EXPERIMENTAL ONCOLOGY

https://doi.org/10.15407/exp-oncology.2023.02.220

Ratsada Praphasawat [1], Sarawoot Palipoch [2], Prasit Suwannalert [3], Witchuda Payuhakrit [3], Paween Kunsorn [3], Sarunya Laovitthayanggoon [4], Sinittra Thakaew [1], Narongsuk Munkong [1], Warangkhana Klajing [5],

[1] Department of Pathology, School of Medicine, University of Phayao, Phayao, Thailand 56000
[2] School of Medicine, Walailak University, Nakhon Si Thammarat, Thailand 80160
[3] Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok, Thailand 10400
[4] Expert Centre of Innovative Herbal Products, Thailand Institute of Scientific and Technological Research, Techno Polis, Khlong Luang District, Pathum Thani, Thailand 12120
[5] Department of Traditional Chinese Medicine, School of Public Health, University of Phayao, Phayao, Thailand 56000
* Correspondence: Email: wangyisheng_23@hotmail.com

RED RICE BRAN EXTRACT SUPPRESSES COLON CANCER CELLS VIA APOPTOSIS INDUCTION/CELL CYCLE ARREST AND EXERTS ANTIMUTAGENIC ACTIVITY

Background. Red rice bran extract (RRBE) contains many biologically active substances exerting antioxidant and anti-inflammatory effects. **Aim.** To evaluate the anticancer potential of RRBE in human colon cancer cells and its mutagenic/antimutagenic effects on nonmalignant cells. **Materials and Methods.** The cytotoxic effect of RRBE was determined by trypan blue exclusion in HCT116, HT29 cell lines and a non-cancerous HEK293 cell line, and its antiproliferative effect using MTS and colony formation assay. The apoptosis induction was evaluated using ELISA, and the apoptotic rate and cell cycle progression were assessed by flow cytometry. The mutagenic/antimutagenic potential of RRBE was analyzed by micronucleus assay in the V79 cell line. **Results.** RRBE caused a dose-dependent reduction of cell viability in colon cancer cells and showed a limited cytotoxicity against HEK293 cells. The treatment with RRBE suppressed proliferation of HCT116 and HT29 cells and induced apoptosis as evidenced by the increased DNA fragmentation and the apoptotic cell counts. Furthermore, RRBE treatment significantly increased the number of cells at the G_2/M phase triggering the arrest of the cell cycle in colon cancer cells. Interestingly, RRBE did not increase the micronucleus frequency in V79 cells but reduced the micronucleus

Citation: Praphasawat R, Palipoch S, Suwannalert P, Payuhakrit W, Kunsorn P, Laovitthayanggoon S, Thakaew S, Munkong N, Klajing W. Red rice bran extract suppresses colon cancer cells via apoptosis induction/cell cycle arrest and exerts antimutagenic activity. *Exp Oncol*. 2023; 45(2): 220-230. https://doi.org/10.15407/exp-oncology.2023.02.220

formation caused by mitomycin C. **Conclusion.** RRBE effectively suppressed proliferation, induced apoptosis, and caused a cell cycle arrest in human colon cancer cells while being non-mutagenic and exerting antimutagenic effects *in vitro*.

Keywords: colon cancer cells, apoptosis, cell cycle arrest, antimutagenicity, red rice bran extract.

Red rice (*Oryza sativa L.*) has become increasingly popular as a functional food due to the high content of valuable phytochemicals compared with white rice [1—3]. It is well known that red rice is a rich source of bioactive compounds, such as phenols, flavonoids, anthocyanins, proanthocyanidins, protocatechuic acid, ferulic acid, γ-oryzanol, and vitamin E [2]. Another distinctive part of red rice is inedible bran, which is a by-product of the rice milling process. It was reported that red rice bran extract (RRBE) exerts a variety of biological activities including antioxidant [4, 5], antidiabetic [6], and anti-inflammatory ones [7 8]. The chemical analysis has shown that RRBE from Hawm or Hawm Dowk Mali Deang varieties has the highest phenolic conteфnt, which leads to an enhanced antioxidant activity when compared with several colored varieties of rice and the potential for anticancer effects [9]. In cancer research, red rice and its bran have been tested *in vitro* and showed the suppression of the proliferation of leukemic cells, cervical, stomach [10], breast [11], and liver cancer cells [12]. However, the anticancer activity of RRBE in colon cancer cells as well as its antimutagenic property have not been studied yet. Therefore, our study sought to determine the anticancer effect and the antimutagenic activity of RRBE in two human colon cancer cell lines (HCT116 and HT29) and hamster lung fibroblast cell line (V79), respectively. Moreover, this study can provide useful information on the safety of RRBE based on the results of the cytotoxicity and the mutagenicity evaluations.

Material and Methods

Cell culture and in vitro experiment. Two human cancer cell lines with different genetic profiles and a non-cancerous cell line were used. Colon carcinoma HCT116 (*TP53*^{wt} gene) and colon adenocarcinoma HT29 (*TP53*^{R273H} gene) cells were supplied from the American Type Culture Collection (ATCC). The colon cancer cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 1% v/v streptomycin and penicillin (Gibco, USA). The Chinese hamster lung fibroblast cell line V79-4 (V79) was kindly supplied by Dr. Prapaipat Klungsupya (Thailand Institute of Scientific and Technological Research) and used in mutagenicity/antimutagenicity assessment in accordance with the Organization for Economic Co-operation and Development guidelines (487) for the testing of chemicals [13]. The cells were cultured in DMEM supplemented with 10% FBS and 1% v/v streptomycin and penicillin. The embryonic kidney cells HEK293 (CRL-1573^{TM}) were purchased from ATCC and cultured in minimum essential Eagle medium (EMEM) (Gibco, USA) containing 10% FBS and 1% penicillin-streptomycin solution. All cell lines were incubated at 37 °C with 5% CO_2. Cell passage was performed once every 3—4 days or at 80—90% confluence.

