatic CRC, which exhibits a mere ~14% 5-year survival rate, continues to pose a significant threat. Unlike early-stage CRC, which can be effectively treated through surgery with or without adjuvant chemotherapy, metastatic CRC presents a formidable challenge. This is attributed to the substantial presence of disseminated cancer cells, comprising therapy-resistant and metastasis-competent cells, making complete eradication unattainable (6).

CRC arises as a result of genetic or epigenetic alterations, which transform normal glandular epithelial cells into benign neoplasms (adenomas) and subsequently into invasive carcinomas and eventually metastatic cancer (7). Epigenetic changes, which are heritable variations in the patterns of gene expression without altering the underlying DNA sequence, can be brought about by exposure to environmental elements such as pollution, toxicants, inflammation, and nutrition (8). Epigenetic changes, including DNA methylation, histone modifications, and noncoding RNAs, have recently been demonstrated in CRC (9,10). Inhibitors of histone deacetylases (HDACs) are highly effective drug compounds capable of promoting histone acetylation at lysine residues. They induce an open chromatin conformation at the loci of tumor suppressor genes, ultimately leading to the suppression of tumors (11).

The Food and Drug Administration (FDA) has approved certain HDAC inhibitors for certain malignancies (11). However, the therapeutic potential of HDAC inhibitors can only be realized through combination treatments (9). Consequently, numerous clinical trials are underway to explore combinations of HDAC inhibitors with DNA methyltransferase inhibitors and histone methyltransferase (11). The utilization of a combination of epigenetic drugs in the treatment of CRC has emerged as an intriguing area of investigation. However, there is currently limited data available regarding their effectiveness. In a previous study, HCT-116 human CRC carcinoma cells were treated with an HDAC inhibitor (entinostat) combined with irinotecan, a topoisomerase-1 inhibitor. It has been observed that the combination considerably raised cell death to 60% in HCT-116 cells (9). A notable recent clinical trial has shed light on this topic. In their study involving CRC patients, a demethylating agent (5-azacitidine) along with entinostat was used. The results indicated a progression-free survival above the median threshold (12). The results of such studies encouraged us to investigate the effect of a combination of entinostat and genistein. Genistein, chemically identified as 5,7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one, is a naturally occurring isoflavone predominantly found in soy products. It shares a similar chemical structure with mammalian estrogens and has garnered interest for its potential health benefits, including protection against osteoporosis, reducing the risk of cardiovascular disease, alleviating postmenopausal symptoms, and exhibiting anticancer properties (13). Genistein was chosen because of its well-known anticancer effects, along with its dimethyl transferase inhibitory activity on CRC (14,15). The suppression of cancer facilitated by dietary polyphenols like genistein is linked to the reactivation of genes through the demethylation process in the promoters of tumor suppressor genes that were silenced by methylation (15).

MATERIALS AND METHODS

Cell lines and culture conditions

The human colorectal adenocarcinoma HT-29 and the human colorectal carcinoma HCT-116 cell lines were purchased from the American Type Culture Collection (ATCC). HT-29 cells were cultivated in Roswell Park Memorial Institute (RPMI; EuroClone, Italy) growth medium and HCT-116 cells in high glucose Dulbecco's Modified Eagle Medium (DMEM; EuroClone, Italy). Both growth media were supplemented with 10% fetal bovine serum (FBS; Gibco, Germany) and 1% penicillin/streptomycin (EuroClone, Italy).

Cell proliferation assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT; Promega, USA) assay was used. Briefly, 10,000 cells/well were seeded into a 96-well plate. After 24 h, cells were treated, in triplicate, with genistein (Tocris Bioscience, UK; 0.78-100 µM), entinostat (MS-275, Tocris Bioscience, UK; 0.78-100 µM),

or their combination. Both entinostat and genistein stock solutions were prepared by dissolving in dimethyl sulfoxide (DMSO) so that their final concentration did not exceed 2%. Cells were incubated for 48 h at 37 ℃ with 5% CO_2. MTT test was performed according to the manufacturer's directions, and the estimated half-maximal inhibitory concentration (IC_{50}) was calculated using GraphPad Prism software version 8. The assessment of synergy resulting from a 1:1 fixed ratio of entinostat and genistein combination was calculated using CompuSyn software (version 1.0). The combination index (CI) values: = 1, > 1, and < 1 indicate additive, antagonistic, and synergistic effects, respectively.

