ผลของสารสกัดข้าวไรซ์เบอรรี่ต่อการยับยั้งการเติบโตและการลุกลามของเซลล์มะเร็งเต้านมชนิดทริปเปิ้ลเนกาทีฟ MDA-MB-231

The Inhibitory Effect of Riceberry Rice on MDA-MB-231 Triple Negative Breast Cancer Cell Growth and Migration

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Abstract

Triple-negative breast cancer (TNBC) has a highly aggressive behavior with different therapeutic responses. Standard forms of therapy such as radiotherapy and chemotherapy still provide the first line of treatment of this disease. However, TNBC patients are found to have an increased chance of recurrence and accumulate normal systemic cytotoxicity after receiving radiotherapy and chemotherapy. Therefore, finding new therapeutic options for TNBC patients is needed in order to suppress the aggressiveness of this cancer. This study aims to investigate the inhibitory effect of Riceberry rice extract on MDA-MB-231 triple negative breast cancer cell migration. In this study, the cell viability of the MDA-MB-231 cells was assessed by MTT assay (IC50=4.84 mg/mL). Cancer cell migration was evaluated by wound healing assay, and cellular oxidant levels were measured by DCFH-DA assay. The results show that at concentrations of 5 and 10 mg/mL, the extract significantly inhibited cancer cell migration to to 78.0±0.11% and 58.1±0.52%, respectively. Additionally, the extract substantially reduced cellular oxidant levels in MDA-MB-231 cells, and the reduction of cellular oxidants was strongly correlated with the inhibitory effect (R^2 = 0.992, p<0.001). Therefore, this study suggests that Riceberry rice extract exhibits an inhibitory effect on MDA-MB-231 triple-negative breast cancer cell growth and migration in association with the role of cellular oxidants.

Keywords: Riceberry rice, Breast cancer, Migration, Cellular oxidants

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Introduction

Worldwide, breast cancer has become the most frequently-diagnosed form of cancer among females (Bray et al., 2018). Despite improvements in the treatment options, the rate of resistance to treatment, incidences of recurrence, and poor therapeutic outcomes are still increasing continually. (Akram et al., 2017; Harbeck et al., 2019). Among the different types of breast cancer, triple-negative breast cancer (TNBC) is the most aggressive and typically with the poorest prognosis (Natrajan & Weigelt, 2016). Additionally, the therapeutic choices for TNBC are limited because of the lack of an identified molecular receptor (Liu, Zhang, & Zhang, 2014). Hence, TNBC patients are mostly treated by standard therapy (surgery, radiation and chemotherapy). However, those options appear to extend the probabilities of breast cancer cell metastasis (Dawood et al., 2009; Kalimutho et al., 2015). Also, previous studies have revealed that distant metastasis is associated with the accumulation of oxidative stress (Aggarwal et al., 2019). Oxidative stress is well-known to play a critical role in many types of cancer (Liao, Chua, & Tan, 2019). An excessive accumulation of reactive oxygen species (ROS) is also a key driver of TNBC migration and invasion, which are the initial steps for distant metastasis (Sarmiento-Salinas et al., 2019). Further, the use of the present types of chemotherapy, for example, platinum-based medication, is reported to cause normal cell toxicities (Odle, 2014). To provides additional treatment options, chemo-free medicine agents are suggested for TNBC drug development (Gullett et al., 2010). Riceberry rice (*Oryza sativa* L.), a registered variety of Thai rice developed through cross-breeding, has the potential to produce high levels of powerful anti-oxidants to support anti-cancer growth and migration due to its previously reported anti-oxidant activity (Rodboon et al., 2020; Tammasakchai et al., 2012). Riceberry rice is abundant in phenolics which are recognized for their preventive progression effects on many cancer types (Chen et al., 2015; Diaconeasa et al., 2018; Pereira-Caro et al., 2013). The targeted phenolic compounds which are abundant in Riceberry rice include ferulic acid, vanillic acid, protocatechuic acid, syringic acid, chlorogenic acid, ferulic acid and p-coumaric acid (Kongthitilerd et al., 2020; Thiranusornkij, Thamnarathip, Chandrachai, Kuakpetoon, & Adisakwattana, 2019). Therefore, this study focusses on determining the inhibitory effect of Riceberry rice on MDA-MB-231 triple negative breast cancer cells growth and migration.

