Thai Journal of Pharmaceutical Sciences (TJPS)

5th International Conference on Pharmaceuticals,
Nutraceuticals and Cosmetic Science (IPNaCS 2017)

Effect of *Gymnema inodorum* on P-glycoprotein Function: an *In Vitro* Study

May Phyu Thein Maw[1], Nusara Piyapolrungroj[1]*

[1] *Department of Biopharmacy, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand*.

* Corresponding author: Tel. +66(0)34255800; Fax. +6634255801; E-mail address: PIYAPOLRUNGROJ_N@su.ac.th

Keywords: P-glycoprotein (P-gp), Herb-drug interaction, *Gymnema inodorum* (Lour.) Decne, Uptake assay

Introduction

P-glycoprotein (P-gp), 170-kDa membrane protein belonging to the ATP-binding cassette (ABC) efflux transporter superfamily, has long been known for its role in developing multidrug resistance (MDR) of tumor cells. However, P-gp also expresses in the plasma membrane of many normal tissues, especially in the brain, liver, kidney, and intestine, where it limits absorption and distribution of drugs. Therefore, the modulation of P-gp can affect the efficacy and safety of structurally diverse drugs such as cytotoxic drugs, cyclosporine, HIV protease inhibitors, and etc. In recent year concomitant administration of herbal medicines with conventional medications has increased.[1] As a result, clinically recognized P-gp mediated herb-drug interactions had been reported with St John's wort, capsaicin, quercetin, curcumin, piperine and others.[2]

Gymnema inodorum (Lour.) Decne (Asclepiadaceae), found ubiquitously in South-eastern Asia, is consumed as food and traditional medicine. GI* is widely consumed as vegetable in diet because of its nutritional value. Apart from its nutritional value, the leaves of this plant also have therapeutic value in diabetes mellitus, rheumatic arthritis and gout. It had been found that the extracts of GI reduce the plasma glucose level by inhibiting the intestinal blood glucose absorption process.[3, 4] The high antioxidant activity and vitamin E content of the GI plant had also been reported.[5] Although there are scientific studies on the pharmacological activities of GI for treatment of diabetes mellitus and as an antioxidant, the modulatory effect on P-gp has not been thoroughly investigated so far. Conventional antidiabetic and lipid lowering drugs such as glyburide, sitagliptin, saxagliptin and glibenclamide are P-gp substrates[6] and they may be concomitantly used with GI. Consequently, P-gp mediated herb-drug interactions can occur when this plant is taken together with P-gp substrate drugs. Hence it is important to know potential herb-drug interactions to minimize clinically relevant P-gp mediated interactions.

The purpose of this study was to investigate the effect of *Gymnema inodorum* leaf extract on the function of P-gp transporter *in vitro*. The effect of GI leaf extract on uptake of calcein-AM, a P-gp substrate, in Caco-2 (a human colonic adenocarcinoma cell line) was evaluated. LLC-PK_1 (a porcine kidney epithelial cell line) and LLC-GA5-COL300 (a human P-gp overexpressed-LLC-PK_1 cell line) were also utilized to confirm its role on P-gp function.

Methods

Preparation of the crude extract from Gymnema inodorum

Gymnema inodorum leaves were collected from a fresh market in Chiang Mai Province, Thailand. The extraction was done by maceration method. The powdered sample of dried leaves (5 g) was shaken with (100 ml) of ethanol at room temperature for 2 days with frequent agitation and the macerate was filtered. The filtrate was evaporated to dryness and kept at -20ºC until use. The dried extract was then diluted in HBSS buffer pH 7.4 with the final DMSO concentration not exceeding 1% in all experiments.

Cell Culture

The Caco-2 cells (ATCC, order no: S0249019, passage 40-60) were routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin, 2mM L-glutamine, 1% non-essential amino acid. LLC-PK_1 cells (passage 38-40) were grown in M199 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-

* Abbreviation
GI - *Gymnema inodorum*

streptomycin. LLC-GA5-COL300 (Riken Cell Bank, air waybill order no: 8462-5956-2849 passage 25-28) cells were cultured in M199 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin and 300 ng/ml colchicine. All of the cells were maintained in a humidified 5% CO_2 atmosphere at 37ºC.

