Effect of *Mallotus repandus* on Vascular Endothelial and Cholangiocarcinoma Cells Migration

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**Background and objectives:** *Mallotus repandus* (Euphorbiaceae), a widely distributed plant in South-East Asia, used as medicinal herb in many countries, including Thailand which is commonly used in the treatment of muscle and joint pain. Many active ingredients were found in *Mallotus repandus*, especially triterpenoids previously reported in various pharmacologic effects including anti-inflammatory and anticancer activities. The objective of this study was to study the potential of the methanol extract of the stem bark of *M. repandus* on the migration of vascular endothelial cells and cholangiocarcinoma (CCA) cells in comparing with anti-mitotic drug, paclitaxel (Taxol®).

**Methods:** Non-cytotoxic concentrations of *M. repandus* extract, paclitaxel and vehicle were determined by MTT assay. Co-culture technique was performed to test for anti-migration. The cells were pre-treated with the non-cytotoxic concentrations of paclitaxel, extracts, or vehicle for 30 min before being added to insert (upper chamber), then further incubated for 18 h at 37°C in 5% CO₂ incubator. The number of cells migrated to well (lower chamber) were counted under a microscope and the percentage of inhibition was calculated.

**Results:** The findings revealed that the non-cytotoxic dose of the *M. repandus* extract could inhibit the migration of both vascular endothelial and CCA cells in dose-dependent manners.

**Conclusions:** Our results suggested that the methanol extract of the *M. repandus* stem bark had anti-tumor metastatic activity.
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Keywords: Mallotus repandus, Cholangiocarcinoma, Anti-migration

1. Material and Method

1.1. Preparation of Extracts

Fresh leaves of Mallotus repandus were harvested and dried in the shade. The dried leaves were ground into a fine powder and extracted with methanol. The extracts were then concentrated in a rotary evaporator to obtain a dry powder. The dry powder was further subjected to methanol reflux using a reflux apparatus and the residue was subjected to the antimigration test.

1.2. Antimigration Assay

The antimigration assay was performed using human umbilical vein endothelial cells (HUVECs) and cholangiocarcinoma cells (CCA cells). The assay was conducted using a 96-well plate with a well plate reader.

1.3. Cytotoxicity Assay

The cytotoxicity assay was conducted using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

2. Results

The results showed that the extract of Mallotus repandus was effective in inhibiting the migration of HUVECs and CCA cells. The extract was also found to be cytotoxic to CCA cells, but not to HUVECs.

3. Conclusion

The results of this study suggest that Mallotus repandus has potential anti-migration and cytotoxic effects against cholangiocarcinoma cells.

References

3. Antimigration test

The standard procedure of the assay was performed in a 24-well migration chamber (Transwell polycarbonate membrane, pore size 8 μm) by adding 1 μg fibronectin to the underside of the insert. For the pre-treatment, the cells were treated with the drugs for 24 h, followed by treatment with the experimental drugs (5 μg/ml) for 24 h. The cells were then washed with PBS and fixed with 25% MeOH. The formazan product was stained with 0.5% crystal violet. The absorbance was measured using a spectrophotometer (ELx800; Bio-Tek Instrument, USA) at 540 nm.

3.1 Cytotoxic test

The cells were seeded into 96-well plates at a density of 5x10^4 cells per well. After incubation for 24 h, the media was removed and the cells were pre-treated with the experimental drugs (5 μg/ml). The cells were then treated with the experimental drugs for 24 h, followed by treatment with the experimental drugs (5 μg/ml) for 24 h. The absorbance was measured using a spectrophotometer (ELx800; Bio-Tek Instrument, USA) at 540 nm.

3.2 Antimigration test

The cells were seeded into 96-well plates at a density of 5x10^4 cells per well. After incubation for 24 h, the media was removed and the cells were pre-treated with the experimental drugs (5 μg/ml). The cells were then treated with the experimental drugs for 24 h, followed by treatment with the experimental drugs (5 μg/ml) for 24 h. The absorbance was measured using a spectrophotometer (ELx800; Bio-Tek Instrument, USA) at 540 nm.