Objective

To investigate the inhibitory effect of Riceberry rice extract on MDA-MB-231 triple negative breast cancer cell growth and migration that associated with cellular oxidants.

Methods

1. Riceberry rice extraction

Riceberry rice (*Oryza sativa* L.), License number 88/2560, was obtained from the Rice Science Center & Rice Gene Discovery Unit, Thailand. Riceberry rice extract was extracted from the Riceberry rice by using 80% ethanol in distilled water (1:4). The mixture was shaken at 300 rpm, 25 ℃ for 4 hours in dark conditions by an incubator shaker (floor type). The mixture was centrifuged to collect the supernatant. Then, the supernatant was filtered through Whatman Filter Paper No.1 (Whatman, UK). The Riceberry rice extract was stored at -20 ℃ until it was used in the cell culture experiments. The extract was sterilized by filtration through a 0.45 μM polytetrafluorethylene (PTEE) nylon filter before being used in sterile experiments.

2. MDA-MB-231 cell culture

MDA-MB-231 triple negative cancer was acquired from the American Type Culture Collection (ATCC® HTB-26™). The cells were maintained in a high-glucose DMEM medium (Sigma, Singapore) supplemented with 10% fetal bovine serum, 1% non-essential amino acid, 1% streptomycin, 1% penicillin and 3.7 mg/mL of $NaHCO_3$. The MDA-MB-231 cells were consistently maintained at 37 ℃ in a humidified atmosphere, supplied with 5% CO_2 in an incubator for all *In vitro* experiments (Park et al., 2014).

3. Cytotoxicity by 3-(4,5-dimethylthaizol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay MTT assay

The MDA-MB 231 cells were treated with the Riceberry rice extract to assess its cytotoxicity. The assay was developed from a prior study (Tsai et al., 2018). The MDA-MB-231 cells at $1x10^5$ cells/mL were seeded in a 96-well plate and incubated for 24 hours. The old media were discarded and the cells were treated with various concentrations of Riceberry rice extract (1, 5, 10, 50 and 100 mg/mL) and incubated for the next 24 hours. Then, 1.2 mM MTT reagaent (Sigma-Aldrish, Singapore) was mixed with the media (1:10) and replaced the old media before being incubated for 2 hours. Next, dimethyl sulfoxide (DMSO) 100 μL (Merck, Kenilworth, USA) was added and the absorbance was analyzed at a wavelength of 530 nm by a microplate reader (1420 Victor 2, Wallac, USA).

4. Cellular oxidants by fluorescent 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA) assay

The MDA-MB 231 cells were treated with the Riceberry rice extract to determine the cellular oxidant levels. The protocol was modified from a previous study (Rodboon, Okada, & Suwannalert, 2020). The MDA-MB-231 cells at $1x10^5$ cells/mL were seeded into a 96-well plate and incubated for 24 hours. The old media were discarded and the cells were treated with various concentrations of Riceberry rice extract (5 and 10 mg/mL), before being incubated for the next 24 hours. Then, 5 μM of DCFH-DA (Merck, Kenilworth, USA) was mixed with the media and incubated for another 25 minutes. The fluorescence was measured by a microplate reader (Spark™ 10M, TECAN, Switzerland) at 495/529 nm.

5. MDA-MB-231 cancer cell migration by wound healing assay

The MDA-MB-231 cell migration was observed by using a scratch-wound healing assay. The assay was modified from a previous study (Aimvijarn, et al,. The MDA-MB-231 cells at $2x10^5$ cells/mL were seeded in a 6-well plate and then incubated until the cell's confluence gained 80-90%. Next, the cells were serum-starved in the media with 2% FBS for 36 hours. After this, the cells were scratched with a 200 μL pipette tip. The migrated areas of the cells were photographed at 0 and 24 hours and were analyzed by ImageJ software-version 1.52q.

6. Statistical analysis

All studies were carried out in triplicate. The results were shown as mean±standard deviation (mean±SD). One-way analysis of variance (ANOVA) with a Tukey's test was used to compare the significant differences between the experiment groups. Data correlation was calculated by Pearson's correlation. Statistical significance was considered at $p \leq 0.05$ with IBM SPSS Statistic version 25-computer software.

