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Piperine suppresses growth and migration of human breast cancer cells through attenuation of Rac1 expression

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ABSTRACT

Objective: To investigate the effect of piperine on human breast cancer cells.

Methods: The effect of piperine on proliferation and migration of human breast cancer cells, MCF-7 and MDA-MB-231, was investigated using colony formation assays, wound healing assays, Matrigel migration assays, flow cytometry, RT-qPCR, and Western blotting assays.

Results: Piperine inhibited the growth of MCF-7 and MDA-MB-231 cells and suppressed colony formation. Cell reduction at the G_0/G_1 phase and cell arrest at the G_2/M phase were observed in breast cancer cells. However, the significant effect was only demonstrated in MDA-MB-231 cells. Moreover, cancer cell migration was suppressed by piperine at low concentration. RT-qPCR and Western blotting assays showed that piperine downregulated Rac1 gene and protein expression.

Conclusions: Piperine could inhibit growth and migration of breast cancer cells by reducing Rac1 gene and protein expression.

KEYWORDS: Piperine; Breast cancer cells; Rac1; Cell cycle; Cell migration; MCF-7; MDA-MB-231

1. Introduction

Piperine, a nitrogenous pungent substance, is an important alkaloid, found in black and long peppers as a main constituent and displays a variety of pharmacological activities over several pathological diseases[1-3]. It was reported to exhibit many pharmacological effects including anti-inflammatory[4], neuroprotective[5], antioxidant[6], cardiovascular protection[7], digestion promotion[8], anti-bacterial and anticancer activities[9]. Piperine remarkably suppressed growth of several cancer cells and migration in both *in vivo* and *in vitro* studies[10]. Induction of apoptosis in cancer cells and attenuated proliferation in colon cancer cells *via* activating stress of endoplasmic reticulum were observed following treatment with piperine[11]. Additionally, piperine could lower lung cancer incidence in animal models[12]. It also synergistically enhanced the anticancer effect of tamoxifen by lowering IC_{50} values and arresting the breast cancer cells in the G_2/M phase[3].

Angiogenesis was reported to be inhibited through repression of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway and migration and invasion of cancer cells could be suppressed by blocking matrix metalloproteinases (MMPs)[2,3]. Previously, we reported that a higher piperine level in a *Piper nigrum* (*P. nigrum*) extract suppressed MCF-7 cell migration and angiogenesis

Significance

Breast cancer is the most common type of malignancy, and the leading cause of cancer-associated female mortality worldwide. This work indicates that piperine suppresses the breast cancer cell proliferation by reducing cell number at the G_0/G_1 phase, and cell arrest at the G_2/M phase and inhibits cell migration. The mechanism of action of piperine against breast cancer cells is through downregulating Rac1 gene and protein expression in breast cancer cells.

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by reducing MMP9 protein level and attenuating the gene expression levels of *MMP2*, *MMP9*, *VEGFA*, and *ICAMP1*[13]. Interestingly, the above studies suggested that piperine might be useful for treating and/or preventing cancer. However, differential effects of piperine on estrogen-positive (MCF-7) and estrogen-negative (MDA-MB-231) breast cancer cells are still unclear.

Ras-related C3 botulinum toxin substrate 1 (Rac1), as a member of mevalonate (MVA) pathway, plays major roles in cancer cell growth and migration[14] through interacting with PI3K/Akt pathway, and in turn activates p21-activated kinase, protein-related cell proliferation[15]. The reduction of Rac1 expression level may cause induction of cancer cell death in many cancer cell types[14]. Further, the depletion of Rac1 level induces cell cycle arrest and apoptosis in human breast cancer cells[16].

Our previous study reported that *P. nigrum* suppressed the Rac1 expression, by which *P. nigrum* inhibited growth and migration of MCF-7[13]. Piperine is one of the main active compounds found in *P. nigrum* extract that has strong anticancer activities against several cancer cell types, including breast cancer. Therefore, the present study aimed to investigate the antiproliferative and anti-migratory potentials of piperine on MCF-7 (estrogen-positive) and MDA-MB-231 (estrogen-negative) and the underlying mechanism of action through investigation of Rac1 gene and protein expression.

2. Materials and methods

2.1. Reagents and cell cultures

The human breast cancer cells MCF-7 and MDA-MB-231, were obtained from American Type Culture Collection (ATCC, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco Life Technologies, Carlsbad, CA, USA; Cat. No. 11885-084) along with 10% fetal bovine serum (FBS, Gibco Life Technologies, Carlsbad, CA, USA; Cat. No. 10270-106) and 1% antibiotic solution (penicillin and streptomycin, Gibco Life Technologies, Carlsbad, CA, USA; Cat. No. 15140-122) in a CO_2 incubator. After cells became confluent (70%-80%), they were sub-cultured using 0.25% trypsin-EDTA (Gibco Life Technologies, Carlsbad, CA, USA; Cat. No. 25200-072), and prepared for the experiments.

2.2. Cytotoxicity assay

Sulforhodamine B (SRB) assay was performed to evaluate the cell viability of MCF-7 and MDA-MB-231 cells after the addition of piperine. Cells (1×10^4 cells/well) were cultured onto a 96-well plate for 24 h, as previously described[17]. Treatment with piperine (0, 8.75, 17.5, 35, 87.5, 175, and 350 μM) or doxorubicin (0, 1, 2.5, 5, and 10 μM), was performed for 24-72 h. After incubation, cancer cells were incubated with 40 μL of 10% trichloroacetic acid for 60 min at 4 ℃, washed with distilled water, and incubated with 0.4% SRB for 30 min. Absorbance was measured at 540 nm using a microplate reader version Gen5 2.07 (BioTek Synergy H1). The IC_{50} values were calculated by GraphPad Prism 5 (GraphPad Software, San Diego, CA).

