ORIGINAL ARTICLE

Effects of *Momordica charantia* Extract on the Expression of *MDR 1* Gene in Human Lung Cancer Cells

T Yuan, H-Y Li

ABSTRACT

Objectives: *Multi-drug resistance (MDR) is a major hurdle in treatment of cancer, contributing to the failure of chemotherapy. Drug resistance is found to be linked to the overexpression of ATP-binding cassette (ABC) drug transporter proteins that include P-glycoprotein (P-gp), causing a reduction in drug accretion inside the cancer cells. In the present study, the effect of the extracts from the fruit peel and pulp of* Momordica charantia *(MCFPE) fruit in modulating the function of P-gp in human small-cell lung cancer (SCLC) cell lines was assessed.*

Methods: The effects of MCFPE were tested on drug-sensitive (H69) and multi-drug resistant (H69/LX4) human SCLC cells. The cell survival percentage was assessed by MTT cytotoxicity assay. The percentage of drug accumulation and drug efflux were assessed by using [^{3}H]-paclitaxel. The expression of MDR1 *gene was analysed by reverse transcription polymerase chain reaction (RT-PCR), and P-gp by western blot analysis.*

Results: *The extract was able to induce death of cancer cells as measured by cell survival percentage as well as improve drug accumulation, as evidenced by intracellular paclitaxel retention. Prior exposure of cells to MCFPE reversed resistance to paclitaxel. Treatment with MCFPE was found to have a significant impact on* MDR 1 *gene expression in H69/LX4 cell line by decreasing its expression. The extract had no influence on expression of* MDR 1 *gene in the drug-sensitive SCLC cell lines. Western blot analysis of P-gp protein in H69 and H69/LX4 cells revealed that the treatment with the extract modulates the expression of* MDR 1 *in H69/LX4 and had negligible effect on H69 cells.*

Conclusion: *The results indicate that MCFPE was able to effectively reverse multi-drug resistance and improve cancer chemotherapy.*

Keywords: Lung cancer, *Momordica charantia,* multi-drug resistance*,* P-glycoprotein

WIMJ Open 2014; 1 (3): 92

INTRODUCTION

Lung cancer is one of the most common type of cancers and a major cause of cancer-related deaths globally. Small-cell lung cancer (SCLC), an aggressive cancer, accounts for nearly 10−15% of all lung cancers (1). Chemotherapy with combinations involving etoposide, doxorubicin, cisplatin and vincristine are employed in the treatment of patients with SCLC (2). The dearth of chemotherapy regimen is the development of multi-drug resistance (MDR) that leads to ineffectiveness of the therapy. In recent years, cancer research has been focussed on the understanding of the fundamental mechanisms that impart drug resistance.

Multi-drug resistance has been observed to be mediated by several mechanisms such as alterations in the apoptotic proteins and pathways, change in the activity of specific enzyme systems involved, active efflux of the chemotherapeutic drugs by ATP-binding cassette (ABC) transporters and others (3). Among the mechanisms that are proposed to initiate MDR, overexpression of ABC transporter proteins has received extensive attention (4).

P-glycoprotein (P-gp), a 170-KD transmembrane glycoprotein encoded by the human *MDR 1* (ABCB1) gene, is a member of the family of ABC transporters. It acts as an ATP-driven efflux pump that decreases intracellular drug accumulation, thereby decreasing the effectiveness of many chemotherapeutic agents (5, 6).

From: Department of Respiratory Diseases, the Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China.

Correspondence: Dr H-Y Li, Department of Respiratory Diseases, the Fifth Affiliated Hospital of Zhengzhou University, No. 3 Kangfu Road, Zhengzhou, Henan 450052, China. E-mail: lihongyun52013@gmail.com

 DOI: 107727/wimjopen.2014.147

The ATPase domains of P-gp are induced by the presence of transport substrates such as vinca alkaloids (vincristine, vinblastine), anthracyclines (doxorubicin, daunorubicin, epirubicin), epipodophyllotoxins (etoposide, teniposide) and taxanes (7, 8). Thus it would be effective if the activity of P-gp mediated drug efflux could be inhibited which may resensitize MDR. As the P-gp efflux pump is an ATP-dependent transport system (9), compounds that are effective inhibitors of ATP-dependent drug transport should mediate the inhibition of drug resistance and increase intracellular accumulation.