Results

1. Effect of Riceberry rice extract on cytotoxicity

To determine the cytotoxicity of Riceberry rice (RB) extract on MDA-MB-231 cells, the cells were treated with the extract at various concentrations (1, 5, 10, 50 and 100 mg/mL) for 24 hours. The results showed the cytotoxic effect of RB treatment in a dose-dependent manner at 50 and 100 mg/mL (Figure 1). The IC50 value was 4.84 mg/mL. These findings indicate that RB extract treatment inhibits MDA-MB-231 breast cancer cell growth.

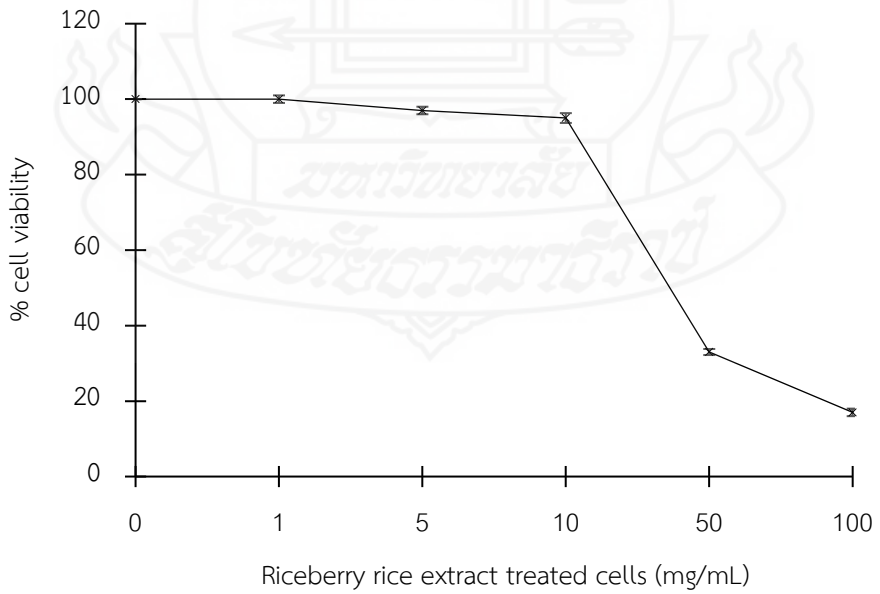
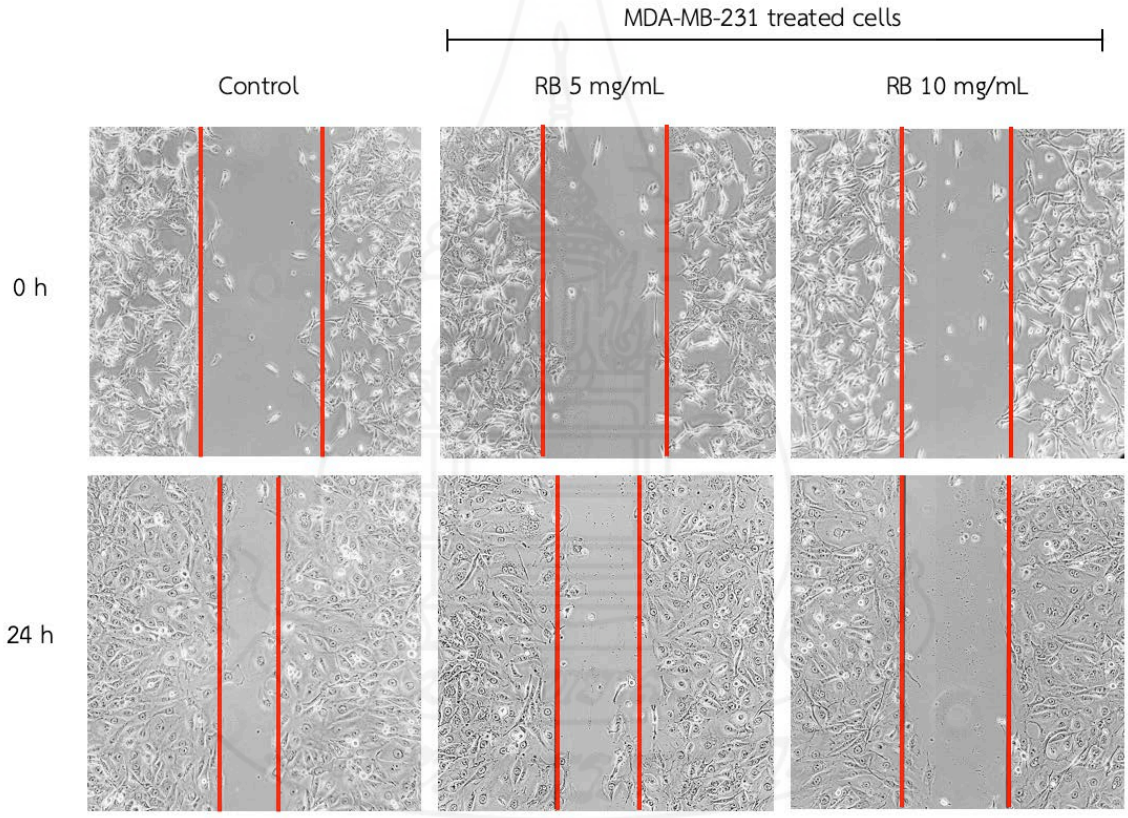