2.3. Colony formation assay

Colony formation assay was performed to evaluate the cell replication of MCF-7 and MDA-MB-231 cells after the treatment with piperine. Cells (500 cells/well) were cultured onto a 6-well plate for 24 h and treated with piperine at different concentrations (0, 17.5, 35, 87.5, 175, and 350 μM) for 24 h. After incubation, the cells were washed several times with PBS, a new complete DMEM medium was added, and re-cultured for 15 d. Methanol (100%) was added and cells were incubated at −20 ℃ for 60 min and stained with 0.5% crystal violet for 60 min. The cells were washed, dried, and the colonies were counted.

2.4. Cell cycle by flow cytometry

Cancer cells were cultured onto 6-well culture plates (2.5×10^5 cells/well) for 24 h. Then, cells were treated with piperine (0, 87.5, 175, and 350 μM) for 24 h and fixed by 70% cold ethanol at −20 ℃. After that, the pellet cells were washed with PBS, suspended in propidium iodide (PI) solution (Cat No. 550825, BD Biosciences, CA, USA) at 4 ℃ for 30 min. The distribution of DNA content was measured using a flow cytometer and the percentage of each phase was calculated using the BD Accuri™ C6 Plus (BD Biosciences, USA).

2.5. Wound healing assay

Wound healing assay was performed to evaluate MCF-7 and MDA-MB-231 migration after treatment with piperine. Cells (2.5×10^5 cells/well) were cultured onto a 24-well plate for 24 h, then they were scratched by 0.2 mL pipette tip, and treated with piperine (0, 35, 87.5, 175, and 350 μM) for 48 h. After incubation, 200 μL of 100% methanol was added and cells were fixed for 60 min at −20 ℃, washed with distilled water, incubated with 0.5% crystal violet for 30 min. The distance of the wound was captured at 0 and 48 h by 4× magnification of inverted microscopy and the remaining wound area was calculated in treatment groups and normalized to the original wounds.

2.6. Matrigel migration assay

Cells (2.5×10^5 cells/well) were seeded onto a 24-well Transwell chamber (8 μM pore size; Corning, Lowell, MA) in the upper chamber with 250 μL of piperine (0, 17.5, 35, 87.5, 175, and 350 μM) in DMEM-free FBS media for 24 h and lower chamber contained DMEM complete media. After incubation time, cancer cells were washed with PBS buffer, and fixed with 100% methanol for 60 min. Then cells were washed with PBS again and incubated

with 0.5% crystal violet for 30 min. The migrated cancer cells were captured at 24 h after incubation with piperine by 20× magnification of inverted microscopy and then counted.

2.7. Gene expression by real–time quantitative PCR (RT–qPCR)

RT-qPCR method was performed to evaluate the gene expression level of *Rac1* in MCF-7 and MDA-MB-231 cells after the treatment with piperine. Cells (2.5×10^5 cells/well) cultured onto a 6-well plate for 24 h were treated with piperine (0, 175, and 350 μM) and incubated for 24 h. After incubation, mRNA was extracted from cancer cells using Trizol® reagent and reverse transcribed into cDNA using the iScript reverse transcription Supermix (Bio-Rad, Hercules, CA, USA). Amplification of the PCR was performed using the target primers for each gene, *Rac1*, *RhoA*, and *β–actin* as an internal control. *Rac1* forward primer 5'ATGTCCGTGCAAAGTGGTATC3', *Rac1* reverse primer 5'CTCGGATCGCTTCGTCAAACA3', *RhoA* forward primer 5'GGA-AAG-CAG-GTA-GAG-TTG-GCT3', *RhoA* reverse primer 5'GGC-TGT-CGA-TGG-AAA-AAC-ACA-T3',