Plant material and preparation of RRBE stock solution. The RRBE was kindly provided by Dr. Narong Munkong and colleagues (School of Medicine, University of Phayao). Briefly, the red rice bran was collected in 2019 from a local market in the Phayao province, Thailand. Then, the red rice bran was extracted and powdered with 50% ethanol. For experiments, the RRBE was dissolved in DMSO (100 mg/mL) (Sigma, USA). The stock solution was prepared, aliquoted, and stored in autoclaved microcentrifuge tubes (1.5 mL) at 4 °C. At the time of the experiment, the stock concentration was diluted with culture

medium to the desired concentration. The final concentration of DMSO was less than 1%.

Cell viability assay. The effect of RRBE on the cell viability was determined using the trypan blue exclusion method. Briefly, a panel of cell lines (HCT116, HT29, V79, and HEK293) was plated at a density of 5×10^4 cells in each well of a 12-well plate. After cell adhesion, the medium was replaced, and the cells were treated with various doses of RRBE (100—1000 μg/mL) along with the negative control. After the treatment completion, the treated cells were harvested. The cells were stained with trypan blue (Gibco, USA) and counted under a light microscope (10x). The IC_{50} value was determined using a linear regression equation utilizing a concentration response curve.

Antiproliferative assay. The RRBE (50—250 μg/mL) effect on cell proliferation was determined by CellTiter 96 Aqueous One Solution Cell Proliferation Assay (MTS, Promega, USA) in accordance with the manufacturer's instruction. The suppression of the proliferation was expressed as the ratio between the absorbance at starting point (T = 0) and after treatment (T = 4 d).

Colony formation assay. Approximately, 300 cells/well was cultured in a 6-well plate. After 48 h of culture, the medium containing RRBE at concentrations of 50 and 100 μg/mL was added. Then, after culturing, the fresh medium was replaced, and cell growth was monitored each day. The cells were cultured for 14 days. The plates were then observed for the formation of colonies. The images were captured using an inverse microscope.

Detection of apoptosis by ELISA and flow cytometry analysis. A Cell Death Detection ELISA PLUS kit (Sigma-Aldrich, Germany) was used for apoptotic detection. This immunoassay allows the specific detection of histone-associated DNA fragments in the cytoplasmic fraction after induced cell death. Briefly, HCT116 and HT29 cells were seeded into a 12-well plate and allowed attaching as described above. The medium was removed and replaced with RRBE solution (0—250 μg/mL). After the incubation, the cells were harvested following the manufacturer's instructions. The absorbance was measured at 405 nm using an ELISA reader (Biotek, USA). For flow cytometric analysis, HCT116 and HT29 cells were plated into 24-well plates and incubated at 37 °C with 5% CO_2 for 48 h. The medium was then removed from each well and replaced with serum-free medium containing RRBE. After 24 h cells were trypsinized, rinsed with phosphate-buffered saline (PBS), and resuspended in a binding buffer. The FITC-conjugated Annexin V and phycoerythrin-conjugated propidium iodide (PI) were added. The cells were washed with PBS, fixed with 1% paraformaldehyde, and then incubated at room temperature in the dark for 15 min. The labeled cells were analyzed using FACScan (Becton Dickinson, USA).

Cell cycle analysis. The cell cycle distribution of RRBE-treated colon cancer cells was determined by flow cytometry. Cells were treated with RRBE in concentrations of 100 and 200 μg/ml for 24 h. Then, cells were harvested, fixed with 70% ethanol and stained with 50 μg/mL propidium iodide (Biolegend, USA) in solution containing 20 μg/mL RNase A (Geneaid, Taiwan). The DNA contents at different cell cycle phases were measured by flow cytometry using an FACScan (Becton Dickinson, USA). BD CellQuest was used for the cell cycle analysis using peripheral blood mononuclear cells (PBMC) as an internal control to cut off the intensity of G_0/G_1 (2N DNA), S-phase, and G_2/mitosis (4N DNA).

Mutagenicity and antimutagenicity assessments by cytokinesis-block micronucleus assay (CBMN). In accordance with OECD guidelines, the cytokinesis-block micronucleus test was used to detect DNA damage. In our experiment, V79 cells were cultured in the presence of RRBE at different concentrations (250 and 500 μg/mL) for 24 h. We selected the concentration based on a viability of above 70% to assess

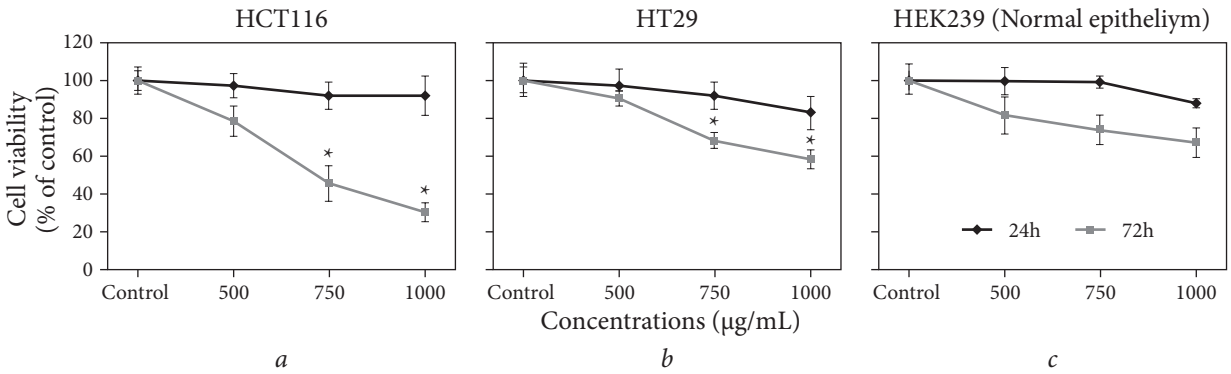

Fig. 1. Effect of exposure time and RRBE concentration on the viability of HCT116 (*a*), HT29 (*b*), HEK293 (*c*) cells. The results are presented as M ± SD from three independent experiments. *$p < 0.05$ compared to the control