Figure 1 Effect of Riceberry rice extract on MDA-MB-231 cancer cell growth by MTT assay. The untreated cells were considered as 100% of control. Data were expressed as mean±SD (n=3).

2. Effect of Riceberry rice extract on cancer cell migration

To investigate the effect of RB extract on MDA-MB-231 cell migration, the cells were treated with non-cytotoxicity concentrations at 5 and 10 mg/mL of the extract for 24 hours. The wound healing results demonstrated that 5 and 10 mg/mL of RB extract treatment inhibited the cell migrated-area to 78.0±0.11% and 58.1±0.52%, respectively, compared with the control (100%) (Figures 2A and 2B). These findings indicate that RB extract significantly inhibits MDA-MB-231 cell migration in a dose-dependent manner.

A)



B)

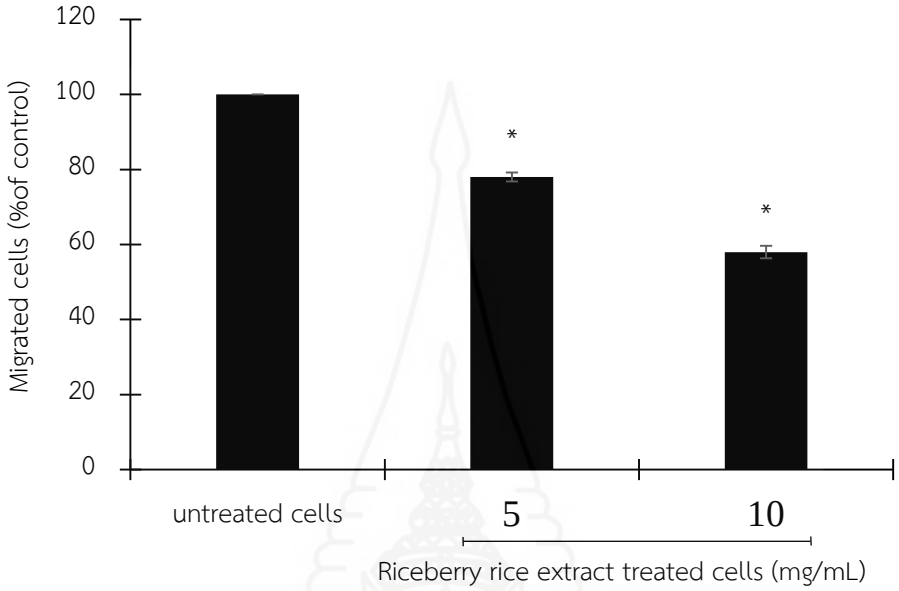


Figure 2 Effect of Riceberry rice extract on cell migration by wound healing assay. **A)** Wound areas represent the migrated area of MDA-MB-231 treated cells. **B)** Percent of the migrated area of MDA-MB-231 cells after treated with the extract. The untreated cells were considered as 100% of control. Data were expressed as mean±SD. *p<0.01, compared with control.

3. Effect of Riceberry rice extract on cellular oxidant levels

To investigate the effect of RB extract on the cellular-oxidant levels, MDA-MB-231 cells were treated with the extract for 24 hours. The results showed that the RB extract at concentrations 5 and 10 mg/mL decreased the cellular oxidant levels to 78.2±1.22% and 70.1±1.15% respectively, compared with the control (100%) (Figure 3A). To further confirm the association between the inhibitory effect of the extract and the cellular oxidants, the correlation coefficient (R-value) was tested. The linear correlation revealed that the decrease of cell migration was strongly correlated with the reduced cellular oxidant levels in the MDA-MB-231 treated cells (R^2 = 0.992, p<0.001). This finding indicates that RB extract exhibits an inhibitory effect on MDA-MB-231 cells associated with cellular oxidant function.