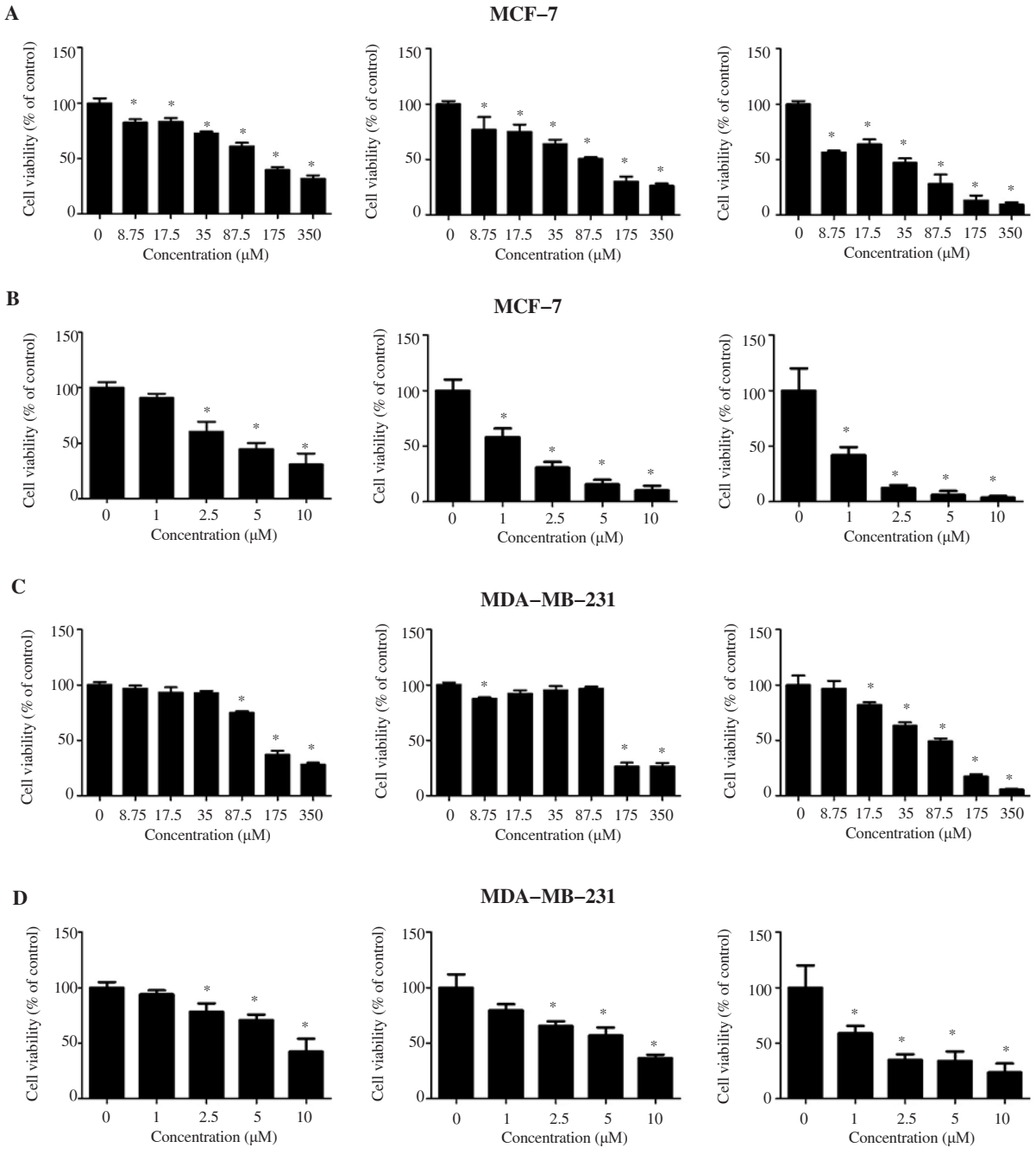

Figure 1. Effect of piperine on viability of human breast cancer cells by sulforhodamine B assay. MCF-7 and MDA-MB-231 cells were treated with various concentrations of piperine (A and C) and doxorubicin (B and D) for 24-72 h. Data are presented as mean±SE. n=3, $^*P$<0.05 compared with untreated control.

β-actin forward primer 5'GTGACGTTGACATCCGTAAAGA3', *β-actin* reverse primer 5'GCCGGACTCATCGTACTCC3'. The PCR was performed in a final volume of 20 μL containing cDNA template, 5 μM of each target primer, or 2.5 μM of each beta-actin primer in SsoFast™ EvaGreen Supermix with low Rox (Bio-Rad, CA, USA). The expression of *Rac1* gene was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.8. Protein expression by Western blotting assay

Western blotting assay was performed to evaluate the protein expression of Rac1 in MCF-7 and MDA-MB-231 cells after treatment with piperine. Cells exposed to piperine (0 and 350 μM) for 24 h were lysed by RIPA lysis buffer and the protein concentration was measured using Bradford reagents (PanReac AppliChem, Darmstadt, Germany, Cat No. A6932). Next, total protein of each group (20 μg) was subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (120 volt, 60 min), transferred to polyvinylidene fluoride (PVDF) membrane, exposed to Rac1 (Cat No. 2465S) and β-actin (Cat No. 4967S) primary antibodies (1:1 000, Cell Signaling Technology, Beverly, MA, USA) overnight at 4 ℃ and then incubated with the anti-rabbit IgG HRP-link antibody (Cat No. 7074S, 1:2 500, Cell Signaling Technology, Beverly, MA, USA) for 2 h. PVDF membrane was washed and visualized using an enhanced Western ECL Substrate.

The densities of the Rac1 bands were normalized against β-actin,

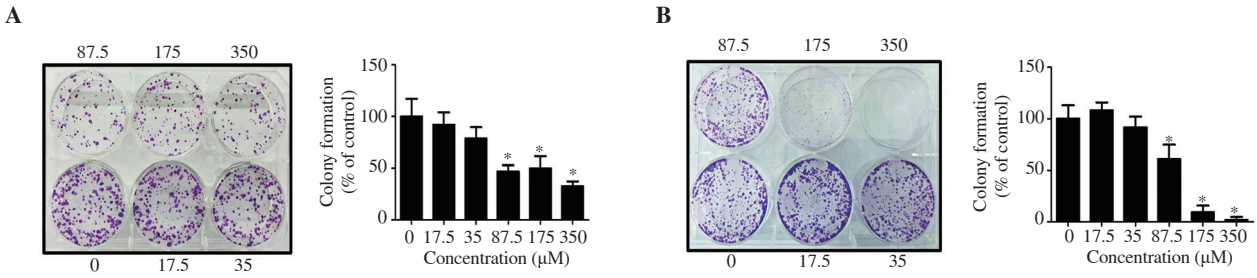

Figure 2. Effect of piperine on cell growth of (A) MCF-7 and (B) MDA-MB-231 cells using colony formation assay. Cells were treated with various concentrations of piperine for 24 h and then cultured further for next ten days for MDA-MB-231 cells and fifteen days for MCF-7 cells. Data are presented as mean±SE. n=3, $^{*}P$<0.05 compared with untreated control.