Cell migration (scratch) assay

HT-29 and HCT-116 cells were seeded in a 12-well plate with a seeding density of 200,000 and 280,000 cells/well for HCT-116 and HT-29, respectively. Cell migration assay was performed as previously described (16). Cells were incubated for 24 h. Then, a line was made using a micropipette tip. Then, HT-29 and HCT-116 cells were treated with a concentration equal to the IC_{50} of genistein, entinostat, or their combination in that particular cell line. After incubation for 48 h, the wound area was measured using Motic software after photographing using an Inverted Nikon Microscope (Japan) equipped with a camera. Closure percent was calculated as follows:

Closure (%) = 100 - (area of wound after 48 h/ area at 0 time) × 100.

Colony-forming assay

HT-29 and HCT-116 cells (600 cells/well) were seeded into a 6-well plate in triplicate and incubated overnight as previously described with slight modifications (17). Then, HT-29 and HCT-116 cells were treated with a concentration equal to the IC_{50} of genistein, entinostat, or their combination in that particular cell line. After 14-day incubation at 37 ℃ and 5% CO_2, the media were removed, and the plates were washed with phosphate-buffered saline (PBS; EuroClone, Italy). Then, cells were fixed by adding 4% paraformaldehyde, stained with 500 µL crystal violet, and colonies were counted.

Gene expression for cell cycle genes

HT-29 and HCT-116 CRC cells were seeded with a seeding density of 1,000,000 cells per flask in 7 mL RPMI and DMEM media, respectively, and incubated overnight at 37 ℃ and 5% CO_2. Then, HT-29 and HCT-116 cells were treated with a concentration equal to the IC_{50} of genistein, entinostat, or their combination in that particular cell line and incubated for 48 h at 37 ℃ and 5% CO_2. After that, cells were washed and pellets were used for RNA extraction.

The treated cells were used for RNA extraction using the Promega (USA) kit, according to the manufacturer's instructions. The extracted RNA was reverse transcribed into complementary DNA (cDNA) using a reverse transcription kit (TaKaRa, China). Ten µL of the mixture was transferred into a PCR tube to produce a final concentration of cDNA (250 ng). The reaction was incubated in a thermal cycler (Applied Biosystem, Singapore). Relative expression level analysis of the genes was done using a quantitative polymerase chain reaction (qPCR) kit (Promega, USA). The test was performed according to the manufacturer's protocol. Real-Time PCR was performed using a QuantStudio (Thermo Fisher, Singapore) with the following parameters: 95 ℃ for 2 min, followed by 95 ℃ for 30 s, 60 ℃ for 30 s, then 72 ℃ for 30 s for 40 cycles. A further melting curve step at 55-95 ℃ was applied. CDC25A and gene β-actin sense and antisense primers were used. All expression data for each sample were normalized by dividing the quantity of target by the amount of β-actin used as an internal control. The sequence of forward and reverse primers is shown in Table 1 as previously described (18).

Table 1. Sequence of amplification primers.

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')
CDC25A	GTGAAGGCGCTATTTGGCG	TGGTTGCTCATAATCACTGCC
β-Actin	CGGGACCTGACTGACTACC	TGAAGGTAGTTTCGTGGATGC

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to compare different parameters in different groups. GraphPad Prism software version 8 was applied to analyze the data. *P*-values < 0.05 were considered statistically significant.

RESULTS

Cytotoxicity assay

The IC_{50} values of genistein were 24.48 µM and 30.41 µM in HCT-116 and HT-29 cells, respectively. For entinostat, the determined IC_{50} values in HCT-116 and HT-29 were 13.65 µM and 20.25 µM, respectively. When combined, the IC_{50} values were 14.55 µM and 19.98 µM for HCT-116 and HT-29, respectively (Fig. 1A and B).

Among all tested combinations of genistein and entinostat, only a combination of 1.56 µM of each drug resulted in a synergistic effect in HT-29 (Table 2, Fig. 2).