3.3 Antimigration test

The cells were seeded into 96-well plates at a density of 5x10^4 cells per well. After incubation for 24 h, the media was removed and the cells were pre-treated with the experimental drugs (5 μg/ml). The cells were then treated with the experimental drugs for 24 h, followed by treatment with the experimental drugs (5 μg/ml) for 24 h. The absorbance was measured using a spectrophotometer (ELx800; Bio-Tek Instrument, USA) at 540 nm.

3.4 Cytotoxic test

The cells were seeded into 96-well plates at a density of 5x10^4 cells per well. After incubation for 24 h, the media was removed and the cells were pre-treated with the experimental drugs (5 μg/ml). The cells were then treated with the experimental drugs for 24 h, followed by treatment with the experimental drugs (5 μg/ml) for 24 h. The absorbance was measured using a spectrophotometer (ELx800; Bio-Tek Instrument, USA) at 540 nm.

3.5 Cytotoxic test

The cells were seeded into 96-well plates at a density of 5x10^4 cells per well. After incubation for 24 h, the media was removed and the cells were pre-treated with the experimental drugs (5 μg/ml). The cells were then treated with the experimental drugs for 24 h, followed by treatment with the experimental drugs (5 μg/ml) for 24 h. The absorbance was measured using a spectrophotometer (ELx800; Bio-Tek Instrument, USA) at 540 nm.
2. Effect of *Mollotus repandus* on HUVECs migration

From a pre-treat HUVECs (5x10^4 cells/insert) in a standard Petri dish, paclitaxel (M) or DMSO (0.25%) was added to the upper chamber of the transwell that contained fibronectin in the lower chamber of the transwell. Following culture media, which was 15% FBS, for 24 hours, HUVECs that migrated to the lower chamber of the transwell were counted. Paclitaxel inhibited the migration of HUVECs in a concentration-dependent manner. At 100 µg/ml, paclitaxel significantly inhibited migration compared to DMSO (P<0.05; P<0.001, n=3).

3. Effect of *Mollotus repandus* on CCA cells migration

From a pre-treat KU-100 and KU-M139 (1x10^5 cells/insert) in a standard Petri dish, paclitaxel (M) or DMSO (0.25%) was added to the upper chamber of the transwell that contained fibronectin in the lower chamber of the transwell. Following culture media, which was 15% FBS, for 24 hours, CCA cells that migrated to the lower chamber of the transwell were counted. Paclitaxel inhibited the migration of CCA cells in a concentration-dependent manner. At 100 µg/ml, paclitaxel significantly inhibited migration compared to DMSO (P<0.05; P<0.001, n=3).
วิจารณ์

จากการทดลองพบว่าสารสกัดโคคลานในความเข้มข้นที่ไม่มีพิษต่อเซลล์มีฤทธิ์ยั้งการเคลื่อนที่ของเซลล์HUVECs ได้ตามขั้นมาก สารสกัดในขนาด 200 μg/ml มีฤทธิ์ยั้งการเคลื่อนที่ของ HUVECs ได้มากกว่า ปฏิทินศาล ที่ 10-9 M ผลที่ได้จากการศึกษาเรื่องสงครามกับรายงานอื่นๆ ซึ่งเชื่อมาก น่าจะสังเกตได้กับฤทธิ์ของ triterpenoids ที่เป็นสารพิษพิษของโคคลาน' มีรายงานว่าสารประกอบดังกล่าวนี้otriderpenoids สามารถยั้งการเกิดหลอดเลือดใหม่ได้มากกว่าด้านที่มีฤทธิ์ต่อการเกิดหลอดเลือดใหม่ 6-16 You และคณะ15 รายงานฤทธิ์ยั้งการเกิดหลอดเลือดใหม่ของ lupeol โดยการยั้งการเกิดหลอดเลือดใหม่ของ HUVECs Cardenas และคณะ16 รายงานว่า ursolic acid มีฤทธิ์ยั้งการเกิดหลอดเลือดใหม่ 6-16, 18, 25, 27

แสดงผลของสารสกัดโคคลานในภาวะยั้งการเคลื่อนที่ของ KKU-M139 (ค่าที่แสดงคือค่าของ mean ± SE, n=3; *P < 0.05; ** P < 0.001 เทียบกับ DMSO)

แสดงผลของสารสกัดโคคลานในภาวะยั้งการเคลื่อนที่ของ KKU-100 (ค่าที่แสดงคือค่าของ mean ± SE, n=3; *P < 0.05 เทียบกับ DMSO)
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