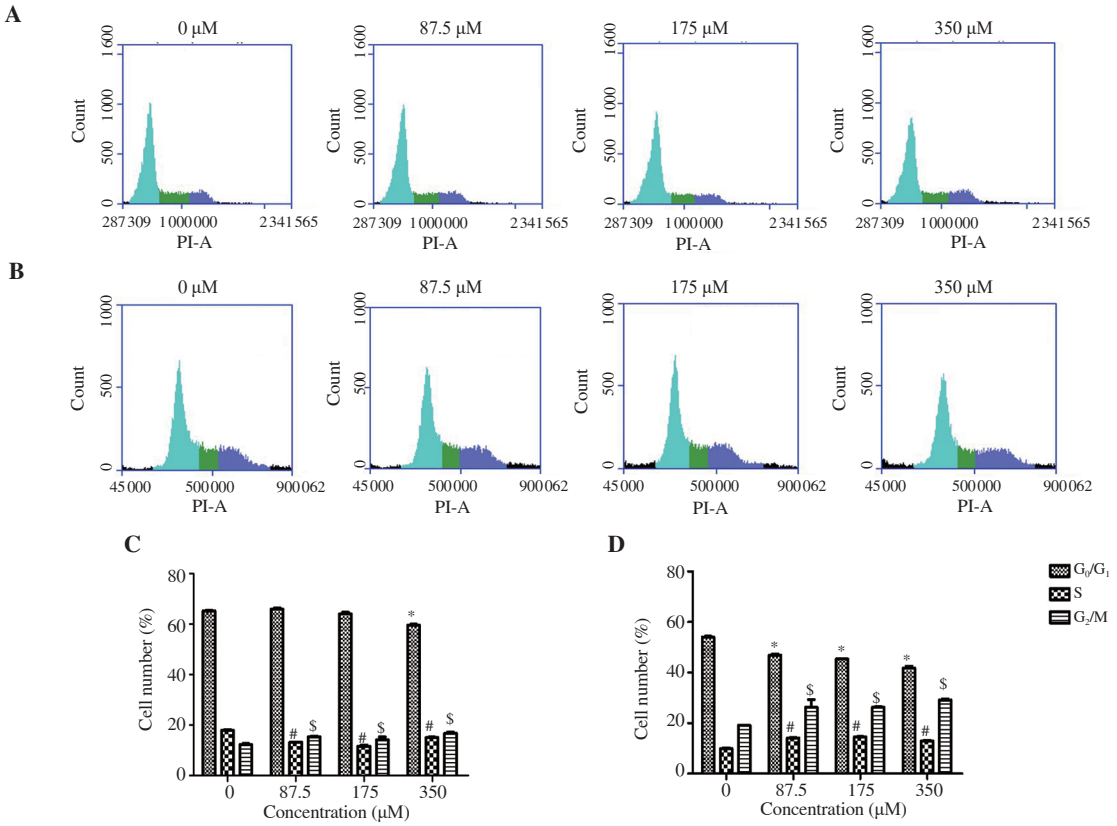

Figure 3. Effect of piperine on cell cycle of (A and C) MCF-7 and (B and D) MDA-MB-231 cells by flow cytometry. Cells were treated with various concentrations of piperine for 24 h. Data are presented as mean±SE. n=3. $^{*}P$<0.05, $^{\#}P$<0.05, $^{\$}P$<0.05 compared with untreated control.

and the mean ratio was calculated using a ChemiDoc™ MP imaging system with Image Lab software (Bio-Rad Laboratories Inc., Hercules, CA, USA).

2.9. Statistical analysis

Data were presented as mean ± standard error (SE). A student unpaired *t*-test was used to compare between treated and untreated groups by GraphPad Prism 5 and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Piperine inhibited cell viability

The cytotoxicity of piperine and doxorubicin, a standard anticancer drug, was shown in Figure 1 and Supplementary Table. Cell viability after treatment with different concentrations of piperine and IC_{50} values and maximum efficacy (Emax) were calculated at each incubation time. The IC_{50} values of piperine for MCF-7 were (117.23±6.78), (72.25±5.82), and (25.40±2.86) μM, and for MDA-MB-231 cells were (179.73±8.14), (165.96±8.97), and (62.94±7.50) μM, for 24, 48, and 72 h, respectively. However, doxorubicin had more potent anticancer effects on MCF-7 cells with IC_{50} values of (4.52±1.12), (1.19±0.19), and (0.56±0.11) μM and on MDA-MB-231 with IC_{50} values of (9.71±2.67), (5.62±1.22), (1.77±0.35) μM for 24, 48, and 72 h, respectively. In terms of Emax values, the results showed that at 72 h, both piperine and doxorubicin showed the best efficacy to inhibit cancer cells.

3.2. Piperine inhibited colony formation

The effect of piperine on colony formation was shown in Figure 2. Piperine attenuated colony formation after culturing for 15 d. The IC_{50} values of piperine against colony formation were approximately (132.33±16.91) and (93.85±2.64) μM for MCF-7 and MDA-MB-231 cells, respectively.

3.3. Piperine affected cell cycle

The effects of piperine on cell cycle in the two breast cancer cells were evaluated using flow cytometry. Piperine caused a reduction in cell number in the G_0/G_1 phase of both cells, with a more significant effect found in MDA-MB-231 cells. Moreover, it also significantly induced cell cycle arrest at the G_2/M phase in MDA-MB-231 cells (Figure 3).