the mutagenicity where the cells were exposed to different concentrations of RRBE for 24 h. For the antimutagenicity evaluation, the cells were treated for 24 h with different concentrations of RRBE in combination with 1.25 μg/mL mitomycin C (MMC) (Sigma, USA). During the treatment, a cytochalasin B solution (Cyt-B) at a final concentration of 6 μg/mL (Sigma, USA) was added to collect the cells at a binucleated stage. At the end of the treatment time, the cells were harvested and prepared as a monolayer on glass slides. Then the cells were fixed with ice-cold methanol and stained with DAPI (Sigma, USA). Micronuclei (MN) formation was scored in 2000 binucleated (BNC) cells under a fluorescence microscope (40x).

Statistical analysis. The experiments were conducted at least three times. The data were presented as the mean ± standard deviation. For statistical analysis, SPSS 20.0 software package was used. One-way ANOVA was used to calculate the statistical difference. Differences with $p < 0.05$ were considered statistically significant.

Results

Effect of RRBE on colon cancer cell viability. When the colon cancer cells were exposed to RRBE for 24 h the highest RRBE dose reduced the viability of HCT116 and HT29 cells by 8% and 18%, respectively (Fig. 1). However, prolonged exposure to RRBE (72 h) induced a dose-dependent cytotoxicity in both colon carcinoma cell lines with a 30—60% loss in cell viability at 1000 μg/mL. IC_{50} for 72 h was calculated as 654.446 μg/mL and 907.448 μg/mL for HCT116 and HT29 cells, respectively. Intriguingly, we observed a significantly high IC_{50} value (1485.984 μg/mL RRBE) for HEK293 cell line while > 71% cells remained alive when exposed to 1000 μg/mL RRBE for 72 h.

RRBE inhibits proliferation of colon cancer cells. The effect of the non-cytotoxic concentrations of RRBE (0—250 μg/mL) on cell proliferation was evaluated in colon cancer cells using MTS assays. The dose-dependent exposure of colon cancer cells to RRBE for 72 h resulted in a distinct morphological change compared to the untreated control, the RRBE-treated cells became rounded and lost the contact with neighboring cells, which indicates a reduction of cell proliferation (Fig. 2, *a*). The RRBE treatment significantly suppresses the proliferation of HCT116 and HT29 cells in a dose-dependent manner (Fig. 2, *b*). To further confirm the antiproliferative potential of RRBE, we investigated the self-renewal properties of these cells by a colony formation assay. After the RRBE treatment, we found a significant decrease in the ability of the cells to form colonies, suggesting

