RESEARCH ARTICLE

Derris scandens Benth Extract Induces Necrosis Rather Than Apoptosis of SW480 Colon Cancer Cells

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Abstract

The extract from *Derris scandens Benth* was previously shown to have anti-proliferative effect against SW480 colon cancer cells. Therefore, the present study was aim to investigate the mechanism of action of the anti-proliferative effect of *D. scandens* extract. Several apoptotic signaling pathways were determined following *D. scandens* treatment. Caspase-3 activity and the expression of Bax pro-apoptotic and Bcl-2 anti-apoptotic proteins were determined. The result showed that *D. scandens* (5-10 μg/ml) slightly increased caspase-3 activity, as well as up-regulated Bax and down-regulated Bcl-2 proteins of SW480 cells. However, these changes were not statistically significant. *D. scandens* extract significantly induced cell necrosis determined by the release of LDH. These results suggest that *D. scandens* primarily mediate SW480 cell death through necrotic rather than apoptotic process.

Keywords *Derris scandens Benth*, apoptosis, colon cancer, SW480 cells

Introduction

Colorectal cancer is a common disease that remains the major cause of cancer-related mortality in developed countries. The incidence rate of colorectal cancer in Thailand is low when compared with other countries and the highest incidence is seen in Bangkok (1). This rate is expected to be rapidly increased in the next decade probably due to the acquisition of Western lifestyle. Diet with high levels of fat and red meat, and low dietary fiber is the major risk factor of colorectal cancer (2). Since diet is definitely important for colon cancer development, dietary interventions are received much attention as one of approaches to prevent this type of cancer. The protective effects of diets rich in fruits and vegetables against colon carcinogenesis are thought to be due to their content of anti-oxidant vitamins and fibers (3,4). Several traditional Thai herbal medicines are believed to have anti-cancer activity but there is limited scientific evident to support their effectiveness. *D. scandens* is one of Asian medicinal plant, local Thai name, Tao-Wan-Priang. Its dried stem has been used as an expectorant, anti-tussive, diuretic and agent for the treatment of muscle aches (5). Based on our previous study, the extract from *D. scandens* showed an effective anti-proliferative activity against SW480 colon cancer cells (IC_{50} = 4.86 μg/ml) (unpublished data). Thus, the aim of this study was to investigate whether *D. scandens* extract drives colon cancer cells to undergo necrosis or apoptosis cell death pathway.

Methods

Preparation of plant extract:

The *D. scandens* powder was prepared and provided by Bangkratum Hospital, Phitsanulok. The dried powder was macerated with 95% methanol for 3 days. The aqueous extract was subsequently filtered and evaporated in a rotavapor at 55-60°C under pressure. The plants extract was kept at -20°C.

Cell culture

The human colorectal cancer cells (SW480) was purchased from the American Type Culture Collection (ATCC). SW480 cells were cultured in DMEM/F-12 supplemented with 10% fetal bovine serum (FBS) and 100 units/ml penicillin and 100 μg/ml streptomycin. Cells were cultured in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Cell viability assay

Cells were exposed to various concentrations of *D. scandens* extract for 24 h. Cells were incubated with 0.5 mg/ml of MTT 2 h before the end of treatment period. Then cells were lysed with DMSO:ethanol (1:1) and the absorbance was read at 595 nm. Lactate dehydrogenase (LDH) released into cultured medium was measured by using pyruvate and NADH as substrates. The reduction of NADH was determined at 340 nm.

Caspase-3 activity

The cells were harvested by trypsinization before the detection of caspase-3 activity by using EnzChek® Caspase-3 assay kit (Molecular Probes). According to the manufacturer's instruction, caspase-3 activity was determined by using rhodamine 110 bis-(*N*-CBZ-L-aspertyl-L-glutamyl-L-valyl-L-aspartic acid amide) (Z-DEVD-R110) as a substrate. The fluorescence of rhodamine 110 (R110) was measured at Ex 488 nm and Em 535 nm.

Expression of Bcl-2 and Bax

Immunoblotting was used to determine the expression of Bcl-2 and Bax proteins. Briefly, proteins in cell lysate were separated on SDS-polyacrylamide gel electrophoresis and transferred onto PVDF membrane. The membrane was then incubated with specific antibody against Bcl-2 or Bax and subsequently secondary antibody conjugated with alkaline phosphatase. The activity was assessed by using nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) as a substrate.

Results

Effect of D. scandens on colon cancer cell viability

The cell viability of SW480 cells in the presence of various concentrations of *D. scandens* was examined. As shown in

Figure 1A, *D. scandens* at 5 and 10 μg/ml dramatically decreased cell viability. At the same concentrations, *D. scandens* induced the substantial release of LDH enzyme indicating necrotic cell death (Figure 1B). These results suggest that *D. scandens* mediates SW480 colon cancer cell death via cell necrotic pathway.

Effect of D. scandens on caspase-3 activity

Caspase-3 is one of executioner caspases which its activity is increased when cell decides to undergo apoptosis (6). The result showed that *D. scandens* extract tended to increase caspase-3 activity in a dose-dependent manner, but no significant difference was observed.

Effect of D. scandens on expression of Bcl-2 and Bax

Apoptosis pathway is controlled by Bcl-2 family proteins. Bcl-2 is the member of a large family of proteins that can be divided into two groups: pro- and anti-apoptotic members such as Bax and/or Bak and Bcl-2 and/or Bcl-X_L respectively (7). After treating cells with *D. scandens* at 10 μg/ml, there was a slight down-regulation of Bcl-2, whereas up-regulation of Bax was observed. However, there was no marked difference in the expression of these two proteins compared to control cells.