Migration/scratch assay

In HCT-116 cells, all treatments significantly reduced wound closure compared to control: 46.3% (genistein), 54.5% (entinostat), 51.7% (combination), versus 70.7% (control) (Fig. 3A). Similarly, in HT-29 cells, genistein and entinostat alone had no effect, but their combination significantly reduced wound closure to 14.5%, compared to 35.2% (genistein), 31.3% (entinostat), and 33% (control) (Fig. 3B).

Table 2. CI of genistein and entinostat combination in HT-29 and HCT-116 cell lines.

Genistein (µM)	Entinostat (µM)	CI in HCT-116	CI in HT-29
25	25	0.98	1.59
12.5	12.5	1.31	1.32
6.25	6.25	1.12	1.07
3.12	3.12	1.29	1.09
1.56	1.56	3.14	0.60

CI, Combination index.

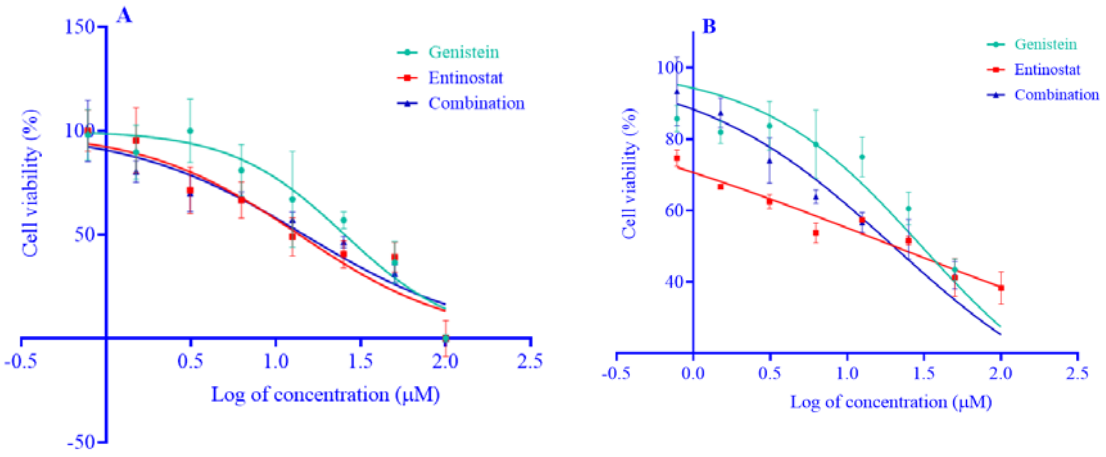

Fig. 1. Determination of cytotoxic effect of genistein, entinostat, and their combination on (A) HCT-116 and in (B) HT-29 cells after 48-h treatment using MTT assay. Values represent mean ± SD and were performed in triplicate.

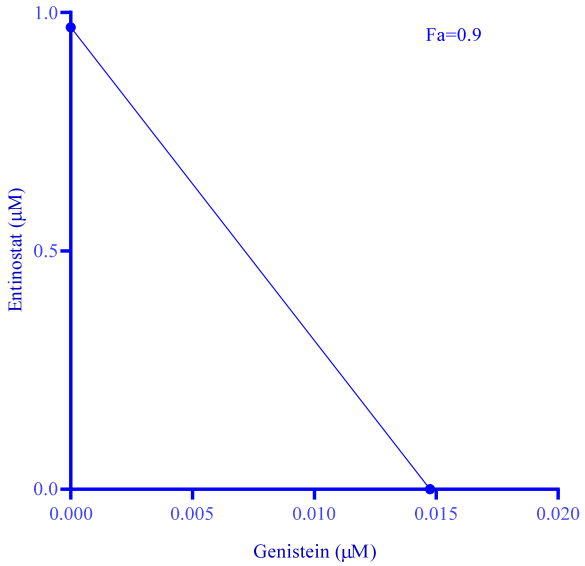

Fig. 2. Isobologram for a 1:1 ratio of entinostat-genistein combination in the HT-29 cell line.