Recent research is now focussing on natural products and plant-derived compounds as MDR-reversing agents. A number of studies demonstrate that natural products derived from plants are potential drugs to overcome MDR in many multi-drug resistant cells (10−12). These studies suggest that plant extracts and derived compounds could be potent agents in reversing MDR and also be chemotherapeutic.

Momordica charantia L (MCFPE; commonly known as bitter melon) is widely consumed as a vegetable. It is also consumed as a folk medicine in Asia. Extracts of bitter melon have been reported to possess antioxidant activities (13), antiviral (14), antidiabetic and immunomodulating properties (15). The present study was undertaken to investigate the effect of the hot water extracts of the peel and pulp of the fruit of *Momordica charantia* on human lung cancer cell lines.

MATERIALS AND METHODS

Chemicals

[^{3}H]-paclitaxel (37.9 Ci/mmol) was purchased from Moravek Biochemicals, Inc (Brea, CA, USA). Verapamil, Roswell Park Memorial Institute (RPMI) medium, fetal bovine serum (FBS), glutamine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) HRP-conjugated goat anti-mouse IgG and the mono-clonal mouse antibody against P-gp, agarose were obtained from Sigma Aldrich (St Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Preparation of bitter melon extracts

The whole fruit of the freshly harvested bitter melon from a local farm was collected, washed well and cleaned. The peel and the pulp alone were collected. The peel and pulp of the bitter melon were shade dried at 30−40 °C. The thoroughly dried samples were finely powdered and extracted with hot water. The powdered sample of 100 g was extracted rigorously with two litres water at 100 °C for six hours. The concoction was filtered and re-extracted with four litres of water. The combined filtrate was re-filtered and concentrated by rotary evaporation and lyophilized to dryness. The MCFPE extract was dissolved in dimethyl sulfoxide (DMSO) and used for experimental purposes, with the final DMSO concentration being 0.1% (v/v) in the culture medium.

Cells and cell culture

Drug-sensitive H69 and multi-drug resistant cell line NCI-H69/LX4 (procured from Sigma Aldrich) were cultured in RPMI medium supplemented with 10% FBS, 2 mM glutamine and 0.4 µg/mL doxorubicin (only for NCI-H69/LX4) at 37 °C in suspension culture in an atmosphere with 5% CO_2. NCI-H69/LX4 has been established by exposure of the parent line H69 to doxorubicin. The cell line hyper-expresses P-glycoprotein and is resistant to doxorubicin and taxanes. When the cells reached around 80% confluence, they were harvested and plated for subsequent passages or for drug treatments**.**

Cytotoxicity assay

H69 and H69/LX4 cells were plated at 3.0×10^3 cells per well in 96-well plates. After 24 hours, various concentrations of MCFPE and paclitaxel were added and the cells were incubated for 72 hours at 37 °C. The cell growth was assessed by means of an MTT colorimetric assay (16). In each experiment, cytotoxicity assessment was carried out in triplicates.

Measurement of [^{3}H]-paclitaxel accumulation

Accretion of paclitaxel in H69 and H69/LX4 cells were measured using [^{3}H]-paclitaxel as described previously (17, 18). The confluent cells in 24-well plates were pre-incubated with verapamil (standard compound that is a known inhibitor of P-gp) and MCFPE for one hour at 37 °C. In order to evaluate the drug accumulation, the cells were then incubated with 0.1 µM [^{3}H]-paclitaxel in the presence as well as absence of 50 µM verapamil and MCFPE for about two hours at 37 °C. The cells were then washed with ice-cold phosphate buffered saline (PBS), trypsinized and the accumulation of paclitaxel was measured in terms of measure of radioactivity. Radioactivity was assessed by using Packard TRI-CARB 1900CA liquid scintillation counter (Packard Instrument, Inc).