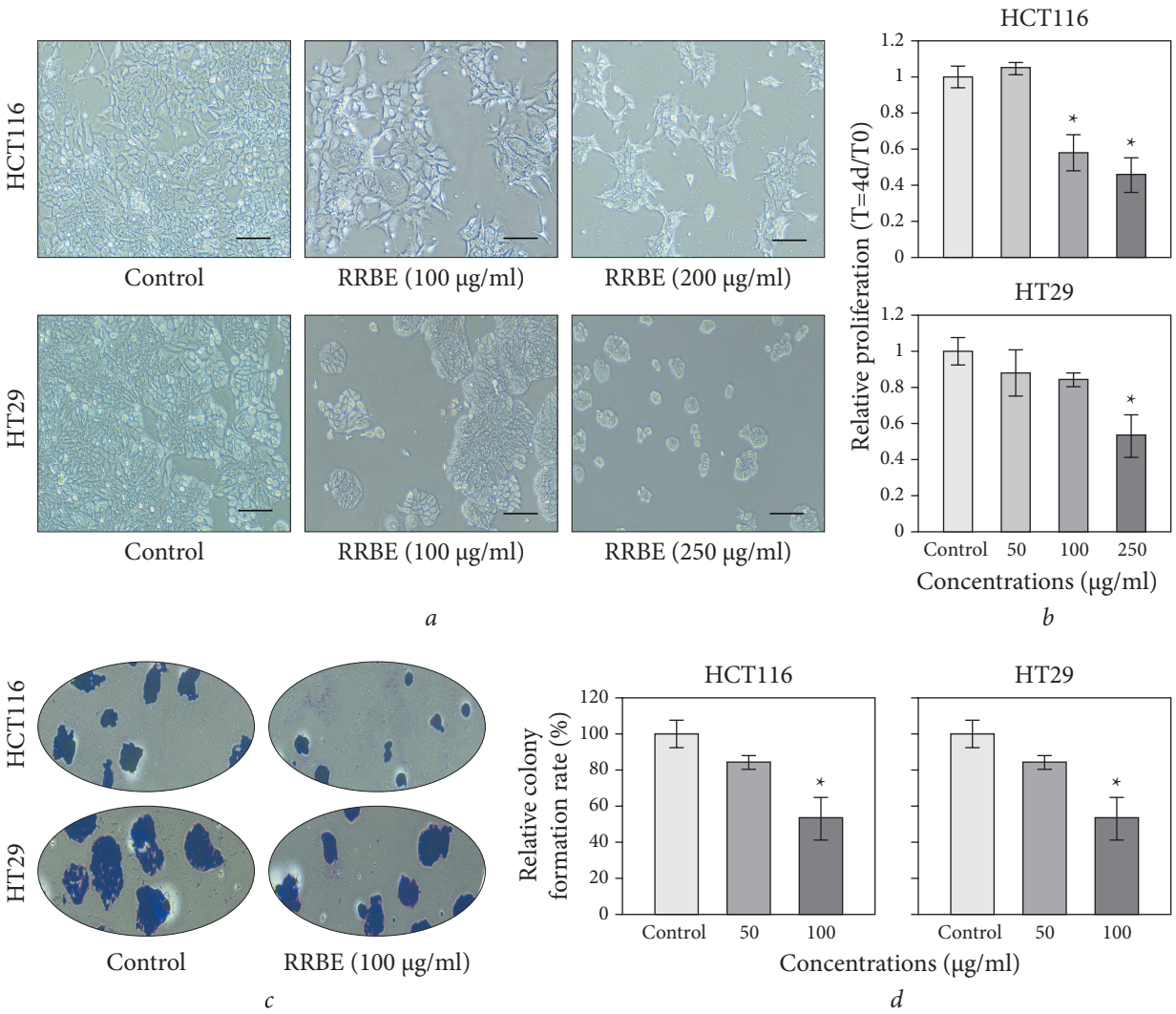

Fig. 2. RRBE inhibits proliferation of colon cancer cells. (*a*) Phase-contrast microscopy shows changes in morphology of HCT116 and HT29 cells. The scale bar is 50 mM at 20x magnification. (*b*) MTS assay shows a dose-dependent inhibition of HCT116 and HT29 cells proliferation upon RRBE treatment for 96 h. (*c*) Representative images show colonies after RRBE treatment of HCT116 and HT29 cells. (*d*) The bar diagram represents the percentage of colonies obtained after RRBE treatment. The values are presented as M ± SD of three independent experiments. *$p < 0.05$ compared to the control (1% DMSO)

that RRBE attenuates the self-renewal in colon cancer cells at concentrations of 50 and 100 µg/mL (Fig. 2, *c*, *d*). These data indicate that RRBE significantly inhibited the proliferation and colony formation of both HCT116 and HT29 colon cancer cells.

RRBE induces apoptosis in colon cancer cells. In order to identify the potential mechanisms responsible for the proliferation-suppressing action of RRBE, we determined the cytoplasmic histone-associated DNA fragments in RRBE-treated HCT116 and HT29 cells as evidence of apoptosis using Cell Death Detection ELISA PLUS. The cells were treated with RRBE (100—250 µg/mL) for 72 h. The doses were chosen depending on the IC50 obtained for each cell line.