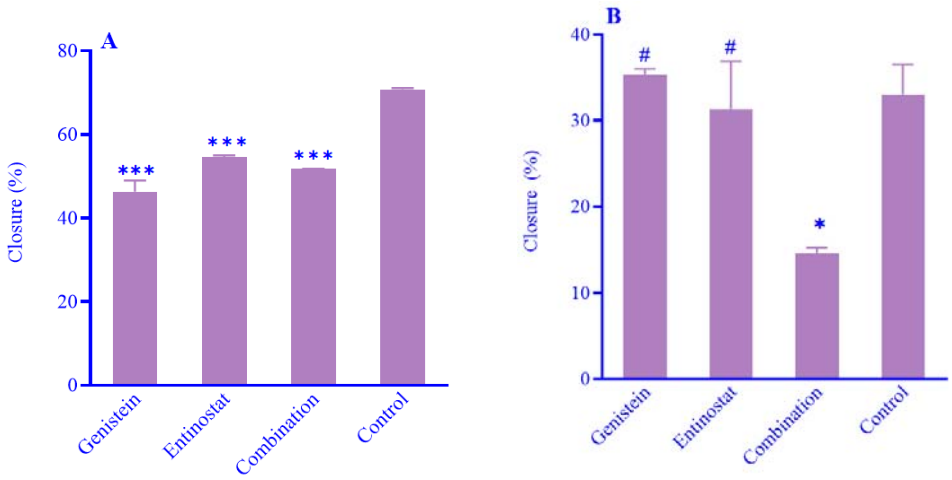

Fig. 3. Results of scratch assay of genistein, entinostat, and their combination in (A) HCT-116 and (B) HT-29 cells. Values represent mean ± SD and were performed in triplicate. *$P < 0.05$ and ***$P < 0.001$ indicate significant differences compared with the control group; $^{\#}P < 0.05$ versus combination.

Colony-forming assay

Genistein was able to reduce the number of colonies in HCT-116 but not in the HT-29 cell line compared to the control group (Fig. 4A and B). In both cell lines, entinostat and its combination with genistein significantly reduced the number of colonies compared to the control. A significant difference in the ability to reduce the number of colonies between genistein and the combination of genistein/entinostat was found in the HT-29 cell line (Fig. 4B).

Gene expression of the cell cycle regulatory protein CDC25 A

Entinostat and the combination of genistein/entinostat downregulated CDC25A expression significantly compared to the vehicle-treated control in both cell lines. A significant difference in the reduction of CDC25A expression between entinostat and the combination of genistein and entinostat in the HT-29 cell line was produced (Fig. 5A and B).

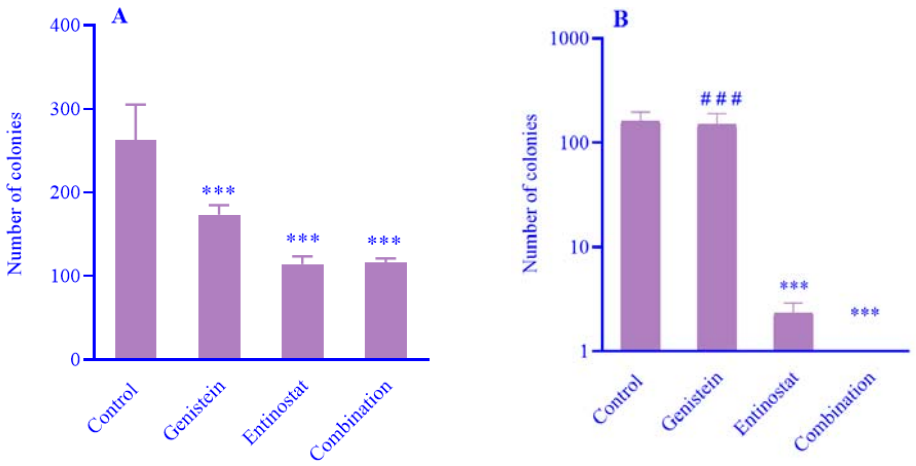

Fig. 4. Colony forming assay for different treatments in (A) HCT-116 and (B) HT-29 cell lines. Values represent mean ± SD and were performed in triplicate. ***$P < 0.001$ indicates significant differences compared with the control group; ###$P < 0.001$ versus combination.