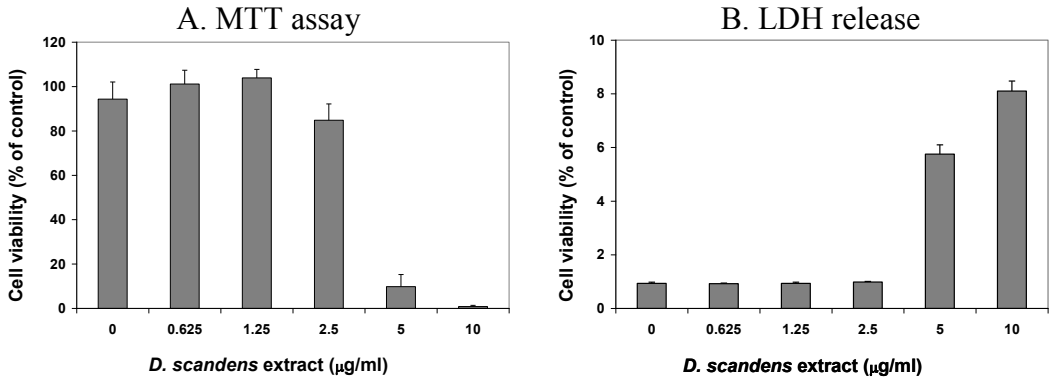

Figure 1 Effect of *D. scandens* extract on cell viability of SW480 colon cancer cells. SW480 cells were treated with various concentrations of the extract for 24 h, cell lysates were prepared for the MTT assay (A) and cultured medium were collected to measure LDH activity (B).

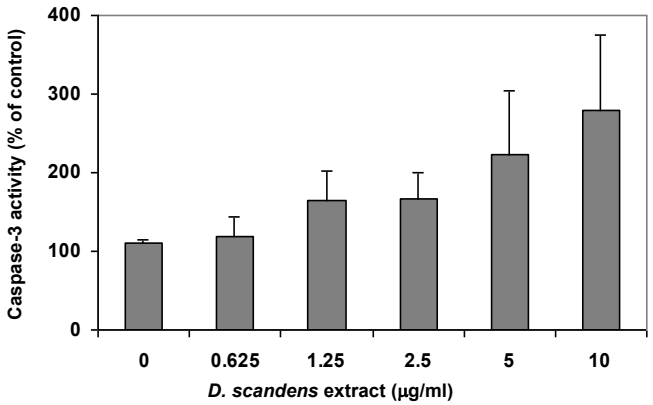

Figure 2 Effect of *D. scandens* extract on caspase-3 activity of SW480 colon cancer cells. Cell lysates were prepared for measuring caspase-3 activity. The data represent mean ± SE from 5 experiments.

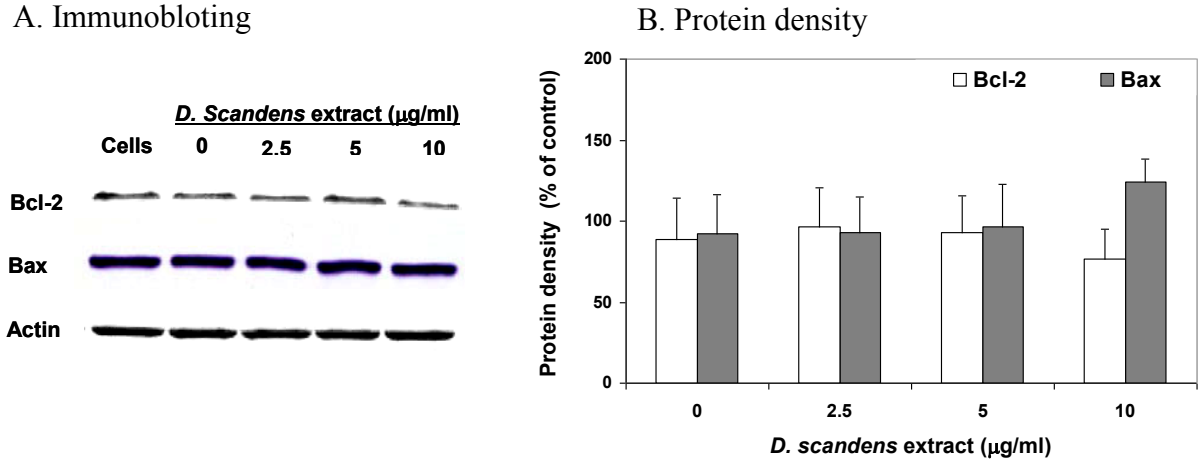

Figure 3 Effect of *D. scandens* extract on the expression of Bcl-2 and Bax in SW480 colon cancer cells. A; immunoblotting and B; the averaged density of protein bands from at least three experiments.

Discussions and Conclusion

From our previous study, the extract from *D. scandens* showed an effective anti-proliferative activity against SW480 colon cancer cells with IC_{50} 4.86 µg/ml (unpublished results). This anti-proliferative effect may be due to certain compounds found in *D. scandens* such as coumarins, isoflavones and isoflavone glycosides which previously showed to have the anti-migration of cancer cells (8). In the present study, we demonstrated that this extract slightly increased caspase-3 activity, up-regulation of Bax pro-apoptotic protein and down-regulation of Bcl-2 anti-apoptotic protein. *D. scandens* leads to substantially release of LDH from SW480 colon cancer cells. Taken all data together, cell necrosis is the major pathway of *D. scandens*-induced cell death. Our finding suggests that the extract of *D. scandens* decreased colon cancer cell viability by induction of cell necrosis rather than cell apoptosis.

Acknowledgements

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