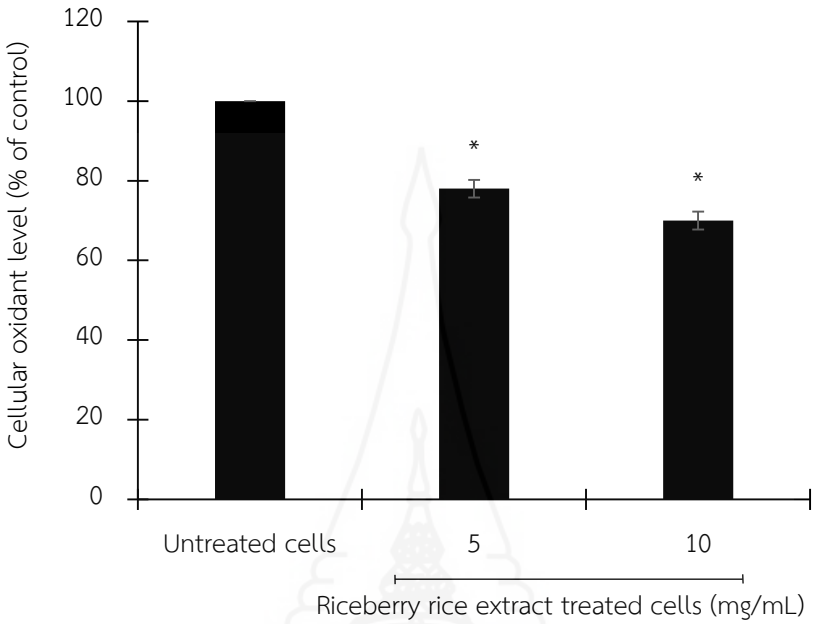


Figure 3 Effect of Riceberry rice extract on cellular oxidants by DCFH-DA assay. The untreated cells were considered as 100% of control. Data were expressed as mean±SD. *p<0.01, compared with control.

Discussions

To date, triple negative breast cancer (TNBC) has been reported as the most aggressive breast cancer due to their aggressive behavior (Bray et al., 2018). Besides, the distant metastasis was the most predictive characters of poor prognosis for TNBC (Al-Mahmood, Sapiezynski, Garbuzenko, & Minko, 2018). Accumulation of cellular oxidant levels also drive disease progression and leading to metastasis (Sarmiento-Salinas et al., 2019). Therefore, the new finding for less-toxic treatment is urgently needed. Recently, natural black rice had been reported on their medical benefits due to the abundance of phytochemical contents (Chen et al., 2015). Phenolic compounds are the most common phytochemicals that constitute in black rice and have been studied on anti-oxidant and anti-cancer effects (Ghasemzadeh, Karbalaii, Jaafar, & Rahmat, 2018). Herein, this study observed the inhibitory effect of Riceberry rice (RB) extract on MDA-MB-231 cells. This present study found that RB extract significantly exhibited an inhibitory effect on TNBC cell growth. The result support previous finding that pigemented rice can inhibit cancer cell growth and induce cancer cell apoptosis (Tammasakchai, Reungpatthanaphong, Chaiyasut, Rattanachitthawat, & Suwannalert, 2012; Wongjaikam, Summart, & Chewonarin, 2014). The extract also inhibited cancer cell migration that associated with cellular oxidants which in agreement with prior studies. The pigemented rice can prevent oxidative stress and suppress cancer cell progression (Chen et al., 2015; Lin, Chen, Chou, & Wang, 2011). In

addition, previous studies also reported the anti-cancer migraton effect of protocatechuic acid, a phenolic acid that present in Riceberry rice, to suppress cell migration and invasion in both B16 melanoma cells and metastasis mice model through the regulation of Ras/Akt/NF-kB pathway (Lin et al., 2011). Also, ferulic acid was also reported to inhibit T medullary thyroid cancer cell line through the regulation of novel gene including CDK4, CDK6, MMP-2, MMP-9, andTIMP-1. (Dodurga et al., 2016).

Further study

This research project benefits the agricultural industry in Thailand. Moreover, further investigation could lead to the development of food supplement with nutrirional and pharmacological values.

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