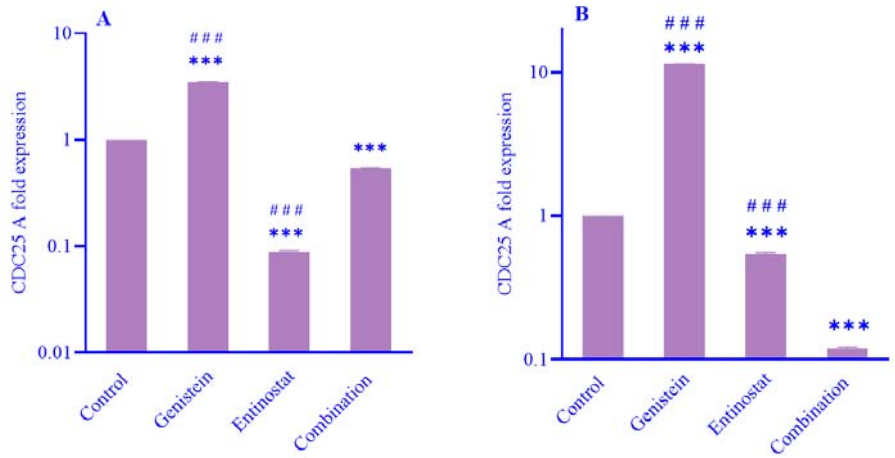

Fig. 5. CDC25 A expression in (A) HCT-116 and (B) HT-29 cell lines. Values represent mean ± SD and were performed in triplicate. ***$P < 0.001$ indicates significant differences compared with the control group; ###$P < 0.001$ versus combination.

DISCUSSION

Combination therapy is a cancer therapeutic strategy that combines two or more pharmaceutical agents. It lowers the possibility of resistance, and it can produce a more effective treatment response (19). In the present study, the cytotoxic effect of entinostat and genistein combination in HCT-116 and HT-29 CRC cell lines was investigated. No synergistic cytotoxic effect was produced by genistein and entinostat combination in HCT-116 cells, while the concentration of 1.56 µM of each drug produced a synergistic anti-proliferative effect in HT-29. If such an effect is produced *in vivo*, lower doses of entinostat can be used for the treatment of CRC, producing the same effects as if genistein was administered concomitantly. The use of lower doses of entinostat causes fewer side effects.

Genistein in the current study decreased the migration in HCT-116 up to 46.3%, which was significantly different from the control, while in HT-29, the effect was not significantly different from the negative control after 48 h. Interestingly, the combination of genistein and entinostat was more efficient than either drug alone in reducing migration in HT-29 cells. In a previous study, genistein (10 µmol/L) demonstrated a significant inhibitory effect on HCT-116 and HT-29 after 24 h of cell migration, with inhibition ranging from 36% to

56% and 32% to 39%, respectively (20). The difference in outcome between studies could be due to the differences in end point at which the wound area was measured, in addition to differences in the passage number of CRC cell lines and growth conditions.

The clonogenic test, sometimes called the colony formation assay, is an *in vitro* cell survival experiment based on the ability of a single cell to proliferate into a colony. In the current study, genistein treatment did not significantly reduce colony formation in HT-29. A similar result was observed in a previous study using a concentration of 10 µM. However, higher concentrations had a significant effect on colony formation inhibition (20). Entinostat, both alone and in combination with genistein, was able to produce almost complete colony formation inhibition in HT-29 cells. The present study, in conjunction with existing research, indicated that entinostat demonstrates superior efficacy in inhibiting colony formation in the HT-29 cell line compared to the HCT-116 cell line (21). It has been reported earlier that HT-29 cells have a less aggressive nature compared to HCT-116, as demonstrated by a lower clonogenic and tumorigenic potential than HCT-116 cells (22).

