

RESEARCH ARTICLE

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Analyzing the AntiCancer and AntiMutagenic Properties of Dinactin, a Macrolide Antibiotic

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Abstract

Background: Produced from *Streptomyces*, Dinactin is one of the most important macrolide antibiotics. Previous studies have found that dinactin exerts inhibiting the cancer stemness potential of lung cancer, making it a promising option for CRC therapy. In this study, we designed experiments to evaluate dinactin's inhibitory effects against two human colorectal cancer cells (HCT116 and HT29) and elucidate the mechanism of its anti-cancer effects. Finally, we provided the first ever investigation of the mutagenic and anti-mutagenic potentials of dinactin. **Methods:** We studied the effects of dinactin as part of an anti-cancer approach using MTT test, clonogenic assay, wound healing assay, and transwell migration assay in HCT116 and HT29 cells. Mechanical studies were conducted using flow cytometry and Cell Death Detection ELISA. The mutagenic and anti-mutagenic properties were demonstrated using the in vitro cytokinesis-blocked micronucleus assay in V79 cells. **Results:** Dinactin was found to have dose-dependent inhibitory effects on the viability of HCT116 and HT29 cells with IC₅₀ values of 1.6 ± 0.4 and 35.0 ± 14 nM, respectively. Furthermore, dinactin showed potent anti-proliferation, anti-migration, and anti-invasion activities and demonstrated similar activity to doxorubicin. Mechanically, treatment with dinactin resulted in increased DNA fragmentation and positive AnnexinV/FITC-PI staining, which demonstrated the induction of apoptosis by dinactin. From the results of the mutagenic assay, dinactin did not produce a significant increase in micronucleus frequency when compared with a negative control ($p > 0.05$). In order to verify dinactin's anti-mutagenic properties, a combination of MMC (a known mutagen) and dinactin were applied to V79 cells. Treatment with dinactin resulted in a significant decrease in the number of micronucleated cells. **Conclusion:** Dinactin inhibits growth and alleviates the progression of CRC cells at nanomolar concentrations. This anti-CRC activity is mediated through the induction of apoptosis. Dinactin did not exhibit mutagenic effects and has the potential to reduce the mutagenic action of MMC.

Keywords: Dinactin, anti-cancer activity, anti-mutagenicity, colorectal cancer

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Introduction

Colorectal cancer is the most common form of cancer incidence worldwide, as well as a leading cause of cancer-related deaths [1–3]. In recent years, while the overall incidence of CRC, particularly rectal and distal colon cancers, has declined in individuals older than 50 years due to effective cancer screening measures, an increase has been observed in the number of young patients diagnosed with colon cancer [4–6]. In Thailand, it was estimated that there were >20,000 new cases of CRC (11.0% of all cases) in 2022 [7]. Genetic and epigenetic modifications are involved in CRC carcinogenesis, leading to transformation from normal epithelial cells to hyperplasia, dysplasia, and in situ cancer until finally developing into malignant neoplasms. Additionally,

advanced metastatic cancer is the final stage and the main cause of mortality of patients with CRC cancer [8–10]. For CRC treatment, using a single method of colonoscopy and colectomy is the most common therapeutic approach but may not be enough to delay cancer development. Thus, a combination with chemotherapy has also been used to fight CRC [11]. However, common resistance to chemotherapy as a consequence of cellular abnormality in apoptosis and toxicity are frequently reported [12, 13]. These are important factors that limit the efficacy of anticancer drugs. Therefore, a new approach using an alternative complementary drug to treat CRC is essential.

Dinactin is a bioactive metabolite isolated from *Streptomyces*. A notable finding of this macrolide dinactin is that it has exhibited strong anti-microbial activity against various bacterial pathogens and pathogenic

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fungi, including *Staphylococcus epidermidis*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Mycobacterium tuberculosis* [14, 15]. In addition, dinactin has been shown to have anti-inflammatory effects through reducing T-cell activation and IL-5 secretion, which is an important factor contributing to pulmonary eosinophilia. These properties would lead to benefits in the treatment of symptoms related to allergic inflammation [16]. In cancer research, the activity of dinactin is associated with anticancer effects as it has been reported to have potent cytotoxic activity against certain cancer cells, such as lung, breast, colon and liver cancer, while being non-toxic to normal cells [17, 18]. Some studies further explored the mechanism of dinactin targeting the *Wnt/β-catenin* signaling pathway and found that it could effectively inhibit cancer progression [18]. Our previous research found that dinactin reduced cell proliferation and the cancer stemness properties of non-small cell lung cancer. Mechanically, dinactin inhibited cell cycle arrest at the G0/G1 phase and reduced the number and size of the tumor spheres as well as down-regulating the expression of cancer stemness markers [17]. In view of our long-standing interest in the antibiotic and anticancer properties of dinactin, we aimed to verify and elaborate the function of dinactin and to determine its underlying mechanism of action through apoptosis and cell cycle effects against colorectal adenocarcinoma. Moreover, we first explored its mutagenic and anti-mutagenic activities in vitro with a cytokinesis-blocked micronucleus test, in the hope that the results would provide essential information to support the clinical studies and potential therapeutic use of dinactin for this type of cancer.