Analysis of [^{3}H]-paclitaxel efflux

To measure drug efflux, the cells were treated in a similar manner as in the drug accumulation experiment; the cells were then incubated for 0, 60 and 120 minutes in the fresh medium at 37 °C in the presence or absence of the reversal agents. After 120 minutes, the cells were then washed thrice with ice-cold PBS followed by trypsinization. The cells were then placed in scintillation fluid to measure the radioactivity in the same way as measurement of drug accumulation. Simultaneously treated, a duplicate set of 24-well plates were used for cell counting.

RNA extraction and reverse transcription polymerase chain reaction

Total RNA was isolated from H69 and H69/LX4 cells treated for 72 hours with MCFPE as well as from untreated control

cells. RNA was isolated using TRIzol (Sigma Aldrich, USA). The isolated RNA was quantified by spectrophotometry and analysed in 1% agarose gel.

Reverse transcription (RT) reactions using 2.5 µg total RNA were performed with oligo-dT primers with M-MLV reverse transcriptase (Sigma Aldrich, USA). The primers specific for *MDR 1* were employed (19−21). The reference gene for the normalization of target gene expression was *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase).

Polymerase chain reaction (PCR) reactions were performed in GeneAmp® PCR System 9700 (Applied Biosystems, USA). The products were separated in 2% agarose gel and were stained with ethidium bromide. The gels were visualized under ultraviolet (UV) light and the densitometry analysis was carried out using Bio-Rad Gel Doc 1000 systems.

Western blot analysis of P-glycoprotein

Plasma membrane from the cells treated with MCFPE at 0.1% for 72 hours was prepared according to the method described by Anuchapreeda *et al* (5). The protein content was measured by the method of Lowry *et al* (22), using bovine serum albumin (BSA) as a standard. Equal amounts of total cell lysates (20 µg of protein/lane) were separated in a 7.5% SDS-PAGE and electrophoretically transferred onto nitrocellulose filters. The filters were incubated sequentially with mouse monoclonal anti-P-gp (23) at dilution of 1:5000 and HRP conjugated goat anti-mouse IgG at a dilution of 1:20 000. Proteins were visualized using a SuperSignal protein detection kit and quantified by scanning densitometry.

Statistical analysis

The results observed are presented as mean ± SD from three individual experiments. Differences between the means were analysed by one-way analysis of variance (ANOVA) and Student *t*-test. Statistical significance was considered when $p < 0.05$. The statistical analyses were performed using SPSS 10.0 software.

RESULTS

Effect of MCFPE on cytotoxicity of paclitaxel in H69 and H69/LX4 cells

To investigate the efficacy of MCFPE on reversing MDR, the effect of MCFPE on the SCLC cells treated with paclitaxel was determined by quantifying the growth inhibition on the cells. The results showed that MCFPE at 0.05%, 0.1% and 0.15% v/v increased sensitivity of H69/LX4 cells to paclitaxel as indicated by a decrease in the cell survival percentage, while similar treatment on H69 cells provided no significant modulating effect. The results obtained were similar on treatment of the cells with 20 µM verapamil (Fig. 1A and B). The highest concentration of the extract was most effective in inhibiting cell survival in SCLC cells.

