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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease with the uncontrolled high blood glucose level. In order to control blood sugar, current treatment plan includes diet restriction, exercise and drug therapy. Anti-diabetic drugs control blood sugar through various mechanisms of action including increase insulin secretion and sensitivity, and limit glucose absorption. Several herbs such as bitter cucumber or mara-kee-nok (*Momordica charantia* L., Family Cucurbitaceae) and cinnamon (*Cinnamomum iners* Reinw. ex Blume Family Lauraceae) have been known for their anti-diabetic action. In our preliminary study, alcoholic extract of four Thai herbs collected from the Plant Genetic Conservation Project area under The Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn potently inhibited intestinal alpha-glucosidase, suggesting their anti-diabetic potential. These plants include *Pterospermum littorale* Craib (or Lam-pang, Family Sterculiaceae), *Dialium cochinchinense* Pierre (Kleng, Family Fabaceae), *Mamecylon plebejum* Kurz. var. ellipsoideum Craib. (Plong-bai-ree, Family Melastomataceae) and *Thespesia populnea* (L.) Soland.ex Corr. (Poe-ta-lay, Family Malvaceae).

Herb-drug interactions can affect efficacy and safety of drug therapy. Interference with the function of P-glycoprotein (P-gp) is one of the most studied transporter-based drug interactions. P-gp is the main energy-dependent efflux transporter protein in the ABC transporter superfamily. This drug efflux pump can be found abundantly at the apical site of several epithelial cells and tissues such as intestine, kidney proximal tubule and lungs[1-3]. Hence, interference with P-gp activity may affect the processes of absorption, distribution and elimination, leading to an alteration of pharmacokinetic behaviors of its drug substrates[4]. For example, piperine in black pepper inhibited P-gp activity, leading to an increase in the plasma concentration of phenytoin and rifampin[5]. The purpose of this study was to determine whether four herbs with anti-diabetic potential (*P. littorale* Craib, *D. cochinchinense* Pierre, *M. plebejum* Kurz. var. ellipsoideum Craib, *T. populnea* (L.) Soland.ex Corr.) could inhibit the function of P-gp transporter, using the *in vitro* model of caco-2 cells.

MATERIALS AND METHODS

Plant materials and preparation of plant extract All medicinal plants were collected in January, 2011 from Chonburi Province, Thailand. Bark of *P. littorale* Craib (PL, 89.56 g), *D. cochinchinense* Pierre (DC, 45.38 g), leaves of *M. plebejum* Kurz. var. ellipsoideum Craib. (MP, 16.9 g) and fruits of *T. populnea* (L.) Soland.ex Corr. (TP, 149.41 g) were cut and macerated separately with 500 ml of 95% ethanol for 5 days at room temperature, and then filtered. The filtrate was evaporated to dryness under vacuum. The weight of each crude extract was 3.26 g for PL, 1.47 g for DC, 1.55 g for MP, and 1.71 g for TP. All the crude extracts were kept at -20 °C until used.

Chemicals The chemicals including verapamil (VER), vinblastine (VBL), calcein acetoxymethyl ester (calcein-AM), Hank's balanced salt solution (HBSS), Bradford reagent were purchased form Sigma chemical company (St. Louis, MO, USA).

Cell cultures The Caco-2 cells (ATCC, Rockville, MD, USA), passage 96 to 112, were cultured in DMEM supplemented with 10% FBS, 1% non-essential amino acid, 1% penicillin-streptomycin, 1% L-glutamine and 10 nM VBL at 37°C in a humidified atmosphere of 5% CO_2. Cells were subcultured every 3-4 days (at approximately 70% confluency). For the uptake study, the cells were seeded onto a 24 well-plate at a density of 13,000 cells/cm^2. The cells were fed with fresh VBL-containing medium every 2 days until they were used in the assay at 21 days after seeding. A day before an uptake study, the medium was switched from VBL-containing medium to VBL-free medium.

Uptake study The Caco-2 monolayers were pretreated with the test extract for 30 minutes, followed by addition of calcein-AM (0.4 μM). After the incubation period of 30 minutes, the cells were washed with ice cold HBSS and lysed with 0.3N NaOH in 1% Triton X-100. The fluorescent intensity of calcein was determined with the microplate reader at an excitation wavelength and an emission wavelength of 485nm and 535 nm, respectively. The amounts of proteins in each sample were determined with Bradford reagent in order to normalize the total protein in each experiment.

Data analysis The results were calculated and expressed as the percentage of the calcein retention/mg protein in relative to those of the control group in each experiment (100%). Data were expressed as the mean ± SEM obtained from at least three separated experiments.

RESULTS

The expression of active P-gp in the Caco-2 monolayers after cultured for 21 days in VBL-containing medium was at the appreciable level. The presence of verapamil (100 μM) and cyclosporine A (10 μM), which are known inhibitors of P-gp, increased intracellular accumulation of calcein by 5 and 6.8 folds, respectively (Figure 1).

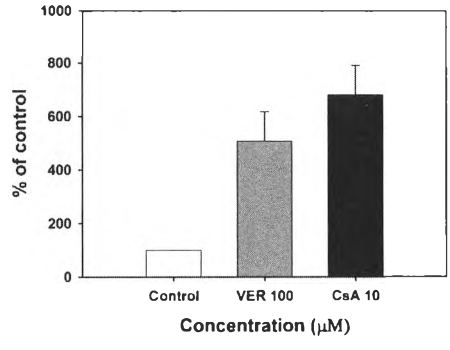

Figure 1 Intracellaular accumulation of calcein in the VBL-resistant Caco-2 monolayers aged 21 days. The effects of verapamil (VER) and cyclosporine A (CsA), the known P-gp inhibitors, were determined and used as our positive control. Data were normalized per mg of proteins and expressed as the percentage of control (untreated group). Values represented the mean ± SEM (n=5-6).