Materials and Methods

Cell culture and treatment

Two human colorectal cancer cell lines, HCT116 and HT29 were obtained from the Department of Pathobiology, Faculty of Science, Mahidol University. V79, a Chinese hamster lung fibroblast cell line, was provided from Thailand Institute of Scientific and Technological Research (TISTR). The HCT116 and HT29 cells were cultured in McCoy's A medium, while the V79 cells were maintained in MEM medium. All media were complemented with 10% Fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). The cells were cultured as the monolayer in tissue culture flasks (Corning, USA), incubated at 37 °C in a 5 % CO₂ humidified atmosphere, and sub-cultured when they reached approximately 80% confluence. Thereafter, dinactin of ≥95.00% purity (Cat. No. C3454) from the APExBio (Boston, MA, USA), suspended in DMSO, was applied for cell treatment. The dilution of the DMSO applied for each treatment was less than 0.1% (v/v).

MTT colorimetric assay for cell viability

The effect of dinactin on the cell viability of the HCT116 and HT29 cell lines was assessed using yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) dye. Briefly, the cells were seeded in 96-well plates at a density of 5000 cells/well and

incubated overnight at 37°C in 5% CO₂. On the following day, the cells were treated with different concentrations of dinactin in 0-100 nM concentrations and incubated for 24 h. In addition, untreated control cells containing medium were also included in each plate. After the incubation time, 50 µL of MTT solution (2 mg/mL in phosphate-buffered saline) was added to each well; the plates were then covered with aluminum foil and incubated for a further 2 h. Formazan crystals were solubilized in DMSO and extinction was measured at 570 nm using a microplate reader (MRX A2000, Korea). MTT assays were performed at least three times using six independent biological replicates. Data were normalized to solvent controls and the graphical representation of the obtained data as well as the calculation of the IC₅₀ values were carried out by GraphPad Prism Software (GraphPad Software, Inc., La Jolla, CA, USA). The impact of dinactin on cell growth was calculated as the percentage of viable cells [19].

Colony formation assay

The HCT116 and HT29 cells were cultured in 6-well plates at a density of 5 x 10² cells per well. After 48 h treatment with dinactin at different concentrations, the cells were incubated for 10 days until colonies were observed. Next, the cells were washed three times with PBS, fixed with 70% methanol, and stained with crystal violet (1%) for 1 h. Colony numbers (>50 cells) were counted under a microscope (100x). Data were signified as colony numbers in dinactin groups relative to the untreated control expressed as a mean ±SD of three independent experiments.

Cell migration and invasion assays

The migratory activity of the HCT116 and HT29 cells were determined using a wound healing assay, as previously described. Those cells were cultured until more than 90% of confluent growth was achieved for each well of 24-well plates, at 37°C in a humidified atmosphere of 5% CO₂. The cell monolayer was carefully wounded by a sterile micropipette tip to make a scratch wound across each well. Following washing and the removal of any cellular debris, the cells were treated with and without different concentrations (0.5 to 2 nM) of dinactin for 24 or 48 h. Cell migration into the wound was photographed and the distances measured with an inverted microscope (Olympus) at each time point (0, 24 or 48 h). The migration rate was calculated as the percentage of wound closure as previously described [19, 20].

$$\text{Wound Closure \%} = [(A_0 - A_t) / A_0] * 100$$

A₀ = 0h is the area of the wound measured immediately after scratching (t = 0 h).

A_t = 24h is the area of the wound measured 24 or 48 h after the scratch is performed.

Cell death detection ELISA

The effect of dinactin on the apoptosis induction was studied using Cell Death Detection ELISA, which recognizes the DNA fragment, a hallmark of apoptosis. The cells were treated with indicated concentrations of dinactin for 24 h. For apoptosis analysis, the cells were

harvested and lysed. A total of 20 μ l lysate was added to the ELISA plate, which was subsequently incubated with an immunoreagent. Then, the immunocomplex was bound with substrate and prepared for analysis at 490 nm by a microplate reader (MRX A2000, Korea) [20, 21].

Annexin-V-FITC/PI assay

The apoptosis was confirmed using the Annexin V/PI double staining assay as it recognized the externalization of phosphatidylserine on the membrane of the apoptotic cells. In brief, 2×10^5 cells per well were seeded in a 12-well plates and treated with and without indicated concentrations of dinactin for 24 h. For apoptosis assessment, the cells were harvested and resuspended in 200 μ l of binding buffer. The cells were stained with each of 2 μ l Annexin V and PI (BioLegend, San Diego, CA) and then incubated at room temperature for 15 min in the dark. Afterwards, the cells were analyzed by a FACSymphony A1 flow cytometer (Becton Dickinson, USA), and the results were processed with FlowJo software.

Cell cycle analysis

The HCT116 and HT29 cells were treated with dinactin (0.1 to 2 nM) for 24 h in complete medium. Subsequently, the cells were subjected to trypsinization, washed by centrifugation with cold PBS, and analyzed for the cell cycle using the Cell Cycle Analysis Kit (Abcam, ab287852). The cells were fixed with cold 70% ethanol for 30 min, then resuspended in buffer and stained with the staining solution. The samples were incubated in the dark at room temperature for 30 min. Cell cycle analysis was then performed using a FACSymphony A1 flow cytometer (Becton Dickinson, USA), and the histograms of the DNA were interpreted with FlowJo software.

