Research Article

Inhibitory effect of *Bridelia ovata* Decne extract on HepG2 cell migration and invasion stimulated by fibroblast–conditioned media

Hifza Baig[1], Penchatr Diskul–Na–Ayudthaya[2], Churat Weeraphan[2], N. Monique Paricharttanakul[2] Jisnuson Svasti[1, 2], Chantragan Srisomsap[2*]

[1] Applied Biological Sciences Program, [2] Laboratory of Biochemistry, Chulabhorn Research Institute, Bangkok 10210

* Corresponding author, E–mail: chantragan@cri.or.th

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Abstract

Migration and invasion are the critical step during tumor metastasis. Therefore, one of the goals for cancer treatment is to suppress the migration and invasion of cancer cells. Here, the effect of *Bridelia ovata* Decne (BO) extract on cell migration and invasion was investigated using human liver cancer cell lines (HepG2) as a model system. Crude ethanol extract is obtained from plant extract using speed vacuum apparatus. The cytotoxic activities of ethanolic extract on HepG2 cell line were evaluated using 3–[4, 5–Dimethyl–2– thiazolyl]–2, 5–diphenyltetrazolium bromide (MTT) assay. *In vitro* migration and invasion assays were performed using a modified Boyden chambers. The results showed that at 80% survival (IC_{20}) with 47 µg/mL as concentration of BO extract inhibited invasion and migration of HepG2 cells. It could suggest that BO extract might be involved in the cytotoxic activity against HepG2 carcinoma cell.

Keywords: *Bridelia ovata* Decne *extract*, human liver cancer cell line, HepG2, cytotoxicity

Introduction

Cancer is the leading cause of death worldwide among which liver cancer is the third leading cause of mortality around the globe. Hepatocellular carcinoma (HCC) is the most common and aggressive type of primary liver tumor. [1] In recent years, cancer has become a major disease causing death of Thai people. [2] HCC, associated with hepatitis B virus, is a major problem in all regions of Thailand, and currently represents the main cause of death in cirrhotic patients. [3]

Migration and invasion are the major properties of cancerous cells which turn a primary tumor into metastatic cancer that spreads from its primary site of origin, and migrates to distant body parts. Metastatic cancer is often asymptomatic thus it cannot be diagnosed at early stages. It is often diagnosed only when the metastatic tumor has grown significantly making it difficult to treat the cancer successfully. [4] Currently, chemotherapy is the most common treatment for advanced stage of cancers.

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However, chemotherapy is not a selective therapy for only cancer cells, and also lethal for normal cells. Nowadays, Thai herbal plants are widely accepted in alternative medicine for treatment patients suffering deleterious diseases such as cancer. *Bridelia ovata* Decne (BO) is a scrambling shrub or small tree with a flattened crown; it can grow up to 8 meters tall, and the trunk can be 10 cm in diameter. BO belongs to the family Euphorbiaceae, and is a traditional medicinal plant in Thailand, which is locally known as Maga in Thai. Moreover, the genus *Bridelia* comprises of about 60 species found throughout Southeast Asia. A decoction of the leaves, stems, roots or barks of this Thai herbal plant has been traditionally used as medicine for many diseases as well as used as a purgative. Recently, extraction of dried BO leaves using hexane, dichloromethane, and methanol has revealed 6 chemical constituents which were identified the mixture of long chain aliphatic hydrocarbons (C(,26)–(,33)), the mixture of long chain aliphatic esters, the mixture of long chain aliphatic alcohols (C(,23), C(,24), C(,32)), friedelin, friedelan–3(...)–ol and stigmasterol. [5] The authors aim to investigate the suppressing migration and invasion properties of BO extracts toward human liver cancer cell lines (HepG2).

Material and Method

Crude extract of *(Bridelia ovata* Decne*)*

The air–dried leaves of BO plant were grinded with a blender into a fine powder. Fifty grams of fine powder of BO leaves were mixed with 150 mL of absolute ethanol, and then incubated for 24 hours on an orbital shaker. The ethanolic extract was filtered, and then concentrated to complete dryness in a speed–vacuum concentrator. The dry extract was dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 250 mg/mL.