Uptake study

For the uptake study, Caco-2 cells were seeded at a cell density of 150000 cells/well onto 24-well plates. Cells used for experiment were cultured for 21 days. LLC-PK_1, 80000 cells/well and LLC-GA5-COL300, 130000 cells/well were seeded onto 24-well plates and grown for 3 days for the studies. Verapamil which is well defined P-gp inhibitor was used as positive control.[7] Hank's balanced salt solution (HBSS) with 1% DMSO was used as the control.

Cells were preincubated in the HBSS in the absence or presence of different concentrations of GI leaf extracts (1.25 µg/ml, 12.5 µg/ml, 62.5 µg/ml and 125 µg/ml) and verapamil (100 µM) for 30 minutes. Then P-gp substrate calcein-AM was added to the final concentration of 1 µM and further incubated for another 30 minutes. Following this, the test solution was removed and washed carefully with ice-cold HBSS. After washing, the cells were lyzed with 0.1% Triton X-100 and the amount of calcein retained in the cells was measured directly on a microplate reader (Fusion, Packard, USA) at an excitation and emission wavelength of 485 and 535 nm, respectively. The amount of calcein accumulated was normalized with the protein concentration and expressed as RFU/mg protein.

$$\% \text{ of control} = \frac{\text{Fluorescence intensity of non-treated cells}}{\text{Fluorescence intensity of treated cell}} \times 100$$

Results

Effect of GI* leaf *extracts on uptake of calcein-AM by Caco-2

All concentrations of the extract used in this study was checked with trypan blue dye exclusion method and exhibited no toxic effect to the cells. To validate P-gp function, the fluorescence intensity of the calcein was firstly measured in the absence and presence of a P-gp positive inhibitor verapamil (100 µM). The calcein accumulation in cells increased to 388.75 ± 52.64 % of control in the presence of verapamil. The effects of different concentrations of extracts on calcein-AM uptake is shown in Figure 1. As seen, the different concentrations of GI leaf extracts (12.5 µg/ml, 62.5 µg/ml and 125 µg/ml) significantly increased the uptake of calcein-AM in Caco-2 cells in a dose-dependent manner by approximately 1.5, 2.9 and 4.7 folds. However, the fluorescence intensity of the lowest concentration of extract (1.25 µg/ml) was almost the same with that of control.

Effect of GI* leaf *extracts on uptake of calcein-AM by LLC-PK_1 and LLC-GA5-COL300

In order to further confirm the role of GI leaf extracts on P-gp function, the uptake of calcein-AM was compared between LLC-PK_1 and LLC-GA5-COL300 cells in the second set of the experiment. The accumulation of calcein in cells was inversely related to the function of the transporter. The uptake of calcein-AM in LLC-PK_1 (4432.33 ± 1228.94 RFU/mg protein) was about 18 times higher than that of LLC-GA5-COL300 (236 ± 112.21 RFU/mg protein). The uptake of calcein-AM in both cell lines elevated when verapamil was added. However, the accumulation was remarkably risen by verapamil (1110.11 ± 320.18 % of control) in LLC-GA5-COL300 compared with that in LLC-PK_1. Similarly, intracellular concentration of calcein also dependently increased with respect to the different concentrations of extracts (12.5 µg/ml, 62.5 µg/ml and 125 µg/ml) by 2.3, 6.9 and 18 folds respectively. Nevertheless, the lowest concentration of extract (1.25 µg/ml) did not alter the uptake of calcein-AM (Figure. 2).