Cytokinesis-blocked MN test (CBMN)

The CBMN was performed to evaluate the mutagenicity and anti-mutagenicity potential of dinactin. From the results obtained with the viability assay, the concentrations of 0.5, 1 and 2 nM of dinactin were selected for the initial evaluation of the genotoxicity of this substance. V79 cells were seeded at a density of 2×10^5 cells in 6-well plates, which were treated with different concentrations of dinactin or Mitomycin C (MMC) at a concentration of 1.25 μ g/ml [22]. MMC was used as a positive control. Following treatment, cytochalasin B (final concentration of 3 μ g/ml) was added to the culture, which was then

incubated for 24 h at 37°C, in an incubator with 5% CO₂. The cells were washed and collected using centrifugation. Then, the treated cells were incubated with hypotonic solution, fixed with cold methanol and stained with 10% Giemsa solution for 30 min. One-thousand binucleated cells per replica were counted and the micronucleus frequency was determined.

For the anti-mutagenicity evaluation, the cells were pre-treated with the different doses of dinactin for 6 h. After this period, the medium was removed and the cells were exposed to a new culture medium containing MMC. Before the harvesting step, the cell cultures were incubated with cyt-B for another 12 h and collected using the methods as described previously.

Statistical analysis

All data were analyzed using SPSS software (version 17). The results were shown as a mean percent value of control $\pm$ standard deviation (SD) and calculated from at least three independent experiments. One-way ANOVA with Tukey's post hoc test was used to assess multi-group comparisons. The p-values are reported as follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Results

Dinactin reduced colorectal cancer cells viability

The effects of dinactin on the cell viability of HCT116 and HT29 cells were assessed (Figure 1). The cells were treated with dinactin at concentrations ranging from 0 to 100 nM for 48 h. Dinactin produced significantly concentration-dependent decreases in the cell viability of both cancer cells. The HCT116 cells were more sensitive to dinactin, with an IC₅₀ value of 1.6 ± 0.2 nM, whereas the HT29 cells exhibited lower sensitivity, with an IC₅₀ value of 35.0 ± 14 nM, indicating a clear difference in responsiveness between the two colorectal cancer cell lines.

Dinactin inhibit self-renewal ability of colorectal cancer cells

We evaluated the proliferation effect of dinactin on HCT116 and HT29 and compared the results with those produced by doxorubicin. As shown in Figure 2, the effect of the doxorubicin treatment groups showed decreased cell colonies of 5.30 ± 0.70 (for HCT116 cells) and 11.51 ± 2.12 (for HT29 cells). After incubation with dinactin, the cell proliferation of those cells was shown to be significantly

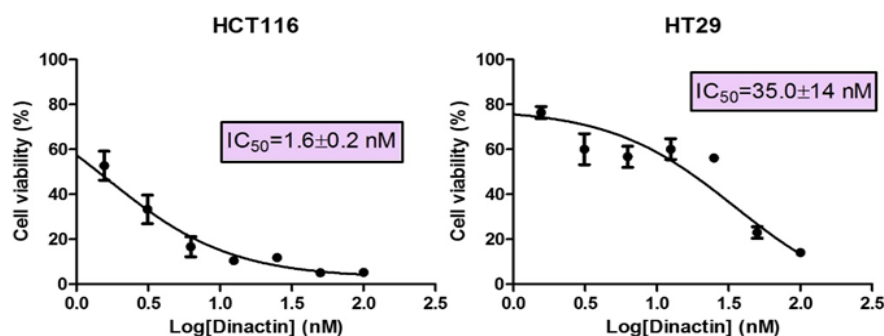


Figure 1. Inhibitory Effect of Dinactin on the Viability of HCT116 and HT29 Cells