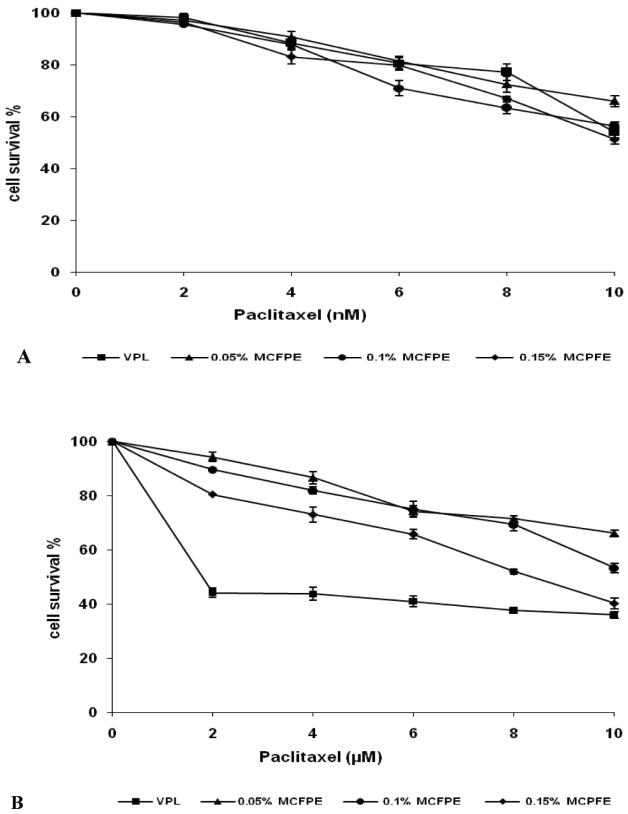

Fig. 1: Effect of *Momordica charantia* (MCFPE) on paclitaxel cytotoxicity in the H69 (A) and H69/LX4 (B) cell lines. The small-cell lung cancer (SCLC) cells were incubated with various concentrations of paclitaxel in the presence or absence of MCFPE and verapamil (VPL). The cell survival percentage with various treatments is marked. The values are expressed as mean ± SD from three individual experiments.

Measurement of [^{3}H]-paclitaxel accumulation

To determine the effects of MCFPE on the function of P-gp as a drug-efflux pump, the accumulation of P-gp substrate [^{3}H]-paclitaxel in the presence or absence of plant extract was measured. The data obtained indicated that the intracellular concentration of [^{3}H]-paclitaxel in P-gp-overexpressing H69/LX4 cells was significantly lower than that in H69 cells (Fig. 2) in the absence of MCFPE or verapamil. After the cells were incubated with MCFPE at 0.05% and 0.1% in DMSO and the standard P-gp inhibitor verapamil for two hours, the intracellular [^{3}H]-paclitaxel accumulation was significantly increased in drug resistant cell line, and MCFPE at 0.1% concentration resulted in highest increase in the intracellular [^{3}H]-paclitaxel which was comparable to that of 10 µM of verapamil. However, the intracellular level of [^{3}H]-paclitaxel as observed in H69 cells was not significantly altered by either MCFPE or verapamil (Fig. 2).

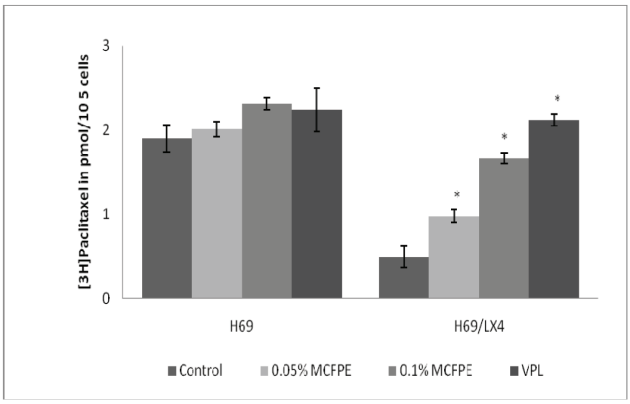

Fig. 2: Effect of *Momordica charantia* (MCFPE) on the accumulation of [^{3}H]-paclitaxel in NCI-H69 and NCI-H69/LX4cells. The accumulation of [^{3}H]-paclitaxel was measured after preincubation with or without the reversal agents (verapamil (VPL) and MCFPE) for one hour at 37 °C and then incubation with 0.1 μM [^{3}H]-paclitaxel in the presence or absence of the reversal agents for two hours at 37 °C. Verapamil was used as a positive control. The values are presented as mean ± SD from three individual experiments.
*indicates statistical significance at $p < 0.05$ for values *versus* those in the control group in H69/LX4 cells.