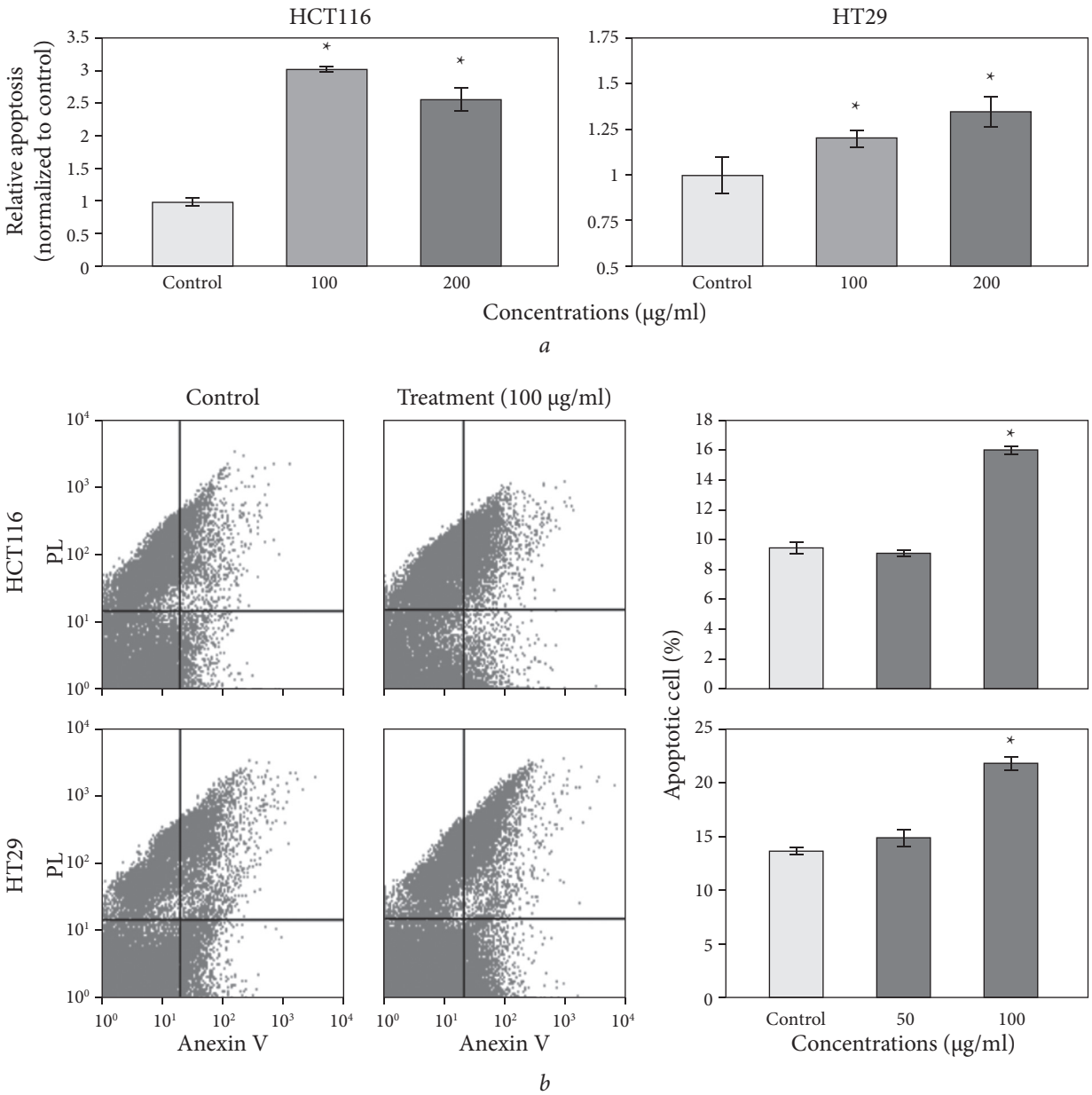

Fig. 3. RRBE induces the apoptosis of HCT116 and HT29 cells. (*a*) Cell death detection ELISA assay shows an increase in apoptotic events after RRBE treatment. (*b*) Following the exposure, the cells were treated and double stained with Annexin V and PI and analyzed by flow cytometry. The results are presented as M ± SD of three independent experiments. *$p < 0.05$ compared to the control

The RRBE treatment results in the enhanced histone release in both cell lines in comparison to the untreated control cells (Fig. 3, *a*).

To confirm these findings, we also used a flow cytometry and found that the percentage of apoptotic cells in both cell lines was obviously higher after RRBE treatment compared to the control (Fig. 3, *b*). These results imply that the inhibition of cell survival upon RRBE treatment is due to the apoptotic mechanism.

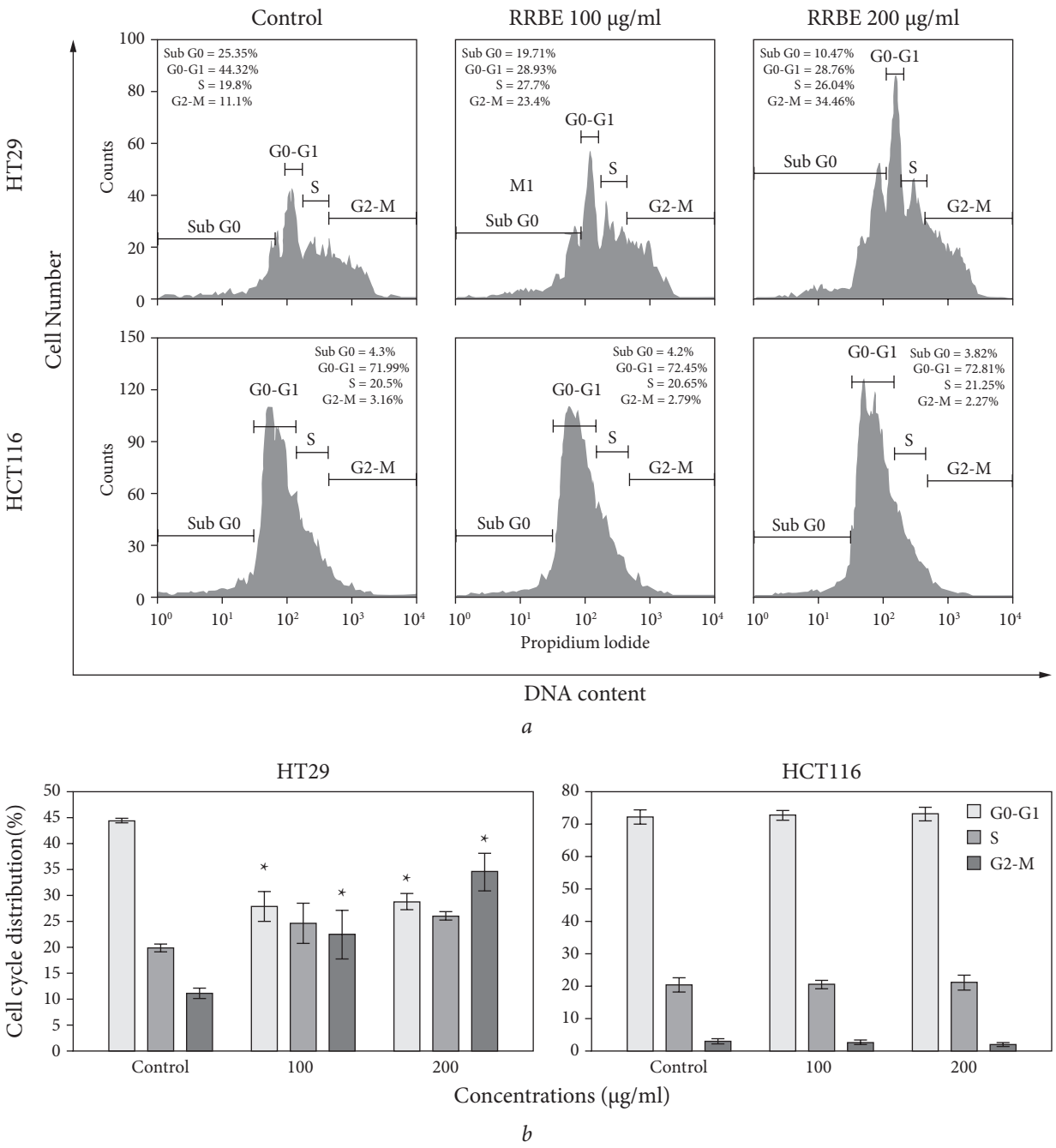

Fig. 4. RRBE induces G_2/M cell cycle arrest. (*a*) Flow cytometry was used to determine the cell cycle profiles of RRBE-treated HT29 and HCT116 cells. (*b*) The bar diagram shows the percentage of cells in each phase of the cell cycle. The values are presented as M ± SD of three independent experiments. *$p < 0.05$ compared to the control