Mitosis and cell proliferation require the sequential progression through the cell cycle's G1, S, G2, and M phases. An important step in cell cycle control is passing through the G1-S phase transition point. When cells reach S phase, they must finish the rest of the cell cycle (23). In the current investigation, the combination of genistein and entinostat decreased the number of colonies and downregulated CDC25A expression significantly compared to control in both cell lines. Studies showed that the administration of entinostat resulted in reduced proliferation followed by cell cycle arrest, as well as substantial tumor apoptosis. Also found that entinostat caused increased histone acetylation and changes in the expression of cell cycle-associated proteins (24). Entinostat, a class I HDAC inhibitor, downregulates CDC25A, a critical regulator of the cell cycle. CDC25A is involved in activating cyclin-dependent kinases that drive cell cycle progression, making it essential for cancer cell proliferation. By inhibiting HDACs, entinostat alters chromatin structure, leading to decreased CDC25A expression, which results in cell cycle arrest and potentially induces apoptosis in cancer cells. This combination therapy can also help overcome drug resistance, particularly in cancers where CDC25A is overexpressed, by preventing cancer cells from bypassing cell cycle checkpoints. The downregulation of CDC25A by entinostat and its combinations offers a targeted approach to disrupt cancer cell proliferation, leading to improved therapeutic outcomes and potentially longer remission periods. Ongoing research continues to explore the full potential of this strategy, aiming to optimize combination regimens for maximum effectiveness in cancer treatment.

CONCLUSION

This study showed for the first time that the genistein and entinostat combination was significantly more efficient than either drug alone in decreasing the viability and migration of the HT-29 cells, as well as decreasing the cell cycle regulator CDC25A gene expression. Therefore, the combination of genistein and entinostat could be a promising treatment for CRC. However, further preclinical and clinical studies are needed.

Conflicts of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

M.A. Abbas and M.M. Abbas conceived and supervised the project. N.A. Alqalalwah performed the experiments, M.M. Abbas analyzed the results, and N.A. Alqalalwah wrote the manuscript in consultation with M.A. Abbas. R. Obeidat and R. El-Rayyes verified the analytical methods. All authors read and approved the finalized article.

REFERENCES

1. Ciardiello F, Ciardiello D, Martini G, Napolitano S, Tabernero J, Cervantes A. Clinical management of metastatic colorectal cancer in the era of precision medicine. CA Cancer J Clin. 2022;72(4):372-401. DOI: 10.3322/caac.21728.