Effects of MCFPE on the efflux of [^{3}H]-paclitaxel

Drug efflux analysis was carried out in order to establish if MCFPE was able to increase the drug accumulation *via* inhibiting P-gp. H69/LX4 cells were observed to release intracellular [^{3}H]-paclitaxel, with increasing time, compared to H69 cells. Upon incubation of the cells with verapamil and 0.1% MCFPE, the drug efflux was significantly reduced when measured at different time intervals (0, 30, 60, 120 minutes) as against the cells not treated with MCFPE or verapamil.

The percentage efflux of the drug [^{3}H]-paclitaxel at time zero minutes was 0%. The percentages of the drug efflux at 30, 60 and 120 minutes were 31.31 ± 1.66%, 43.32 ± 2.14 % and 62.08 ± 1.26%, respectively, in the H69/LX4 cells in the absence of plant extract or verapamil. H69/LX4 on incubation with MCFPE at 0.1% of the efflux of the drug was found to be 20.12 ± 21%, 31.19 ± 2.04% and 39.88 ± 2.10% at 30, 60 and 120 minutes, respectively (Fig. 3B). On treatment with verapamil, the drug efflux in the H69/LX4 cells was observed as 11.21 ± 0.52%, 15.39 ± 0.94%, and 19.12 ± 0.70% at 30, 60 and 120 minutes, respectively. In H69 SCLC cells, there was no significant modulation in the accumulation of paclitaxel in the presence or absence of plant extract or verapamil (Fig. 3A).

Effect of MCPFE on the expression of *MDR 1* gene in the SCLC cells

MDR 1 gene was amplified in order to study the level of expression in the SCLC cells. Treatment with MCFPE was found to have a significant impact on *MDR 1* expression in the H69/LX4 cell line, decreasing its expression (Fig. 4).

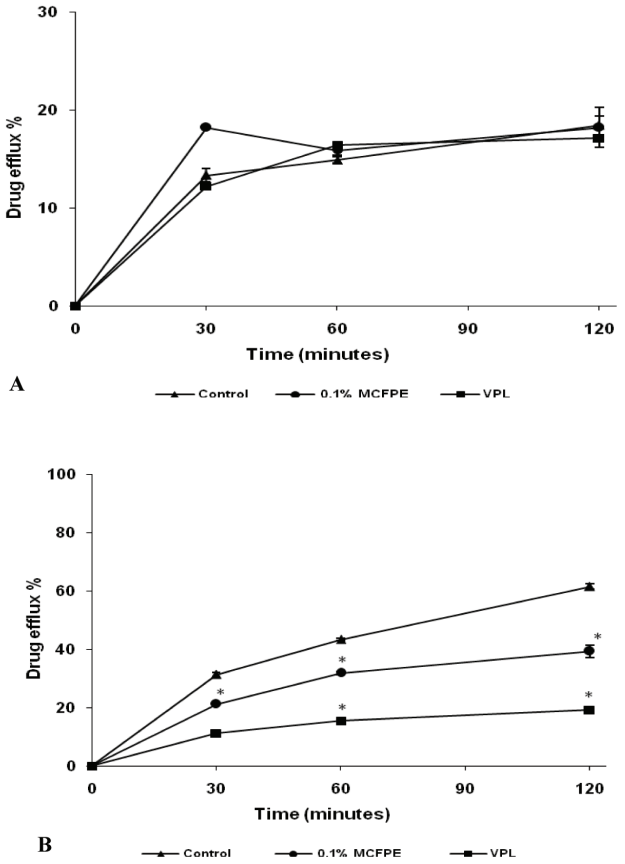

Fig. 3: Effect of *Momordica charantia* (MCFPE) on the efflux of [^{3}H]-paclitaxel from H69 (A) and H69/LX4 (B) cells. The small-cell lung cancer (SCLC) cells were treated with MCFPE and verapamil (VPL) and the levels of [^{3}H]-paclitaxel that was effluxed out were determined at time periods of 0, 30, 60 and 120 minutes. Control represents SCLC cells untreated with MCPFE or VPL. Values are represented as mean ± SD from three individual experiments.
*indicates statistical significance at $p < 0.05$ for values *versus* those in the control group in H69/LX4 cells.