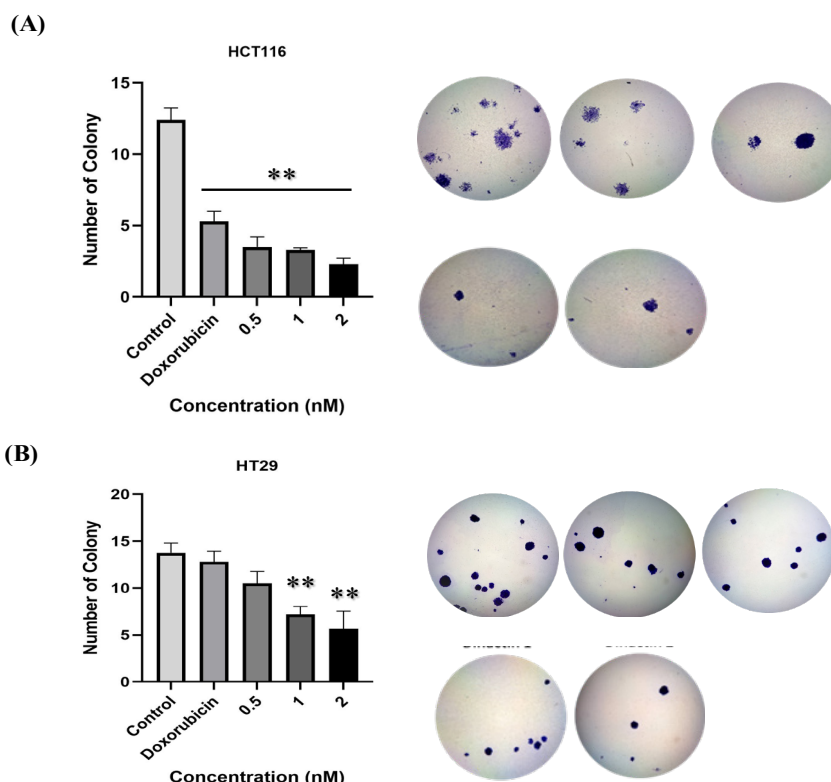


Figure 2. The Effect of Dinactin on Cell-Renewal Ability. The treatment of HCT116 (A) and HT29 cells (B) with varying doses of dinactin compared with untreated and doxorubicin controls. The bar diagram represents the quantification of colonies per field. ** $p < 0.01$.

decreased following testing with clonogenic assay ($p < 0.01$). Interestingly, after treatment with dinactin at concentrations of 0.5, 1 and 2 nM, the cell colonies of HCT116 showed the greatest concentration-dependent decrease in the number of cellular colonies to 3.51 ± 0.70 , 3.33 ± 0.14 , and 2.30 ± 0.42 , respectively. In the HT29 cells, the number of cell colonies was 10.50 ± 1.27 , 7.20 ± 0.84 , and 5.67 ± 1.89 , respectively.

Dinactin Inhibited the Migration and Invasiveness of colorectal cancer cells

Cell migration and invasion are important steps in cancer progression. Therefore, the effects of dinactin on cell movement were examined on HCT116 and HT29 cells. From the results of wound-healing migration assay, our data showed that dinactin inhibited the migration rate of both HCT116 and HT29 cells compared with untreated cells (Figure 3A -3B). The control group exhibited signs of cell migration resulting in a greater percentage of gap closure. In the HCT116 cells, the percentage of gap closure in the control was 60.10%, while the percentage of gap closure in dinactin-treated cells at concentrations of 0.5 and 2 nM was 38.06% and 35.59%, respectively. For the HT29 cells, the results confirmed that the migration ability of cells was significantly diminished at 23.79% and 13.19% in dinactin-treated cells at concentrations of 0.5 and 2 nM, respectively. Moreover, dinactin showed a similar inhibition rate of migration as the doxorubicin positive control.

Furthermore, the invasion ability of colorectal cancer cells was assessed by transwell chamber invasion assay.

As expected, the penetration of HCT116 and HT29 cells through the extracellular matrix-coated filter was suppressed in the presence of dinactin. As shown in Figure 3C, the invasion ability of the HCT116 cells treated with dinactin at concentrations of 0, 0.5 and 2 nM was 62.41 ± 1.29 , 30.50 ± 4.94 and 20.50 ± 4.95 respectively. Moreover, dinactin was also found to decrease the invasion ability of the HT29 cells to 14.20 ± 2.18 , 14.83 ± 4.47 and 11.0 ± 1.41 after treatment with dinactin at concentrations of 0, 0.5 and 2 nM, respectively (Figure 3D). In addition, dinactin showed a similar trend of inhibiting invasion when compared with doxorubicin.

Dinactin induced apoptotic death of colorectal cancer cells

To determine whether the apoptosis induction plays a role in dinactin-induced proliferative suppression of colorectal cancer cells, we performed a quantitative analysis of DNA fragments of apoptotic cells as demonstrated by ELISA. The result revealed that the appearance of round cells like apoptosis was higher in both HCT116 and HT29 cells treated with dinactin compared to untreated control cells (Figure 4A -4B). The apoptosis analysis showed that the apoptosis induction significantly increased following treatment with dinactin at a concentration of 0.5 nM in both the HCT116 and the HT29 cells ($p < 0.01$).

To further confirm the apoptosis effect induced by dinactin, we assessed the number of Annexin-V/PI positive apoptotic cells with Flow cytometry. As shown in Figure 4C -4D, the increase of apoptotic