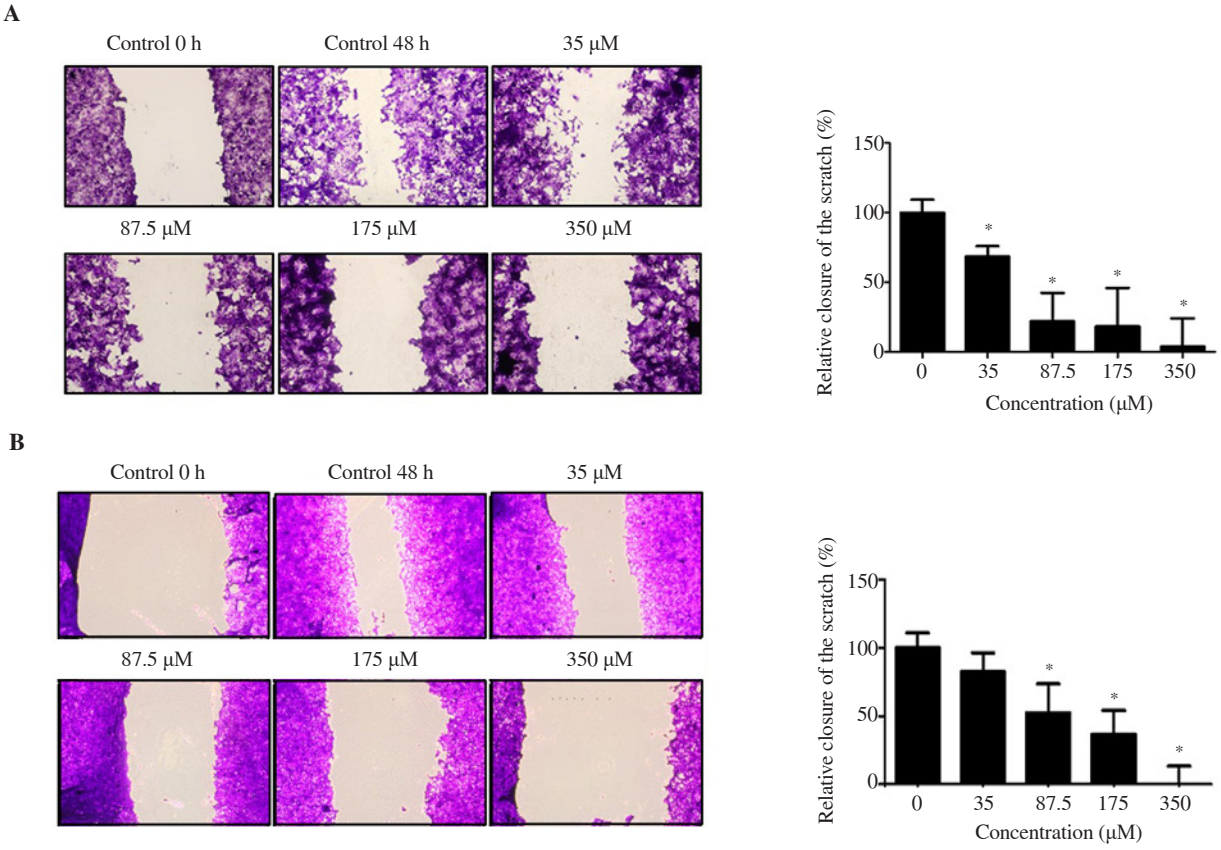

Figure 4. Effect of piperine on cell migration of (A) MCF-7 and (B) MDA-MB-231 cells by wound healing assay. Cells were scratched and then treated with various concentrations of piperine for 48 h (4× magnification). Data are presented as mean±SE. n=3, $^*P$<0.05 compared with untreated control.

3.4. Piperine suppressed cell migration

As shown in Figure 4, piperine suppressed migration of MCF-7 and MDA-MB-231 cells in a dose-dependent manner compared with the control group, and this suppression was significant at 35, 87.5, 175, and 350 μM for MCF-7 cells and 87.5, 175, and 350 μM for MDA-MB-231 cells.

Similar results were obtained from the Matrigel migration assay. After treatment with piperine, the cancer cell number in the lower chamber of the membrane was significantly decreased in a dose-dependent manner (Figure 5).

3.5. Piperine decreased on Rac1 and RhoA expression

Rac1 and RhoA gene and protein expression were evaluated after treating with piperine for 24 h by RT-qPCR and Western blotting, respectively. At high concentration (350 μM), piperine exerted a significant inhibitory effect on gene and protein expression of Rac1 in MCF-7 and MDA-MB-231 cells (Figure 6). On the other hand, *RhoA* gene expression was decreased only in MCF-7 cells at the dose of 350 μM (Supplementary Figure). Therefore, we did not detect the RhoA protein expression in this study.