Each reaction was performed in duplex since a reference gene *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) gene expression was included. The extract had no influence on the expression of the *MDR 1* gene in the drug-sensitive SCLC cell lines.

Influence of MCFPE on P-gp

Reversal of P-gp-mediated MDR could be achieved by either decreasing P-gp expression or by inhibiting P-gp function. To evaluate the effect of MCFPE on P-gp expression, H69/LX4 cells were treated with MCFPE for 72 hours. Western blotting analysis was performed after separation of the proteins by SDS-PAGE. The P-gp antibody was employed to detect the expression levels of P-gp. The result shown in Fig. 5 indicates that the extract had no effect on the protein expression levels in the drug-sensitive H69 cells. Treatment

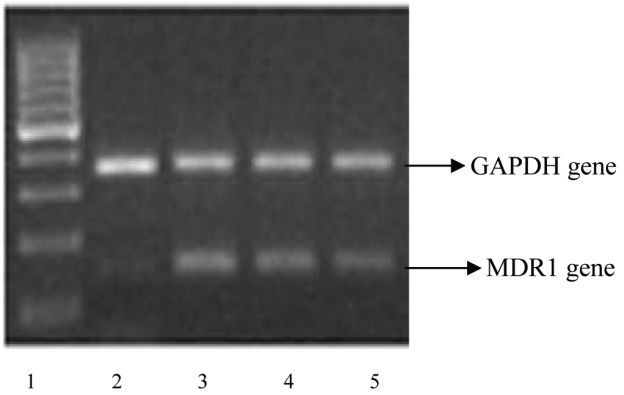

Fig. 4: The isolated mRNA from the H69 and H69/LX4 cells were amplified by reverse transcription polymerase chain reaction (RT-PCR) and the products were analysed for *MDR 1* expression. *GADPH* gene was used as the reference. The PCR products were run beside a 100 bp DNA ladder. Lane 1 represents the ladder. Lane 2 indicates the expression of the *GAPDH* but not the *MDR 1* expression in the H69 cells. Lane 3 represents the expression patterns in H69/LX4 cells. The pattern reveals the intense expression of *MDR 1* (around 250 bp). The treatment of the H69/LX4 cells with *Momordica charantia* (MCFPE) has suppressed the expression of *MDR 1* as presented by lanes 4 and 5. The extract at higher concentration (0.1%) reveals more inhibition (lane 5) on gene expression.

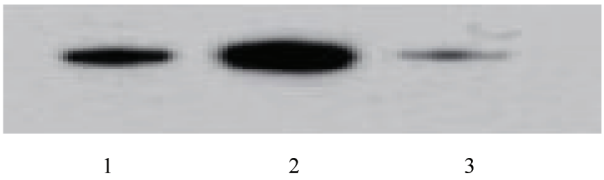

Fig. 5: The analysis of P-glycoprotein (P-gp) by western blot analysis in H69 and H69/LX4 cells. The expression of P-gp, a 170 kDa protein, was under the detectable range in the H69 cells (lane 1). The intense band in lane 2 indicates the hyperexpression of P-gp in the H69/LX4 cells untreated with *Momordica charantia* (MCFPE). The intensity of the expression of P-gp in the H69/LX4 (lane 3) is reduced significantly after treatment with MCFPE as compared to the expression in untreated H69/LX4 cells.

with MCFPE was found to inhibit the expression of P-gp as compared with the H69/LX4 cells that were not treated with MCFPE.