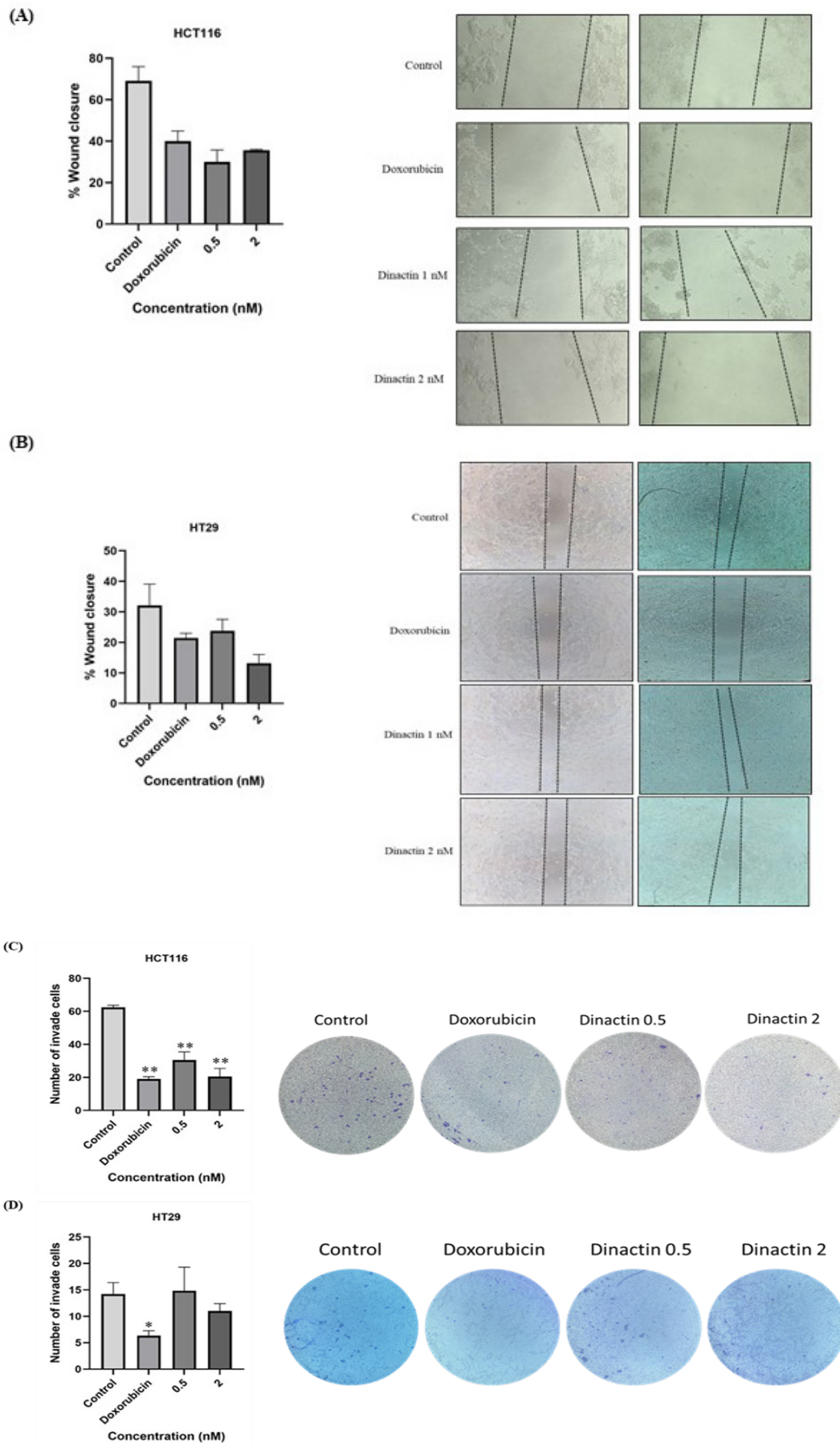


Figure 3. Dinactin Abrogates Cell Migration and Invasion of HCT116 and HT29 Cancer Cells. The migration activity of cells exposed to varying doses of dinactin along with untreated and doxorubicin controls (A, B). Transwell assay was performed to determine HCT116 and HT29 cell invasion after exposed to dinactin along with untreated and doxorubicin controls (C, D). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