2. Nsairat H, Jaber AM, Faddah H, Ahmad S. Oleuropein impact on colorectal cancer. Future Sci OA. 2024;10(1):FSO,1-13. DOI: 10.2144/fsoa-2023-0131
3. Al-Husein BA, Mhaidat NM, Alzoubi KH, Alzoubi GM, Alqudah MA, Albsoul-Younes AM, *et al*. Pentoxifylline induces caspase-dependent apoptosis in colorectal cancer cells. Inform Med Unlocked. 2022;31:100997. DOI: 10.1016/j.imu.2022.100997.
4. Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag C, 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. DOI: 10.1136/gutjnl-2022-327736.
5. Clinton SK, Giovannucci EL, Hursting SD. The world cancer research fund/American institute for cancer research third expert report on diet, nutrition, physical activity, and cancer: impact and future directions. J Nutr. 2020;150(4):663-671. DOI: 10.1093/jn/nxz268.
6. Shin AE, Giancotti FG, Rustgi AK. Metastatic colorectal cancer: mechanisms and emerging therapeutics. Trends Pharmacol Sci. 2023;44(4):222-236. DOI: 10.1016/j.tips.2023.01.003.
7. Pritchard CC, Grady WM. Colorectal cancer molecular biology moves into clinical practice. Gut. 2011;60(1):116-129. DOI: 10.1136/gut.2009.206250.
8. Zhou Z, Rajasingh S, Barani B, Samanta S, Dawn B, Wang R, *et al*. Therapy of infectious diseases using epigenetic approaches. In: Tollefsbol TO. Epigenetics in Human Disease. Academic Press; 2018. pp. 689-715. DOI: 10.1016/B978-0-12-812215-0.00022-4.
9. Marx C, Sonnemann J, Beyer M, Maddocks ODK, Lilla S, Hauzenberger I, *et al*. Mechanistic insights into p53-regulated cytotoxicity of combined entinostat and irinotecan against colorectal cancer cells. Mol Oncol. 2021;15(12):3404-3429. DOI: 10.1002/1878-0261.13060.
10. Chu DT, Ngo AD, Wu CC. Epigenetics in cancer development, diagnosis, and therapy. Prog Mol Biol Transl Sci. 2023;198:73-92. DOI: 10.1016/bs.pmbts.2023.01.009.
11. Ramaiah MJ, Tangutur AD, Manyam RR. Epigenetic modulation and understanding of HDAC inhibitors in cancer therapy. Life Sci. 2021;277:119504,1-19. DOI: 10.1016/j.lfs.2021.119504.
12. Azad NS, El-Khoueiry A, Yin J, Oberg AL, Flynn P, Adkins D, *et al*. Combination epigenetic therapy in metastatic colorectal cancer (mCRC) with subcutaneous 5-azacitidine and entinostat: a phase 2 consortium/stand up 2 cancer study. Oncotarget. 2017;8(21):35326-35338. DOI: 10.18632/oncotarget.15108.
13. Goh YX, Jalil J, Lam KW, Husain K, Premakumar CM. Genistein: A review on its anti-inflammatory properties. Front Pharmacol. 2022;13:820969,1-23. DOI: 10.3389/fphar.2022.820969.
14. Qin J, Teng J, Zhu Z, Chen J, Huang W-J. Genistein induces activation of the mitochondrial apoptosis pathway by inhibiting phosphorylation of Akt in colorectal cancer cells. Pharm Biol. 2016;54(1): 74-79. DOI: 10.3109/13880209.2015.1014921.
15. Supic G, Jagodic M, Magic Z. Epigenetics: a new link between nutrition and cancer. Nutr Cancer. 2013;65(6):781-792. DOI: 10.1080/01635581.2013.805794.
16. Abbas MM, Al-Rawi N, Abbas MA, Al-Khateeb I. Naringenin potentiated β-sitosterol healing effect on the scratch wound assay. Res Pharm Sci. 2019;14(6):566-573. DOI: 10.4103/1735-5362.272565.
17. He L, Lu N, Dai Q, Zhao Y, Zhao L, Wang H, *et al*. Wogonin induced G1 cell cycle arrest by regulating Wnt/β-catenin signaling pathway and inactivating CDK8 in human colorectal cancer carcinoma cells. Toxicology. 2013;312:36-47. DOI: 10.1016/j.tox.2013.07.013.
18. Ma Y, Wang R, Lu H, Li X, Zhang G, Fu F, *et al*. B7-H3 promotes the cell cycle-mediated chemoresistance of colorectal cancer cells by regulating CDC25A. J Cancer. 2020;11(8):2158-2170. DOI: 10.7150/jca.37255. eCollection 2020.
19. Hanahan D, Bergers G, Bergsland E. Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. J Clin Invest. 2000;105(8):1045-1047. DOI: 10.1172/JCI9872.
20. Xiao X, Liu Z, Wang R, Wang J, Zhang S, Cai X, et al. Genistein suppresses FLT4 and inhibits human colorectal cancer metastasis. Oncotarget. 2015;6(5):3225-3239. DOI: 10.18632/oncotarget.3064.
21. Bracker TU, Sommer A, Fichtner I, Faus H, Haendler B, Hess-Stumpp H. Efficacy of MS-275, a selective inhibitor of class I histone deacetylases, in human colon cancer models. Int J Oncol. 2009; 35(4):909-920. DOI: 10.3892/ijo_00000406.
22. 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;107(8):3722-3727. DOI: 10.1073/pnas.0915135107.
23. Carragher NO. Calpain inhibition: a therapeutic strategy targeting multiple disease states. Curr Pharm Des. 2006;12(5):615-638. DOI: 10.2174/138161206775474314.
24. Marques AEM, do Nascimento Filho CHV, Marinho Bezerra TM, Guerra ENS, Castilho RM, Squarize CH. Entinostat is a novel therapeutic agent to treat oral squamous cell carcinoma. J Oral Pathol Med. 2020;49(8):771-779. DOI: 10.1111/jop.13039.