DISCUSSION

The efficacy of chemotherapy in lung cancer treatment is highly restricted by the progress of resistance in cancer cells. Tumour cells that have acquired MDR are generally cross-resistant to a broad variety of chemotherapeutic agents that are chemically and functionally diverse. Multi-drug resistance is often associated with the overexpression of ATP-dependent drug efflux proteins belonging to the superfamily of ABC transporters. P-glycoprotein, which belongs to the family of ABC transporters, is encoded by *MDR 1* gene and is associated with MDR. Various approaches have aimed to reverse MDR and to sensitize the tumour cells to anti-tumour drugs. Verapamil and cyclosporine A have been found to be the most effective P-gp inhibitors *in vitro*, but they have been limited due to side effects. Recent studies are focussed on plant products to find new reversing agents that do not have the undesirable toxic effects.

In the present study, the effects of MCFPE on the cytoxicity of the anticancer drug paclitaxel were analysed in the NCI-H69 drug-sensitive cell line and NCI-H69/LX4 drug-resistant cell line. The results (Fig. 1) indicated that the extract was able to modulate the uptake and thereby increase the cytotoxic effects of the drug paclitaxel as evidenced by the decrease in the cell survival percentage. The P-gp inhibitor verapamil also showed a similar effect. The effect of MCFPE was found to increase with an increase in the concentration of the extract.

The influence of MCFPE on the accumulation of paclitaxel inside the cancer cells was analysed. The extract at two different concentrations – 0.1% and 0.05% – significantly improved the accumulation of [^{3}H]-paclitaxel, a known chemotherapeutic substrate of P-gp transporter in the multi-drug resistant cell line. Similar effects were evidenced on treatment with the drug verapamil in multi-drug resistant H69/LX4 cells. The accumulation of the drug increased nearly four-folds on incubation with MCFPE as compared to untreated H69/LX4 cells. The restoration of the paclitaxel sensitivity of resistant cells could be suggested as resulting from enhanced intracellular accumulation of paclitaxel. The drug accumulation percentage in the H69 cells indicated that the extract and verapamil had negligible effects on the cells.

The drug efflux analysis at different time intervals (30, 60 and 120 minutes) were calculated as a measure of MCFPE efficacy in inhibition of the [^{3}H]-paclitaxel efflux. The percentage of the drug effluxed was found to be increased with time in the H69/LX4 cell lines. The incubation of the cells with 0.1% MCFPE decreased the efflux two times as compared to the untreated cells. In the drug sensitive H69 SCLC cells, the drug efflux was not much altered on exposure to verapamil or the plant extract, indicating the specificity of the agents toward P-gp activity. Previous studies have reported that increased drug efflux leads to decreased drug accumulation (24, 25), thereby reducing the efficacy of the anticancer drugs.

The [^{3}H]-paclitaxel accumulation and efflux analysis provides an understanding of the potency of the extract on improving [^{3}H]-paclitaxel sensitivity. Similar results were reported by Abraham *et al* (26). Limtrakul *et al* (27) reported that the flavonoids quercetin and kaempferol were able to increase the accumulation of paclitaxel and vinblastine dose-dependently as well as decrease the efflux of the paclitaxel and vinblastine in multi-drug resistant human cervical carcinoma cell lines.

Previous studies (28, 29) have demonstrated that the overexpression of P-gp is a major cause for developing MDR, and reduced expression of P-gp is one of the

modalities in reversing multi-drug resistance. In this study, the expression levels of P-gp were analysed both at the transcriptional and at the protein levels. The mRNA isolated from the SCLC cell lines was amplified by RT-PCR and the result of the analysis, presented in Fig. 4, reveals the expression patterns. The band density was observed to be significantly reduced in the H69/LX4 cells on exposure to MCFPE as compared to the untreated H69/LX4 cells. The band in lane 2 reveals the nearly nil expression of P-gp mRNA in the H69 cells that further supports that *MDR 1* gene overexpression contributes to MDR. The decrease in the intensity of the expression band in lanes 4 and 5 (H69/LX4 cells treated with MCFPE) suggests that the extract harbours the potency to reduce the expression of *MDR 1* gene. The results are in line with the recent studies with the flavonoid quercetin. Quercetin is capable of decreasing P-gp expression in a dose-dependent manner (27).