RRBE induces cell cycle arrest at the G_2/M phase. To further investigate the inhibitory effect of RRBE on the proliferation of colon cancer cells, we examined the cell cycle distribution of HCT116 and HT29 cells after RRBE treatment. We found a substantial number of HT29

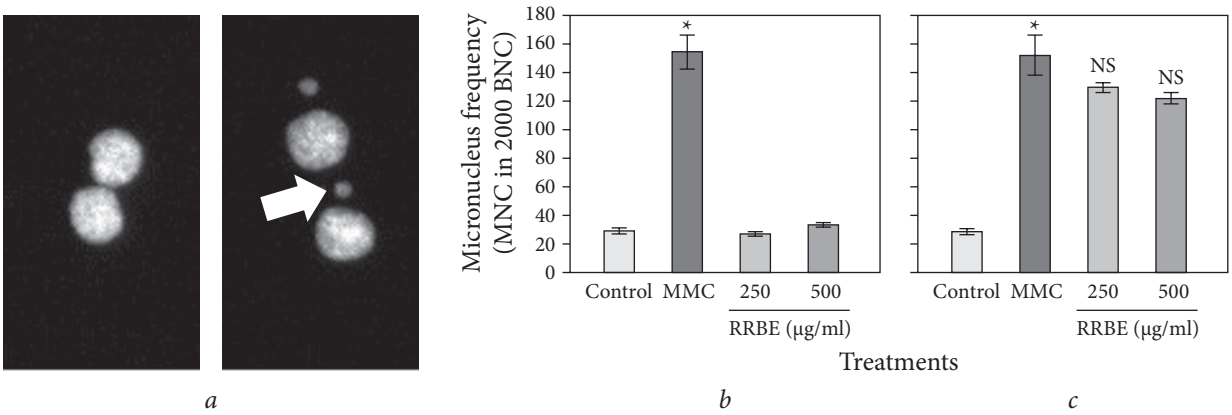

Fig. 5. CBMN test of V79 cells exposed to different concentrations of RRBE. (*a*) Representative images of BNC and BNC with MN (arrow). (*b*) The bar chart shows the frequencies of MN in V79 cells after exposure to different doses of RRBE (250 and 500 μg/mL). The control — untreated cells, whereas 1.25 μg/ml MMC serves as positive control. (*c*) MN frequency in V79 cells treated with RRBE and MMC combination. The values are presented as M ± SD of three independent experiments. *$p < 0.05$ compared to the control, NS indicates non-significant ($p > 0.05$) compared to the positive control.

cells arrested in the G_2/M phase. In untreated cells, 44.32% of HT29 cells were in the G_0/G_1 phase, 19.8% in the S phase, and 11.1% in the G_2/M phase. After RRBE treatment, the G_0/G_1 phase population decreased by about 28.76%, while the number of cells in the S phase increased by 26.04%, and the G_2/M phase population significantly increased by 34.46% (Fig. 4). These findings suggest that RRBE could effectively induce cell cycle arrest in the G_2/M phase.

Antimutagenic potential of RRBE. We investigated the antimutagenic properties of RRBE on V79 fibroblast cell lines in the micronucleus (MN) assay used for detection of the DNA damage resulting from clastogenic and aneugenic activities, which leads to MN formation in the cytoplasm during mitosis. Therefore, we analyzed the MN formation in BNC to ensure that those cells had undergone division (Fig. 5, *a*). Prior to the antimutagenic assessment, the mutagenicity was evaluated at non-toxic doses of RRBE. Our results revealed that the negative control exhibited a baseline mean MN frequency of 29.17 ± ± 1.83 MN/2000 BNC, while in the mutagen-treated cells, the induction of MN obviously increased to 154.23 ± 11.87 MN/2000 BNC. After RRBE treatment, the frequency of MN was similar to that in the negative control (27.00 ± 0.58 and 28.50 ± 0.29 MN/2000 BNC at 250 and 500 μg/mL of RRBE, respectively) (Fig. 5, *b*).

To assess the antimutagenic effects of RRBE, V79 cells were treated with RRBE and MMC. The results showed that treatment with MMC only resulted in a drastic MN formation up to 154.13 ± 14.43 MN/2000 BNC, compared to 28.79 ± 2.00 MN/2000 BNC in the negative control. Interestingly, we found that the combined treatment with RRBE and MMC resulted in a decrease in the MNfrequency (Fig. 5, *c*) — 131.34 ± 3.33 and 123.59 ± 4.31 MN/2000 BNC for RRBE doses of 250 and 500 μg/mL, respectively. These results indicate that while RRBE is not mutagenic for V79 cells, it demonstrates an antimutagenic activity in this cell line.

Discussion

In the present study, we have shown that RRBE possesses an antiproliferative effect through the induction of apoptosis and cell cycle arrest in colon cancer cells, while being low cytotoxic to-

ward non-cancerous cells and exhibiting an antimutagenic effect in the hamster's lung fibroblast cell line.