Cell culture

Liver cancer cell line HepG2 was purchased from the American Tissue Culture Collection (ATCC). HepG2 was grown in Dulbecco's Modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal bovine serum, 1% of penicillin–streptomycin (Gibco, Invitrogen, USA). Cells were grown in uniform culture conditions to maintain humidity and atmospheric pressure at a 37°C, 5% CO_2 incubator, and culture media was changed twice a week.

Cytotoxicity assay

Cell cytotoxicity was determined by using MTT assay. Cells were plated in duplicate in 96–well plates at $2x10^5$ cells per well, and grown for 24 hours. BO extract was then added in serial dilution to reach a final volume of 200 µL/well. Control wells contained 100% DMSO without BO. Plates were incubated for 24 hours incubation at a 37°C with 5% CO_2. After reaction with MTT solution which was dissolved in phosphate buffered saline (PBS) solution (1 mg/mL) for 4 hours, and then DMSO was added to solubilize the reduced tetrazolium salts. Absorbance measurements at 550 nm in a microliter plate reader were performed, and background was subtracted at 650 nm.

Migration Assay

Inhibitory effects of BO ethanolic extract on HepG2 cell migration were observed using a modified Boyden chamber inserted with polyethylene terephthalate filter membrane, containing 8–µm pores in 24–Transwell plate

(Costar, Cambridge, MA). In brief, HepG2 cells were pretreated for 30 minutes in DMEM media containing 47 µg/mL BO extract or DMSO only as the control treatment. HepG2 cells (2×10^5 cells) were then placed in the top chamber. Fibroblast–conditioned media containing 45 µg/mL BO extracts or DMSO only were placed in the bottom chamber. After 24–hour incubation at 37°C with 5% CO_2, cells residing on the inner portion of the top chamber were removed with a cotton bud, and then were fixed with 25% methanol for 15 minutes. Cells that migrated across the filter membrane were stained with crystal violet for 15 minutes, and five random phase contrast images were taken of the underside of each top chamber. Cells were counted under magnification of 200x.

Invasion assay

The *in vitro* cell invasion assay was performed by using a modified Boyden chamber inserted with polyethylene terephthalate filter membrane containing 8–µm pores in 24–transwell plate (Costar, Cambridge, MA). The filter membranes were coated with 30 µg Matrigel (BD Bioscience, Bedford, MA). In brief, HepG2 cells were pretreated for 30 minutes in DMEM media containing 47 µg/mL BO extract or DMSO only as the control treatment. HepG2 cells (2×10^5) were then placed in the top chamber. Fibroblast–conditioned media containing 45 µg/mL BO extracts or DMSO only were placed in the bottom chamber. After 24–hour incubation at 37°C with 5% CO_2, cells residing on the inner portion of the top chamber were removed with a cotton bud, and then fixed with 25% methanol for 15 minutes. Cells that migrated across the filter membrane were stained with crystal violet for 15 minutes, five random phase contrast images were taken of the underside of each top chamber and counted under magnification of 200x.

Results and Discussion

Cytotoxicity assay

The purpose of doing MTT assay was to determine the cytotoxic effect of BO extract on HepG2 cells at 24–hour incubation. In this experiment, the concentration of BO used ranged from 31.25 µg/mL to 500 µg/mL. The concentration at which 50% survival (IC_{50}) and 80% survival (IC_{20}) of cells occurred are 92.8 ± 6.7 µg/mL and 47.0 ± 0.9 µg/mL, respectively as shown in Figure 1. The concentration at IC_{20} was further used to do migration and invasion assays. According to the results obtained from the cytotoxicity assay, HepG2 cells were highly sensitive to BO ethanolic extract.