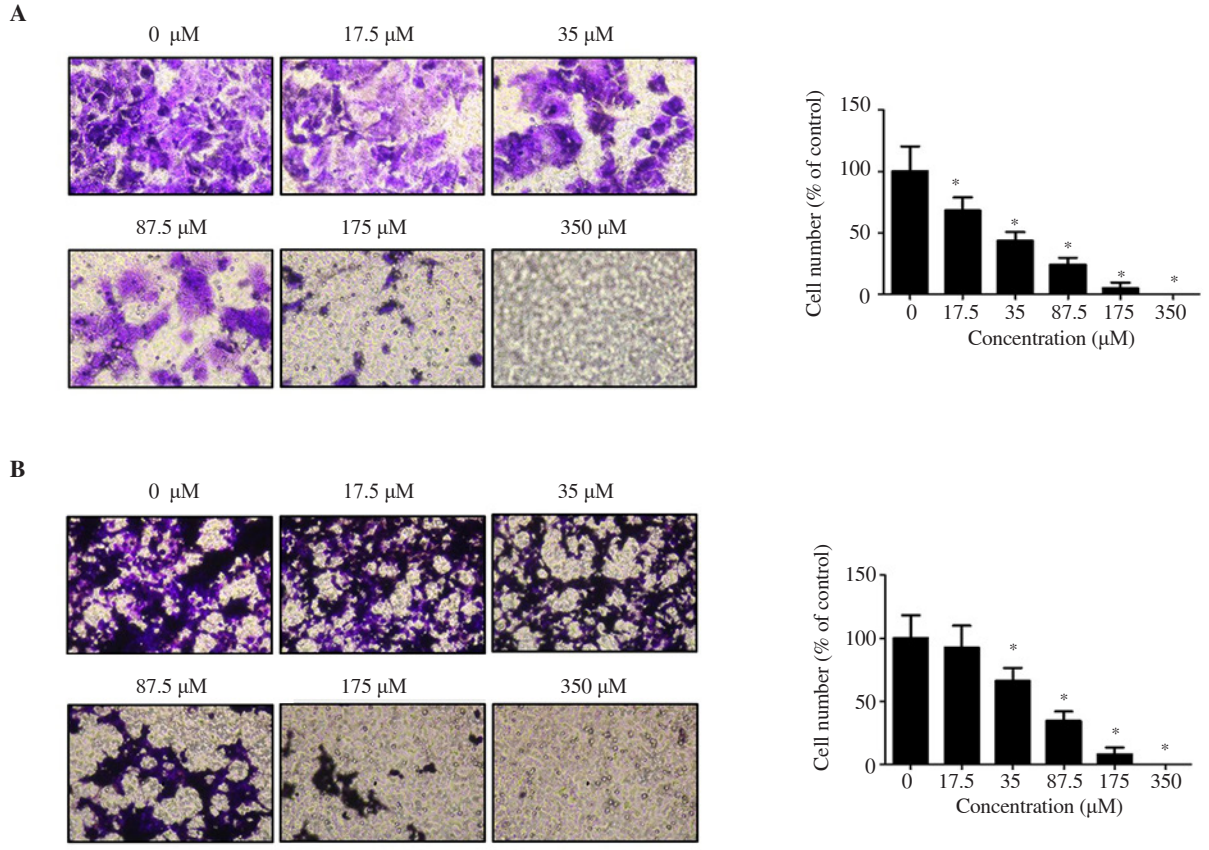

Figure 5. Effect of piperine on cell migration of of (A) MCF-7 and (B) MDA-MB-231 cells by Matrigel migration assay. Cells were seeded onto insert-wells and treated with various concentrations of piperine for 24 h (20× magnification). Data are presented as mean±SE. n=3, $^{*}P$<0.05 compared with untreated control.

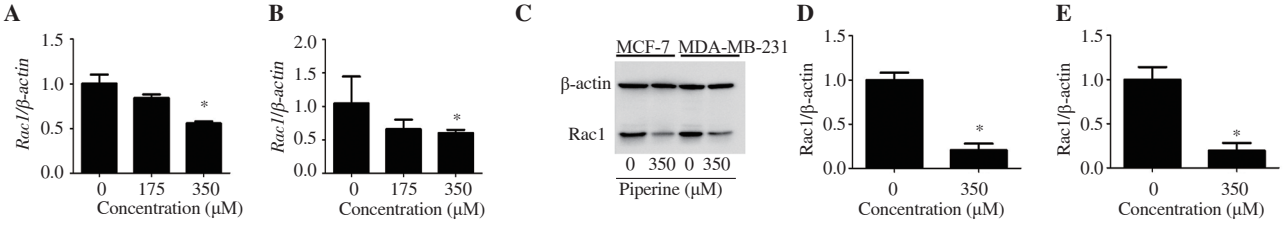

Figure 6. Effect of piperine on Rac1 gene (A: MCF-7; B: MDA-MB-231) and protein expression (D: MCF-7; E: MDA-MB-231) by RT-PCR and Western blotting assays, respectively. Data are presented as mean±SE. n=3, $^{*}P$<0.001 compared with untreated control.

4. Discussion

Currently, piperine is one of the most active compounds from *P. nigrum* and it has been accepted as an anticancer agent for several cancer cells. Nevertheless, its anticancer effect and mechanism on the human breast cancer cells remains elusive. The study herein demonstrated that piperine possessed significant cytotoxic effect against MCF-7 (estrogen-positive) and MDA-MB-231 (estrogen-negative) cells. It was shown that piperine at a dose of 350 μM arrested MDA-MB-231 cells at the G_2/M phase. Furthermore, piperine significantly suppressed cancer cell migration as revealed by wound healing and Matrigel migration assays. We also found that piperine at a dose of 350 μM significantly downregulated Rac1 gene and protein expression in MCF-7 and MDA-MB-231 cells. Therefore, piperine could induce breast cancer cell death and inhibit their migration by attenuating Rac1 expression, but this needs further investigations using Rac1 gain and loss function to confirm this hypothesis.

Piperine had high potential against several cancer cell types including breast[18], colorectal[19], melanoma[20], and cervical cancer[21]. Previous reports indicated that piperine could inhibit cancer cell growth through a different mechanism. Piperine can suppress breast cancer cell growth and metastasis *in vitro* and *in vivo*, through arresting the cell cycle at the G_2/M phase and subsequent downregulation of cyclin B1 expression[18]. Our data indicated that estrogen-positive cells (MCF-7) were more sensitive to piperine than estrogen-negative cells (MDA-MB-231). Similarly, *Ardisia crispa* has a more cytotoxic effect on MCF-7 than MDA-MB-231[5]. Piperine-free *P. nigrum* also inhibited the growth of MCF-7 more than MDA-MB-23 through induction of apoptosis[22]. Therefore, piperine and *Piper* extracts have more cytotoxic effects against estrogen-positive than estrogen-negative breast cancer. Some experiments demonstrated that piperine induced apoptosis *via* increasing cleaved-caspase 3 protein expression and reducing phospho-ERK1/2 protein levels[23]. However, the differential apoptotic effects of piperine on MCF-7 and MDA-MB-231 cells have not been elucidated yet.