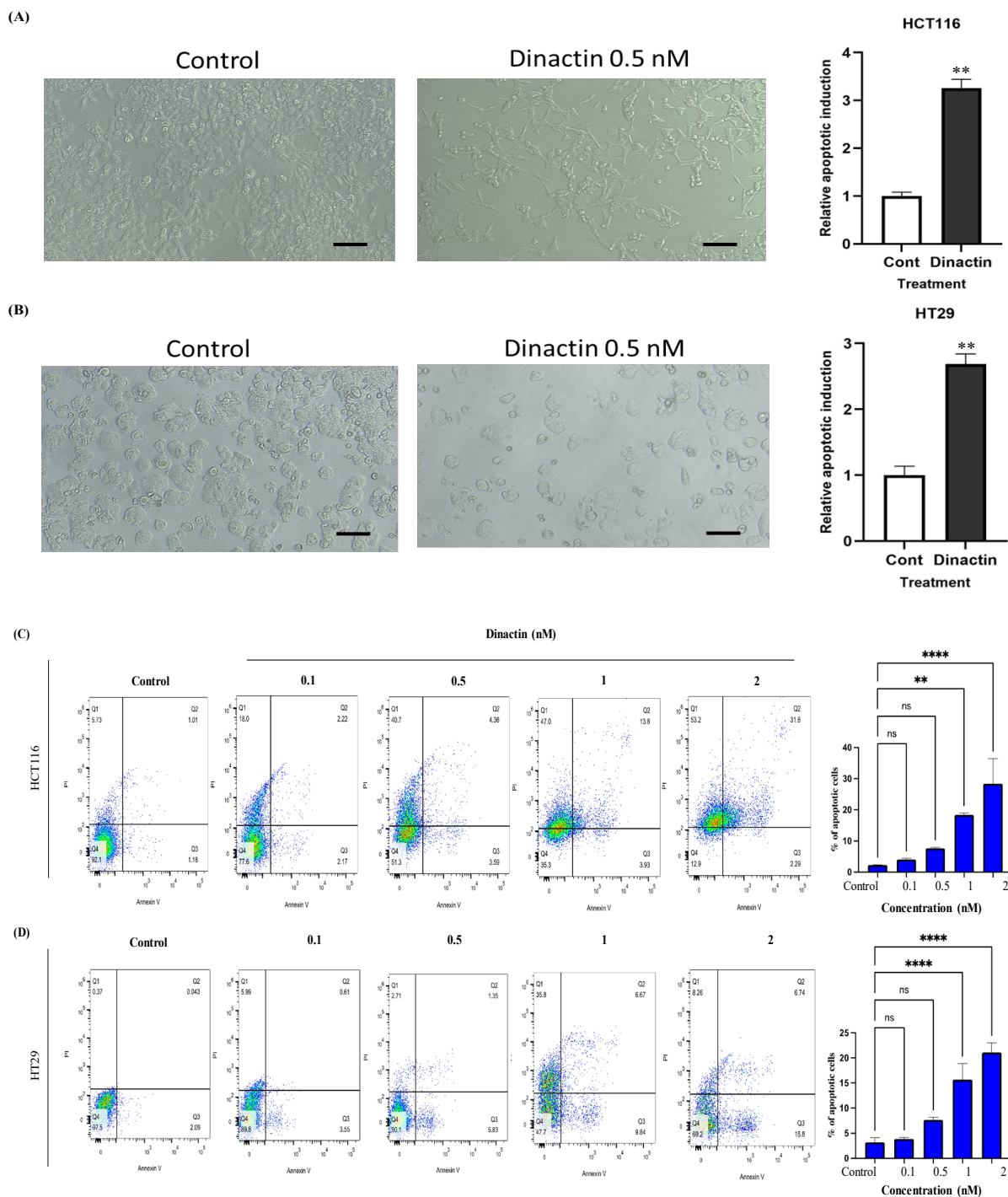


Figure 4. Dinactin Induced Apoptotic Cell Death of Colorectal Cancer Cells. HCT116 and HT29 were treated with varying doses of dinactin and compared with an untreated control. The apoptotic analysis used ELISA (A, B). The apoptotic populations induced by dinactin were determined using flow cytometer-based Annexin V assay (C, D). ** $p < 0.01$, **** $p < 0.0001$.

cell death (Q2+Q3) was observed in both HCT116 and HT29 following treatment with dinactin at various concentrations (0, 0.1, 0.5, 1 and 2 nM), suggesting the dose-dependent apoptosis effect of dinactin.

Cell cycle distribution analysis of dinactin

We examined the effect of dinactin on the cell cycle distribution of colorectal cancer cells. As shown in Figure 5, HCT116 and HT29 cells were treated with dinactin (0.1-2 nM) for 48 h. Flow cytometry analysis

revealed no significant treatment-related cell cycle change from dinactin at any concentration compared with the untreated control group in both HCT116 and HT29 cells ($p > 0.05$). Therefore, dinactin treatment at the indicated concentrations might not affect the cell cycle's ability to suppress proliferation.

Mutagenicity and anti-mutagenicity evaluation

In order to investigate the mutagenicity and anti-mutagenicity effects of dinactin in V79 cells, concentrations

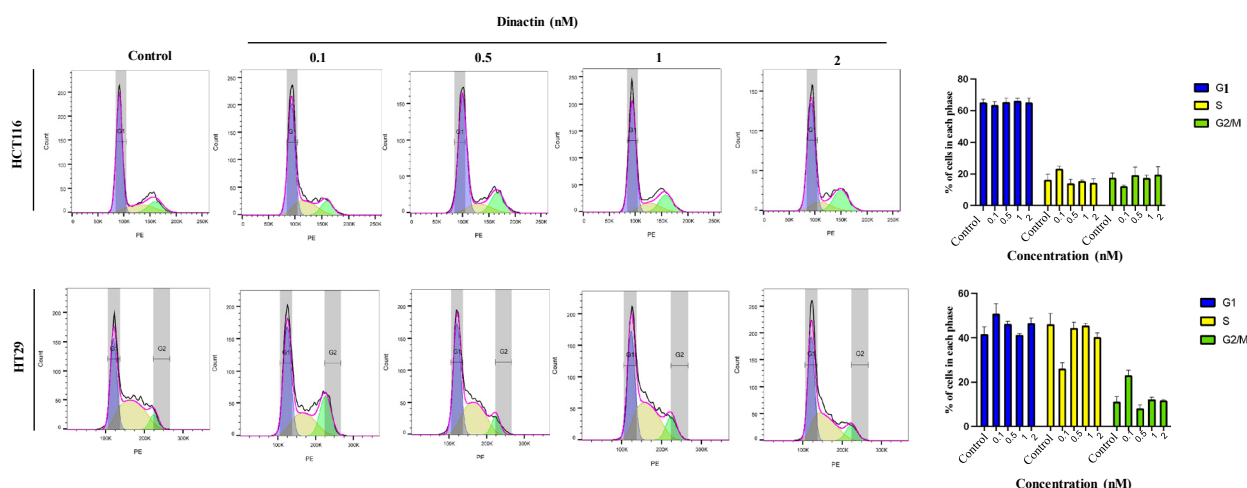


Figure 5. Cell Cycle Evaluation of HCT116 and HT29 Cells Treated with Dinactin. Histogram showing the effects of dinactin on the cell cycle in HCT116 (A) and HT29 (B) cells treated with different concentrations (0, 0.1, 0.5, 1, and 2 nM) for 24 h using flow cytometry. Bar graph representing the percentage of cells in each phase of the cell cycle. Data are presented as mean $\pm$ standard deviation (Mean $\pm$ SD) from three independent experiments (n=3).