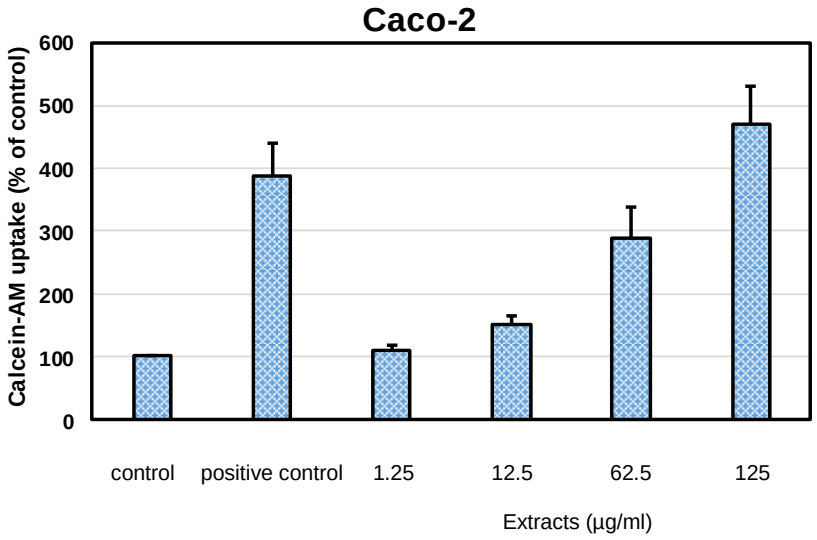

Figure 1 Effects of GI leaf extract on calcein-AM uptake in Caco-2 cells. Data are presented as the mean ± SEM, n = 3, positive control: 100 µM verapamil.

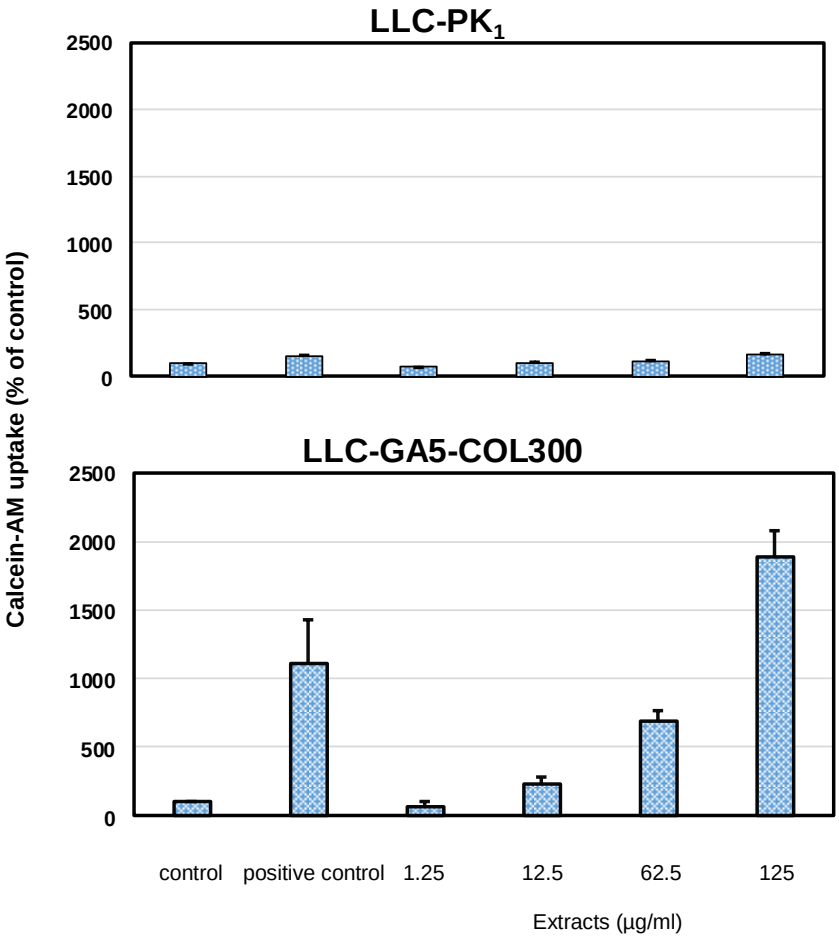

Figure 2 Effects of GI leaf extract on calcein-AM uptake in LLC-PK_1 and LLC-GA5-COL300 cells. Data are presented as the mean ± SEM, n = 3, positive control: 100 µM verapamil.