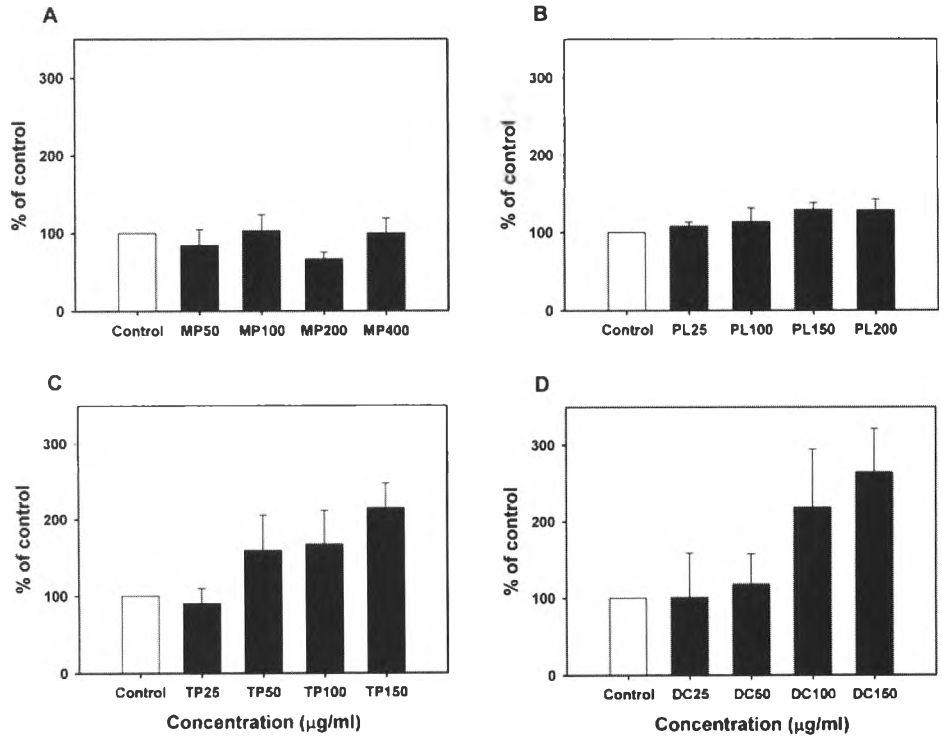

Figure 2 Effect of alcoholic extracts (MP, PL, TP, and DC) on P-gp function in the VBL-resistant Caco-2 monolayers aged 21 days. The calcein accumulation was expressed as the percentage of control. Values represented the mean ± SEM (n=3-6).

Crude extracts of TP and DC were able to increase intracellular calcein retention in the VBL-resistant Caco-2 cells, suggesting their inhibitory action against P-gp activity (Figure 2). At the maximal concentration of 150 μg/ml, TP and DC increased calcein retention by 2.14 and 2.64 folds, respectively. The inhibition was apparently concentration-dependent. Neither MP nor PL extract could inhibit P-gp activity in our uptake assay (Figure 2). It should be noted that the maximal concentration of each crude extract in this study was the highest soluble concentration in 1%DMSO. In addition, all the crude extracts had no significant effects on the viability of VBL-resistant Caco-2 cells.

DISCUSSION

In this study, the function of P-gp was assessed by measuring the intracellular calcein accumulation in the VBL-resistant Caco-2 monolayers cultured for 21 days. The Caco-2 cell is an epithelial human colon adenocarcinoma cell line which has been widely accepted as a model of the intestinal barrier for predicting drug absorption[6]. Upon being cultured in optimum condition, the Caco-2 monolayers express various drug transporters in the ATP binding cassette superfamily including P-gp[7-8]. Although the Caco-2 cells are able to express the appreciable levels of P-gp, several factors such as passage number and subculture process can cause the variation in the number of functional active protein[9]. In order to increase the expression of active P-gp, we cultured the Caco-2 cells in the presence of vinblastine (10 nM) as described in the previously reported protocols[10-12]. Our VBL-resistant Caco-2 cells steadily expressed high levels of functional active P-gp, even when the high passage numbers (96-112) of the cells were used.

Four Thai herbs in the Plant Genetic Conservation Project area under The Royal Initiative of HRH Princess Maha Chakri Sirindhorn were chosen for this study based on their ability to inhibit alpha-glucosidase, which is a known drug target for diabetic control. We demonstrated that extracts of *M. plebejum* and *P. littorale* at the highest concentration in this study were unable to alter P-gp activity. On the other hand, extracts of *D. cochinchinense* and *T. populnea* at their maximal soluble concentration inhibited the P-gp function in comparable potency. The inhibition could be observed at the concentration as low as 50 μg/ml (TP) and 100 μg/ml (DC). Upon increasing the concentrations of the two extracts, their inhibitory action increased. It was likely that certain chemical constituents in crude alcoholic extracts of MP and DC were potent inhibitors of P-gp. Hence, concomitant use of either *D. cochinchinense* or *T. populnea* and P-gp drug substrates might cause the problem regarding P-gp-mediated drug interactions through the direct inhibition of this efflux pump. Further studies in this regard are needed. In addition, isolation and identification of the compounds being P-gp inhibitors in these two herbs should be pursued.

CONCLUSION

Crude alcoholic extracts of *Thespesia populnea* (TP) and *Dialium cochinchinense* (DC) were able to inhibit the P-glycoprotein function.

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