of 0.5, 1 and 2 nM were used. The results of MN are shown in Figure 6 and a total of 1,000 binucleated cells per concentration were analyzed for each individual experiment. Also, the numbers of cells with one, two, three, or four nuclei were recorded. For the mutagenicity evaluation, the frequency of MN in the group treated with 0.5–2 nM of dinactin, which were $10.13 (\pm 8.01)$, $10.03 (\pm 7.14)$ and $16.92 (\pm 4.82)$ respectively, showed no significant difference from the untreated group: $8.18 (\pm 0.97)$, whereas the MMC showed a statistically

significant increase in the frequency of MN (46.55 ± 5.67 , $p < 0.001$), as shown in Figure 6B. The anti-mutagenic activity of dinactin is shown in Figure 6C. The results show that MMC alone significantly induced MN formation in V79 cells (45.83 ± 3.33) ($p < 0.01$) as compared to the negative control group, whereas the MMC-induced MN formation was significantly inhibited by the treatment of MMC with dinactin at concentrations of 0.5, 1, and 2 nM with 15.19 ± 0.69 , 13.88 ± 2.03 , and 19.73 ± 1.14 of micronucleated cells, respectively when compared with

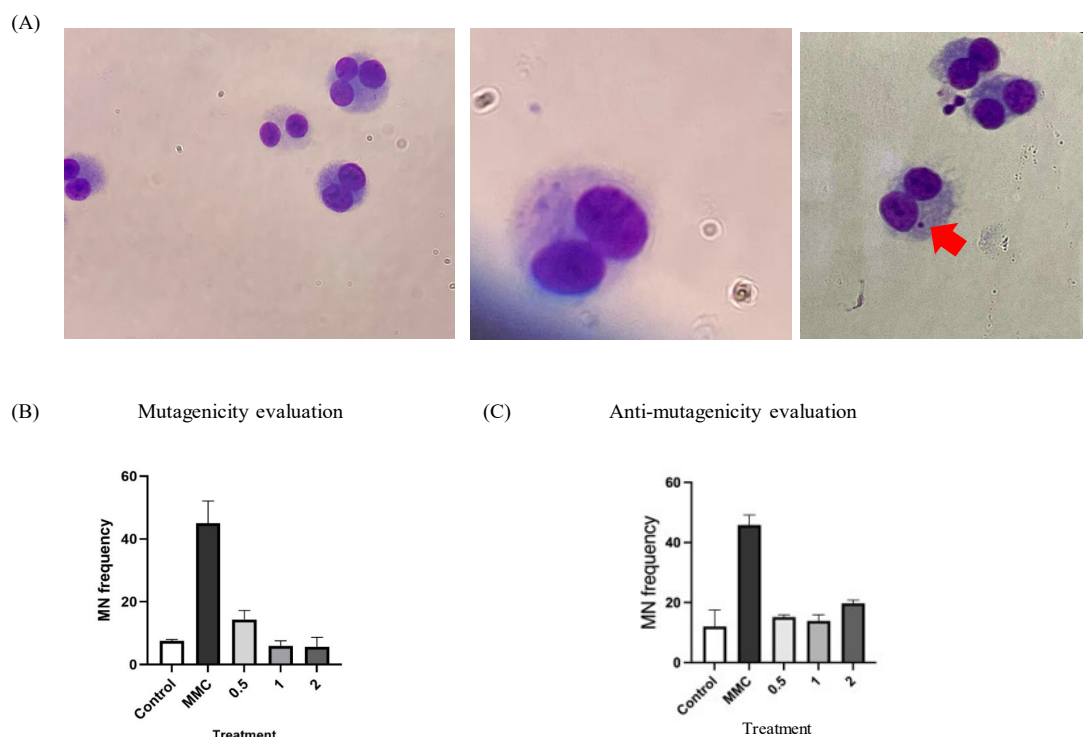


Figure 6. *In vitro* Cytokinesis-Blocked Micronucleus Assay in V79 Cells. (A) A photograph of V79 cells showing cell distribution and micronuclei (arrow) in binucleated cells. (B) Frequency of micronuclei obtained in binucleated cells after exposure to dinactin at indicated concentrations (0.5, 1 and 2 nM) or MMC (1.25 $\mu\text{g/ml}$) as a positive control. (C) Frequency of micronuclei obtained in binucleated cells after exposure to dinactin at indicated concentrations (0.5, 1 and 2 nM) in combination with MMC. ** $p < 0.01$ compared to negative control, a $p < 0.01$ compared to MMC group.

the group treated with MMC alone ($p < 0.01$).