Metastasis is the key pathway for cancer cells migrating to the distant organs to create secondary tumor. If piperine could inhibit migration of cancer cells, formation of the secondary tumor could be suppressed. In this study, piperine suppressed the metastasis in the two cancer cells as revealed by wound healing and Matrigel migration assays. Similarly, piperine had a powerful anti-migratory effect on osteosarcoma cells through inhibiting the MMP 2/9 activity and increasing tissue inhibitor of metalloproteinase-1/-2 levels[24]. In the present study, we did not detect the MMP-9 gene or protein expression to explore the effect of piperine on MCF-7 and MDA-MB-231 cells. However, our previous study demonstrated that crude extract of *P. nigrum* inhibited migration of MCF-7 cells through the reduction of MMP-9 expression along with suppression of the gene expressions of *MMP−2*, *MMP−9*, *VEGFA*, and *ICAMP1*[13]. On the other hand, piperine reduced DU145 lung cancer cells migration by suppressing p-Akt, MMP-9, and p-mTOR expressions[25]. Moreover, an *in vivo* study demonstrated that piperine (2.5 and 5 mg/kg) suppressed the migration of breast cancer 4T1 cells to the lung[18]. It could also inhibit the migration of melanoma cells to the lungs, and increase the life span of the metastatic tumor-bearing animals[12]. In future experiments, we will explore the mechanism of anti-migratory activity of piperine against two breast cancer cells and also determine protein- and gene-related migration.

Rac1 in MVA pathway regulates the transformation, differentiation, proliferation, migration, and invasion of cells[26]. Piperine inhibited Rac1 gene and protein expression in MCF-7 and MDA-MB-231 cells. A previous report indicated that *P. nigrum* suppressed MVA products in both Rac1 and RhoA expression, which led to the inhibition of growth and migration in breast cancer cells[13]. The present study confirmed that piperine significantly reduced Rac1 gene and protein expression in both MCF-7 and MDA-MB-231 cells; however, there was no change in RhoA expression except at 350 μM in MCF-7 cells (Supplementary Figure). Piperine and *P. nigrum* extract caused a reduction of Rac1 gene and protein expression; however, RhoA expression was decreased in only *P. nigrum* extract. Interestingly, RhoA may be affected by the other compound in *P. nigrum* extract which needs further exploration. Importantly, Rac1 is related to migration, metastasis, and invasion of cancer cells[27] and down-regulated Rac1 level can inhibit the migration and invasion of cancer cells in several cancer cells including breast cancer. It induced rearrangement of the actin cytoskeleton and sensitized cancer cells toward anticancer drugs[28]. The results in this study suggested that inhibition of Rac1 by piperine is related to potential molecular targets to attenuate migration in breast cancer cells. In addition, piperine may serve as a promising anticancer agent for breast cancer by blocking the Rac1 expression. The limitations of this study are that the toxic effect of piperine was not evaluated and *in vivo* study was performed. Thus, the antitumor effect of piperine should be further verified using *in vivo* study.

In conclusion, this study revealed that piperine had inhibitory effects on growth and metastasis of MCF-7 and MDA-MB-231 cells. It also arrested MDA-MB-231 cells at the G_2/M phase and downregulated Rac1 gene and protein expression in MCF-7 and MDA-MB-231 cells. Piperine may be useful for treating breast cancer.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Authors' contributions

BB and MJ designed the study. BB performed experimental analysis. BB and MJ wrote the manuscript.

References

[1] Verma VC, Lobkovsky E, Gange AC, Singh SK, Prakash S. Piperine production by endophytic fungus *Periconia* sp. isolated from *Piper longum* L. *J Antibiot (Tokyo)* 2011; **64**(6): 427-431.

[2] Doucette CD, Hilchie AL, Liwski R, Hoskin DW. Piperine, a dietary phytochemical, inhibits angiogenesis. *J Nutr Biochem* 2012; **24**(1): 231-239.

[3] Khamis AAA, Ali EMM, El-Moneim MAA, Abd-Alhaseeb MM, El-Magd MA, Salim EI. Hesperidin, piperine and bee venom synergistically potentiate the anticancer effect of tamoxifen against breast cancer cells. *Biomed Pharmacother* 2018; **105**: 1335-1343.

[4] Bae GS, Kim JJ, Park KC, Koo BS, Jo IJ, Choi SB, et al. Piperine inhibits lipopolysaccharide-induced maturation of bone-marrow-derived dendritic cells through inhibition of ERK and JNK activation. *Phytother Res* 2012; **26**(12): 1893-1897.

[5] Chonpathompikunlert P, Wattanathorn J, Muchimapura S. Piperine, the main alkaloid of Thai black pepper, protects against neurodegeneration and cognitive impairment in animal model of cognitive deficit like condition of Alzheimer's disease. *Food Chem Toxicol* 2009; **48**(3): 798-802.

[6] Mittal R, Gupta RL. *In vitro* antioxidant activity of piperine. *Methods Find Exp Clin Pharmacol* 2000; **22**(5): 271-274.