We have shown that RRBE elicited significantly high cytotoxicity against colon cancer (HCT116 and HT29) cell lines. with the IC_{50} of 654.446 and 907.448 μg/mL, respectively The extract used in this study is more cytotoxic than that studied in the earlier research where IC_{50} values of 175.0 and 151.0 mg/mL for MCF-7 and MDA-MD-231 breast cancer cell lines, respectively were determined [11]. However, the discrepancy between these values may be influenced by the varieties of rice (collection site, cultivation process, etc.) or the type of cancer cell lines. Additionally, we observed an incredibly low cytotoxic effect of RRBE at the same dose and treatment duration against normal cells of the HEK293 line. We found a 50% reduction of the HEK293 cell survival at a dose of 1485.984 μg/mL, and survival rate was > 71% at 1000 μg/mL of RRBE. Our findings are in accordance with the criteria of cytotoxic activity for crude extracts, as established by the National Cancer Institute, which states that crude herbal extracts that do not decrease the viability of normal cells by more than 30% are safe for human consumption [14].

Also, we have found that RRBE suppresses the proliferative activity and induced apoptosis and cell cycle arrest at the G_2/M phase in HCT116 and HT29 cell lines. Our results are consistent with the current research and indicate the potent antiproliferative activity of red rice bran against MCF-7 and MDA-MB-231 cells [11]. The authors suggest that the high content of phytochemicals and the antioxidant activity of red rice bran can contribute to its antiproliferative activity. Moreover, the study performed by Supranee Upanan et al. [12] showed that the proanthocyanin-rich fraction obtained from red rice bran inhibits cell proliferation, induces cell apoptosis, and causes cell cycle arrest the G_2/M phase in HepG2 cells. Similarly, Ming-Hsuan Chen et al. [10] reported that RRBE exhibited strong inhibitory effects on various cancer cell types including leukemia, cervical cancer, and stomach cancer cells. They also found that the high concentrations of proanthocyanidins were identified in red rice bran and correlated with their inhibitory activity. However further research on the relationship between active compounds involved in the antiproliferative activity of RRBE is still required.

We observed that the levels of response between two cell lines used in our study were different. We found that the p53-wild type HCT116 cells were more sensitive to apoptosis induction by RRBE than $p53^{R273H}$ HT29 cells. *TP53* is a tumor suppressor gene coding the p53 tumor suppressor protein, which is one of the main players in apoptosis [15, 16]. This gives us the reason to assume that the apoptosis induction caused by RRBE treatment probably involves activation of the p53 pathway.

In addition, there was no evidence of the mutagenic potential of RRBE reported in previous research. Therefore, we evaluated the mutagenicity and the antimutagenicity of RRBE using the CBMN assay under the OECD guidelines [13]. The MN assay allows one to detect a structural numerical chromosomal damage and evaluate the genotoxic potential of substances [17, 18]. Our results revealed the absence of mutagenicity of RRBE in the V79 cell line but showed the antimutagenic effect when using CBMN. Our results concur with the previous findings reported by Treetip Ratanavalachai et al. [19] who have demonstrated that the water extract of red rice bran (Sangyod rice) possess an antigenotoxic potential against genotoxic damage (by doxorubicin) using the sister chromatid exchange assay in human lymphocytes.

Funding

This work was supported by the University of Phayao, Thailand (Grant no. FF64-RIM030, FF65-RIM-091 and FF64-RIB008).

REFERENCES

1. Tyagi A, Shabbir U, Chen X, et al. Phytochemical profiling and cellular antioxidant efficacy of different rice varieties in colorectal adenocarcinoma cells exposed to oxidative stress. *PloS One*. 2022;17:e0269403. doi: 10.1371/journal.pone.0269403
2. Goufo P, Trindade H. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food Sci Nutr*. 2014;2:75-104. doi: 10.1002/fsn3.86
3. Gunaratne A, Wu K, Li D, Bentota A, Corke H, Cai YZ. Antioxidant activity and nutritional quality of traditional red-grained rice varieties containing proanthocyanidins. *Food Chem*. 2013;138:1153-1161. doi: 10.1016/j.foodchem.2012.11.129
4. Sapna I, Jayadeep A. Influence of enzyme concentrations in enzymatic bioprocessing of red rice bran: A detailed study on nutraceutical compositions, antioxidant and human LDL oxidation inhibition properties. *Food Chem*. 2021;351:129272. doi: 10.1016/j.foodchem.2021.129272
5. Ghasemzadeh A, Baghdadi A, Z E Jaafar H, et al. Optimization of flavonoid extraction from red and brown rice bran and evaluation of the antioxidant properties. *Molecules*. 2018;23:E1863. doi: 10.3390/molecules23081863
6. Boue SM, Daigle KW, Chen MH, et al. Antidiabetic potential of purple and red rice (Oryza sativa L.) bran extracts. J Agric *Food Chem*. 2016;64:5345–5353. doi: 10.1021/acs.jafc.6b01909
7. Tan XW, Kobayashi K, Shen L, et al. Antioxidative attributes of rice bran extracts in ameliorative effects of atherosclerosis-associated risk factors. *Heliyon*. 2020;6: e05743. doi: 10.1016/j.heliyon.2020.e05743
8. Limtrakul P, Yodkeeree S, Pitchakarn P, Punfa W. Anti-inflammatory effects of proanthocyanidin-rich red rice extract via suppression of MAPK, AP-1 and NF-κB pathways in Raw 264.7 macrophages. *Nutr Res Pract*. 2016;10:251-258. doi: 10.4162/nrp.2016.10.3.251
9. Surarit W, Jansom C, Lerdvuthisopon N, et al. Evaluation of antioxidant activities and phenolic subtype contents of ethanolic bran extracts of Thai pigmented rice varieties through chemical and cellular assays. *Int J Food Sci Technol*. 2015;50:990-998. doi: 10.1111/IJFS.12703
10. Chen MH, Choi SH, Kozukue N, et al. Growth-inhibitory effects of pigmented rice bran extracts and three red bran fractions against human cancer cells: relationships with composition and antioxidative activities. *J Agric Food Chem*. 2012;60:9151-9161. doi: 10.1021/jf3025453
11. Ghasemzadeh A, Karbalaii MT, Jaafar HZE, Rahmat A. Phytochemical constituents, antioxidant activity, and antiproliferative properties of black, red, and brown rice bran. *Chem Cent J*. 2018;12:17. doi: 10.1186/s13065-018-0382-9
12. Upanan S, Yodkeeree S, Thippraphan P, et al. The proanthocyanidin-rich fraction obtained from red rice germ and bran extract induces HepG2 hepatocellular carcinoma cell apoptosis. *Molecules*. 2019;24:813. doi: 10.3390/molecules24040813
13. OECD. Test No. 487: In Vitro Mammalian Cell Micronucleus Test [Internet]. Paris: Organisation for Economic Co-operation and Development; 2016 [cited 2022 Aug 21]. Available from: https://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264264861-en
14. World Health Organization. WHO traditional medicine strategy: 2014-2023 [Internet]. World Health Organization; 2013 [cited 2022 Aug 21]. 76 p. Available from: https://apps.who.int/iris/handle/10665/92455
15. Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. *Nat Rev Clin Oncol*. 2020;17:395-417. doi: 10.1038/s41571-020-0341-y
16. Lowe SW, Lin AW. Apoptosis in cancer. *Carcinogenesis*. 2000;21:485-495. doi: 10.1093/carcin/21.3.485
17. Araldi RP, de Melo TC, Mendes TB, et al. Using the comet and micronucleus assays for genotoxicity studies: A review. *Biomed Pharmacother*. 2015;72:74-82. doi: 10.1016/j.biopha.2015.04.004
18. Fenech M. Cytokinesis-block micronucleus cytome assay. *Nat Protoc*. 2007;2:1084-1104. doi: 10.1038/nprot.2007.77
19. Ratanavalachai T, Thitiorul S, Tanuchit S, et al. Antigenotoxic activity of Thai Sangyod red rice extracts against a chemotherapeutic agent, doxorubicin, in human lymphocytes by sister chromatid exchange (SCE) assay in vitro. *J Med Assoc Thail Chotmaihet Thangphaet*. 2012;95:109-114. Suppl 1:S109-S114