Discussion

P-gp, one of the well-known efflux transporters, has an important role in multidrug resistance (MDR) and bioavailability of conventional drugs. It can be modulated by herbal constituents possibly leading to clinical herb-drug interactions which is either beneficial or detrimental to the patient.[8]

In vitro effect of GI leaf extracts on P-gp function was firstly investigated in Caco-2 cells. Caco-2 cells, human colon carcinoma derived cells, are widely employed as *in vitro* culture models for absorption studies of drugs with a good expression of P-gp.[6] The study of P-gp function was performed by the calcein-AM uptake assay. The non-fluorescent calcein-AM was converted into the fluorescent calcein after being hydrolyzed by the intracellular esterase in the cells. In the presence of verapamil (100 µM), a known inhibitor of P-gp, the accumulation of calcein increased, indicating that the finding correlated with the observed transporter activity in these cells. The different concentrations of extracts (12.5-125 µg/ml) significantly increased the fluorescence intensity of the Caco-2 cells in a concentration dependent manner similar to verapamil function. This result suggests that the extract from GI leaf has potential inhibitory effect on efflux transporter, especially P-gp.

To further confirm the role of GI leaf extract on P-gp, the uptake study was performed on LLC-PK_1 which express very low level of P-gp and LLC-GA5-COL300, which overexpress human P-gp. The uptake of calcein-AM in LLC-GA5-COL300 was about 19 times lower than that of LLC-PK_1, which is in accordance with the higher expression levels of P-gp in LLC-GA5-COL300 cells and the accumulation of calcein remarkably increased when verapamil was added. The uptake of calcein-AM in LLC-GA5-COL300 markedly increased in the concentration dependent manner in the presence of different concentrations of the extracts (12.5-125 µg/ml). These results confirmed the role of GI leaf extracts on P-gp function *in vitro*. However, more studies including the *in vivo* experiment should be further investigated to confirm these results.

Conclusion

In conclusion, the present study indicates that *Gymnema inodorum* leaf extract could inhibit the function of efflux transporter P-gp *in vitro*.

Acknowledgements

The authors would like to thank the Research and Creative Fund, Faculty of Pharmacy, Silpakorn University for the financial support.

References

1. Ampasavate C, Sotanaphun U, Phattanawasin P, Piyapolrungroj N. Effects of Curcuma spp. on P-glycoprotein function. Phytomedicine. 2010;17(7):506-12.
2. Chieli E, Romiti N, Rodeiro I, Garrido G. In vitro modulation of ABCB1/P-glycoprotein expression by polyphenols from Mangifera indica. Chemico-Biological Interactions. 2010;186(3):287-94.
3. Shimizu K, Ozeki M, Iino A, Nakajyo S, Urakawa N, Atsuchi M. Structure-activity relationships of triterpenoid derivatives extracted from Gymnema inodorum leaves on glucose absorption. Japanese journal of pharmacology. 2001;86(2):223-9.
4. Shimizu K, Ozeki M, Tanaka K, Itoh K, Nakajyo S, Urakawa N, et al. Suppression of Glucose Absorption by Extracts from the Leaves of Gymnema inodorum. Journal of Veterinary Medical Science. 1997;59(9):753-7.
5. Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chemistry. 2005;92(3):491-7.
6. Oga EF, Sekine S, Shitara Y, Horie T. P-glycoprotein mediated efflux in Caco-2 cell monolayers: The influence of herbals on digoxin transport. Journal of Ethnopharmacology. 2012;144(3):612-7.
7. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y. Increased accumulation of vincristine and adriamycin in drug-resistant P388 tumor cells following incubation with calcium antagonists and calmodulin inhibitors. Cancer research. 1982;42(11):4730-3.
8. Liang Y, Zhou Y, Zhang J, Liu Y, Guan T, Wang Y, et al. In vitro to in vivo evidence of the inhibitor characteristics of Schisandra lignans toward P-glycoprotein. Phytomedicine. 2013;20(11):1030-8.