[7] Taqvi SI, Shah AJ, Gilani AH. Blood pressure lowering and vasomodulator effects of piperine. *J Cardiovasc Pharmacol* 2008; **52**(5): 452-458.

[8] Ononiwu IM, Ibeneme CE, Ebong OO. Effects of piperine on gastric acid secretion in albino rats. *Afr J Med Med Sci* 2002; **31**(4): 293-295.

[9] Boros C, Stermitz F. Iridoids: An updated review. Ⅱ. *J Nat Prod* 1991; **54**(5): 1173-1246.

[10]Turrini E, Sestili P, Fimognari C. Overview of the anticancer potential of the "King of Spices" *Piper nigrum* and its main constituent piperine. *Toxins (Basel)* 2020; **12**(12): 747.

[11]Yaffe PB, Power Coombs MR, Doucette CD, Walsh M, Hoskin DW. Piperine, an alkaloid from black pepper, inhibits growth of human colon cancer cells *via* G_1 arrest and apoptosis triggered by endoplasmic reticulum stress. *Mol Carcinog* 2014; **54**(10): 1070-1085.

[12]Pradeep CR, Kuttan G. Effect of piperine on the inhibition of lung metastasis induced B16F-10 melanoma cells in mice. *Clin Exp Metastasis* 2002; **19**(8): 703-708.

[13]Buranrat B, Boontha S. Effect of *Piper nigrum* ethanolic extract on human breast cancer cell growth and cell migration. *Phar Mag* 2019; **15**(65): 538-546.

[14]Wong WW, Dimitroulakos J, Minden MD, Penn LZ. HMG-CoA reductase inhibitors and the malignant cell: The statin family of drugs as triggers of tumor-specific apoptosis. *Leukemia* 2002; **16**(4): 508-519.

[15]Roy M, Kung HJ, Ghosh PM. Statins and prostate cancer: Role of cholesterol inhibition *vs.* prevention of small GTP-binding proteins. *Am J Cancer Res* 2011; **1**(4): 542-561.

[16]Yoshida T, Zhang Y, Rivera Rosado LA, Chen J, Khan T, Moon SY, et al. Blockade of Rac1 activity induces G_1 cell cycle arrest or apoptosis in breast cancer cells through downregulation of cyclin D1, survivin, and X-linked inhibitor of apoptosis protein. *Mol Cancer Ther* 2010; **9**(6): 1657-1668.

[17]Buranrat B, Senggunprai L, Prawan A, Kukongviriyapan V. Simvastatin and atorvastatin as inhibitors of proliferation and inducers of apoptosis in human cholangiocarcinoma cells. *Life Sci* 2016; **153**: 41-49.

[18] Lai LH, Fu QH, Liu Y, Jiang K, Guo QM, Chen QY, et al. Piperine suppresses tumor growth and metastasis *in vitro* and *in vivo* in a 4T1 murine breast cancer model. *Acta Pharmacol Sin* 2012; **33**(4): 523-530.

[19] de Almeida GC, Oliveira LFS, Predes D, Fokoue HH, Kuster RM, Oliveira FL, et al. Piperine suppresses the Wnt/beta-catenin pathway and has anti-cancer effects on colorectal cancer cells. *Sci Rep* 2020; **10**(1): 11681.

[20] Yoo ES, Choo GS, Kim SH, Woo JS, Kim HJ, Park YS, et al. Antitumor and apoptosis-inducing effects of piperine on human melanoma cells. *Anticancer Res* 2019; **39**(4): 1883-1892.

[21]Jafri A, Siddiqui S, Rais J, Ahmad MS, Kumar S, Jafar T, et al. Induction of apoptosis by piperine in human cervical adenocarcinoma *via* ROS mediated mitochondrial pathway and caspase-3 activation. *EXCLI J* 2019; **18**: 154-164.

[22]Sriwiriyajan S, Tedasen A, Lailerd N, Boonyaphiphat P, Nitiruangjarat A, Deng Y, et al. Anticancer and cancer prevention effects of piperine-free *Piper nigrum* extract on *N*-nitrosomethylurea-induced mammary tumorigenesis in rats. *Cancer Prev Res (Phila)* 2016; **9**(1): 74-82.

[23]Fofaria NM, Kim SH, Srivastava SK. Piperine causes G_1 phase cell cycle arrest and apoptosis in melanoma cells through checkpoint kinase-1 activation. *PLoS One* 2014; **9**(5): e94298.

[24]Zhang J, Zhu X, Li H, Li B, Sun L, Xie T, et al. Piperine inhibits proliferation of human osteosarcoma cells *via* G_2/M phase arrest and metastasis by suppressing MMP-2/-9 expression. *Int Immunopharmacol* 2015; **24**(1): 50-58.

[25]Zeng Y, Yang Y. Piperine depresses the migration progression *via* downregulating the Akt/mTOR/MMP9 signaling pathway in DU145 cells. *Mol Med Rep* 2018; **17**(5): 6363-6370.

[26]Jaffe AB, Hall A. Rho GTPases: Biochemistry and biology. *Annu Rev Cell Dev Biol* 2005; **21**: 247-269.

[27]Price LS, Collard JG. Regulation of the cytoskeleton by Rho-family GTPases: Implications for tumour cell invasion. *Semin Cancer Biol* 2001; **11**(2): 167-173.

[28]Chen QY, Xu LQ, Jiao DM, Yao QH, Wang YY, Hu HZ, et al. Silencing of Rac1 modifies lung cancer cell migration, invasion and actin cytoskeleton rearrangements and enhances chemosensitivity to antitumor drugs. *Int J Mol Med* 2011; **28**(5): 769-776.