Submitted: October 03, 2022

Ратсада Прафасават [1], Саравут Паліпоч [2], Прасіт Суванналерт [3], Вітчуда Паюхакріт [3], Павін Кунсорн [3], Сарунья Лаовіттхаанггун [4], Сінітра Тхакаєв [1], Наронсук Мунконг [1], Варангхана Кладжинг [5]

[1] Медичний факультет, Університет Пхаяо, Пхаяо, Таїланд
[2] Медичний факультет, Університет Валайлак, Накхон Сі Таммарат, Таїланд
[3] Факультет природничих наук, Університет Махідол, Бангкок, Таїланд
[4] Експертний центр інноваційних рослинних продуктів, Таїландський інститут наукових і технологічних досліджень, Технополіс, Патхум Тані, Таїланд
[5] Відділ традиційної китайської медицини, Школа громадського здоров'я, Університет Пхаяо, Пхаяо, Таїланд

ЕКСТРАКТ ІЗ ВИСІВОК ЧЕРВОНОГО РИСУ ПРИГНІЧУЄ ПРОЛІФЕРАЦІЮ КЛІТИН РАКУ ТОВСТОЇ КИШКИ ШЛЯХОМ ІНДУКЦІЇ АПОПТОЗУ І ЗУПИНКИ КЛІТИННОГО ЦИКЛУ ТА ПРОЯВЛЯЄ АНТИМУТАГЕННУ АКТИВНІСТЬ

Стан питання. Екстракт із висівок червоного рису (ЕВЧР) містить велику кількість біологічно активних речовин, які характеризуються антиоксидантною та протизапальною дією. **Мета.** Оцінити протипухлинний ефект ЕВЧР на клітини раку товстої кишки людини та його мутагенну/антимутагенну дію на нормальні клітини. **Матеріали та методи.** Дослідження проведене на клітинах ліній раку товстої кишки (HCT116, HT29) і немалігнізованих клітинах лінії HEK293. Вплив ЕВЧР на життєздатність клітин досліджуваних ліній визначали забарвленням трипановим синім. Цитотоксичну та антипроліферативну дію ЕВЧР визначали за допомогою MTS-тесту та аналізом формування колоній. Індукцію апоптозу оцінювали за допомогою ELISA, а рівень апоптозу та розподіл за клітинним циклом — за допомогою проточної цитометрії. Мутагенний/антимутагенний потенціал ЕВЧР аналізували за допомогою мікроядерного аналізу в клітинах V79. **Результати.** Встановлено дозозалежне зниження життєздатності клітин ліній раку товстої кишки під дією ЕВЧР за обмеженій цитотоксичності для клітин HEK293. ЕВЧР пригнічував проліферацію клітин HCT116 і HT29 і індукував апоптоз в них, про що свідчить підвищена фрагментація ДНК і поява апоптотичних клітин. Продемонстровано збільшення кількості клітин раку товстої кишки у фазі G_2/M під дією ЕВЧР. Зауважимо, що ЕВЧР не збільшив частоту мікроядер у клітинах V79 і понад те, зменшив утворення мікроядер, спричинене мітоміцином С. **Висновки.** ЕВЧР ефективно інгібує проліферацію, індукує апоптоз і викликає зупинку клітинного циклу в клітинах раку товстої кишки людини, не будучи мутагенним і виявляючи антимутагенну дію *in vitro*.

Ключові слова: клітини раку товстої кишки, апоптоз, зупинка клітинного циклу, антимутагенність, екстракт із висівок червоного рису.