Discussion

Dinactin has recently been a popular subject of biological research due to it being a secondary metabolite compound produced by *Streptomyces* sp. and functionally exhibiting strong anti-microbial activity against bacterial pathogens including *Mycobacterium tuberculosis* [14, 15]. Dinactin's potent antineoplastic activity has also been attracting increasing interest in the development of an anti-cancer drug from these small molecules because of its effectiveness in targeting certain cancer cell types [17, 18, 23]. To support the growing body of anti-cancer evidence, the present study evaluated the anti-cancer effects of dinactin on two colorectal cancer cells with different genetic profiles, namely HCT116 and HT29 cells. The study also explored both the mutagenicity and the anti-mutagenicity of this compound in V79 cells.

Our previous studies have shown that dinactin specifically inhibited the proliferation of non-small cell lung cancer (NSCLC), including Lu99 and A549 cells. We also showed that dinactin is able to block cell cycle at the G0/G1 phase and reduce the number and size of the tumor spheres, leading to dinactin inhibiting cancer stemness in NSCLC cells [17]. In the present study, we demonstrated that dinactin inhibits colorectal cancer cells as validated by reducing cell viability and clonogenic renewal and inhibiting cell migration and invasive abilities. We also investigated the mechanisms that could potentially suppress proliferation. Interestingly, our results show that inducing apoptosis may be part of the process leading to the suppression of the proliferation of colorectal cancer cells. The results of the present study are consistent with the finding from previous studies, which reported that dinactin exerted cancer inhibition against various cancer types in the in vitro MTT assay and reduced cellular clonogenic proliferation as well as migration in HCT116 cells (23). Previous studies also further reported the efficacy of dinactin in inhibiting cancer cell proliferation and inducing G1/S cell cycle arrest by blocking the *Wnt/β-catenin* signaling pathway in cancer cells [18]. However, the present study found that treatment with dinactin could induce cancer cell death through the induction of apoptosis in those colorectal cancer cell lines. In addition to inhibiting proliferation, dinactin was also shown to inhibit cell migration and invasion abilities, which play important roles in the spread of cancer cells spread and lead to increased mortality rates. However, we could not find the impact of cell cycle arrest caused by dinactin at the concentrations used in this study. It could be hypothesized that cells seem to use different pathways for cell deaths.

Finally, the impact of dinactin on chromosomal stability is not yet known. Therefore, we first explored both the mutagenicity and the anti-mutagenicity triggered by dinactin, using the cytokinesis-blocked micronucleus (CBMN) assay. This assay detects structural and numerical chromosomal damage, making it suitable to evaluate the chromosomal mutations of compounds [22, 24–26]. In the present study, we measured the genotoxic response of

dinactin in V79 cells using CBMN assay in accordance with the OECD guidelines no.487 [27]. The OECD protocols recommended using the cytochalasin-b to inhibit cytokinesis during cell division. The advantages of doing this are that it decreases the number of false-negative results and ensures the analysis of micronucleate cells which have completely undergone cell division after exposure. Based on the data presented, the incidence of micronucleated cells in V79 cells exposed to all doses of dinactin were similar to the negative control group, except for the values observed in the MMC positive control group. These data reveal that no evidence indicating the mutagenic potential of dinactin was detected in the V79 cells. Moreover, we evaluated the anti-mutagenic potential of dinactin against a well-known mutagen mitomycin C using CBMN assay. Interestingly, the mean incidence of micronucleated cells tended to decrease with dinactin treatment in a dose dependent manner. This finding may imply that dinactin would be able to act against mutagens via intracellular and extracellular mechanisms. Therefore, it possesses antimutagenic ability as well as demutagenic modes of action which capable of preventing the action of damage-inducing agents and lesions to DNA [28–31]. However, we suggested that a molecular mechanism upon which apoptosis induction and anti-mutation needed to be investigated.

In conclusion, our results indicate that dinactin possesses anti-colorectal cancer properties. These findings demonstrate that dinactin induces apoptosis on both types of cancer cells (HCT116 and HT29) which could lead to the suppression of their proliferation. We compared the efficacy of dinactin against a chemotherapeutic agent, namely doxorubicin. We showed that dinactin produced similar effects on cell growth and movement inhibitions at concentrations of 0.5 to 2 nM. Additionally, we showed that treatment with dinactin did not cause mutagenicity but that it possessed potent anti-mutagenic properties in the cytokinesis-blocked micronucleus assay. These data can provide a new perspective of dinactin for biological therapy of colorectal carcinoma and may be effective drug targeting colorectal cancer in the future.

Author Contribution Statement

Ratsada Praphasawat: Conceive the study and Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Resources, Writing - original draft, Funding acquisition. Anchalee Rawangkan: Conceive the study and Investigation, Methodology, Interpreted the results, Writing the results and original draft, Funding acquisition. Grissana Pook-in, Tomoko Takahashi: Methodology and Validation. Sinithra Takaew, Wichuda Payuhakrit: Methodology, Formal analysis, Validation. All authors have read and approved the final manuscript.

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General

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Ethics statement

Ethical approval was not required based on this research. No human or animal subjects were involved.

Availability of data and materials

All data of the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest related to this manuscript.

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