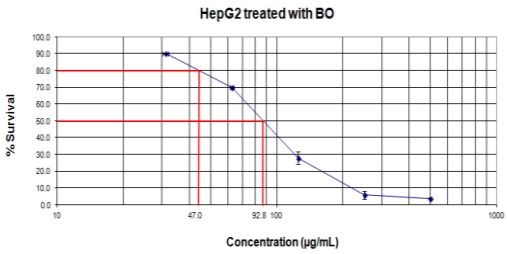
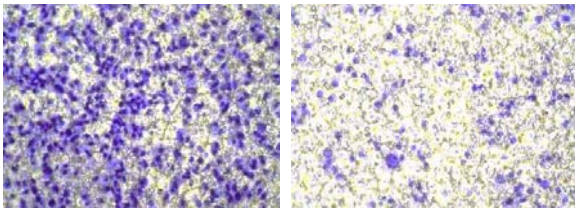


Figure 1 Cell cytotoxicity of BO extract on HepG2 cells for 24 hours was determined by MTT assay. The concentration of BO extract at 50% survival (IC_{50}) and 80% survival (IC_{20}) of cells were 92.8 ± 6.7 µg/mL and 47.0 ± 0.9 µg/mL, respectively.

Inhibition of Cell Migration

From our experiment, *Bridelia ovata* Decne (BO) is an interesting Thai herb because its ethanolic extract has the ability to inhibit both metastasis and invasive properties of liver cancer

cells. In this study, we demonstrated that the BO extract could inhibit the *in vitro* migration and invasion ability of liver cancer cells. HepG2 cells are relatively not aggressive. According to our hypothesis, HepG2 cells can be induced with fibroblast–conditioned media to establish a model of aggressive cell line in order to evaluate the potential inhibitory effect of BO extract on cell migration. The results demonstrated that BO extract has substantially inhibitory effects on HepG2 cells as shown in Figure 2. Figure 2A shows that cell migration was significantly high in the control chamber. Figure 2B shows that inhibition of cell migration in BO treated chamber was significantly reduced. The average cell count was significantly different in the control (DMSO) and treated (BO) chambers of HepG2 cells. There was a 4.4–fold difference in cell counts between treated and untreated cells.

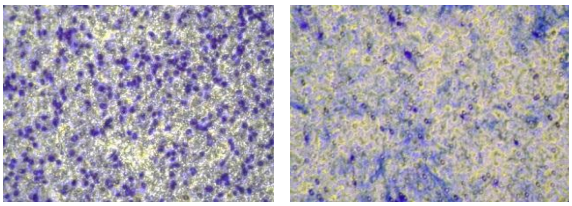


(A) Control (DMSO) (B) Treated (BO extract)

Figure 2 Effect of BO extract on the migration of HepG2 cells *in vitro*. Crystal violet stained microscopy of cell counts random field (A) untreated control DMSO (B) treated with BO extract of HepG2 cells at magnification 200X.

Reduction in Cell Invasion

BO extract–treated cells reduced invasion of aggressive HepG2 cells in Matrigel invasion assay. After transfection as described in Materials and Methods section, HepG2 cells were allowed to invade through the Matrigel up to 24 hours. Invaded cells were stained with crystal violet and counted. Untreated control clearly showed invasive properties of HepG2 cells (Figure 3A) while the treated chamber has distinct inhibition in invasiveness of HepG2 cells after induction with fibroblast–conditioned media (Figure 3B). The average cell count was significantly different in the control (DMSO) and treated (BO) chambers of HepG2 cells. There was a 25.8–fold different in cell counts between treated and untreated cells.



(A) Control (DMSO) (B) Treated (BO extract)

Figure 3 Effect of BO extract on the invasion of HepG2 cells *in vitro*. Crystal violet stained microscopy of cell counts random field (A) untreated control DMSO (B) treated with BO extract of HepG2 cells at magnification 200X

In conclusion, In vitro BO extract inhibit invasion of HepG2, and it could be suggested that BO extract might be involved in the cytotoxic activity against HepG2 carcinoma cell.

Acknowledgements

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References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90.

2. Vatanasapt V, Sriamporn S, Vatanasapt P. Cancer Control in Thailand, Jpn J Clin Oncol. 2002;32 (Suppl 1):S82–91.
3. Worns MA, Galle PR. Future Perspectives in hepatocellular carcinoma. Dig Liver Dis. 2010;42 (Suppl 3):S302–9.
4. Alphonso A, Alahari SK. Stromal cells and integrins: Conforming to the needs of the tumor microenvironment. Neoplasia. 2009;11(12):1264–71.
5. Thongkon N. Chemical Constituents of the Leaves of Bridelia ovata Decne [Dissertation]. Bangkok: Chulalongkorn University; 1994.