Western blot analysis showed that the 170 kDa P-gp was expressed in H69/LX4 cells untreated with MCFPE (lane 2). The expression of P-gp in the H69 cells was far below the detectable level (lane 1). Upon exposure to MCFPE, the P-gp expression of the H69/LX4 cells was remarkably reduced as seen in lane 3 (Fig. 5). The observed results further support the capacity of the extract in modulating MDR by targeting the expression and activity of P-gp.

The reduction of *MDR 1* expression probably at both translational and transcriptional levels has been put forth as one of the possible means by which some compounds reverse MDR (3).

Thus, in this study, our results showed that MCFPE distinctly raised the cytotoxicity of paclitaxel in paclitaxel-resistant P-gp overexpression observed in H69/LX4 cells but faintly in paclitaxel-sensitive H69 cells. This indicates that MCFPE may act by reverses MDR by mediating over-expressed P-gp that is further supported by the results of [^{3}H]-paclitaxel accumulation and efflux analysis. Furthermore, as reported by Krishna and Mayer (3), the results of the RT-PCR and western blotting suggest that MCPFE acts at inhibiting the expression of *MDR 1* and thus reverses MDR. Similar results were obtained on treatment of drug-resistant cancer cells by cucurmin (30).

Much effort is being taken recently toward identifying natural compounds from plant origins that inhibit P-gp, reverse the MDR phenotype, and sensitize cancer cells to conventional chemotherapy (31, 32). The present study thus is an attempt to suggest that phytochemicals could suppress MDR. In this study, it could be suggested that the secondary metabolites present in the extracts of *Momordica charantia* were able to potentially modulate MDR.

In conclusion, the extract of the peel and pulp of *Momordica charantia* fruits represent potential reversal agents for the treatment of MDR in P-gp-overexpressing SCLC tumours.

REFERENCES

1. Govindan R, Page N, Morgensztern D. Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. J Clin Oncol 2006; **24:** 4539−44.
2. Pass HI, Carbone DP, Johnson DH, Minna JD, Scagliotti G, Turrisi AT, eds. Principles and Practice of Lung Cancer. Philadelphia: Lippincott Williams Wilkins; 2010.
3. Krishna R, Mayer LD. Multidrug resistance (MDR) in cancer—mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. European J Pharma Sci 2000; **11:** 265–83.
4. Litman T, Druley TE, Stein WD, Bates SE. From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. Cell Mol Life Sci 2001; **58:** 931–59.
5. Anuchapreeda S, Leechanachai P, Smith MM, Ambudkar SV, Limtrakul PN. Modulation of P-glycoprotein expression and function by curcumin in multidrug-resistant human KB cells. Biochem Pharmacol 2002; **64:** 573–82.
6. Larsen AK, Escargueil AE, Skladanowski A. Resistance mechanisms associated with altered intracellular distribution of anticancer agents. Pharmacol Ther 2000; **85:** 217–29.
7. Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Ann Rev Pharmacol Toxicol 1999; **39:** 361−98.
8. Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. Nat Rev Drug Discov 2006; **5:** 219−34.
9. Horio M, Lovelace E, Pastan I, Gottesman MM. Agents which reverse multidrug-resistance are inhibitors of [3H] vinblastine transport by isolated vesicles. Biochim Biophys Acta 1991; **1061:** 106−10.
10. Duraj J, Zazrivcova K, Bodo J, Sulikova M, Sedlak J. Flavonoid quercetin, but not apigenin or luteolin, induced apoptosis in human myeloid leukemia cells and their resistant variants. Neoplasma 2005; **52:** 273–9.
11. Skupien K, Osmianski J, Kostrzewa-Nowak D, Tarasiuk J. In vitro antileukaemic activity of extracts from berry plant leaves against sensitive and multidrug resistant HL60 cells. Cancer Lett 2006; **236:** 282−91.
12. Skupien K, Kostrzewa-Nowak D, Oszmianski J, Tarasiuk J. In vitro antileukaemic activity of extracts from chokeberry (*Aronia melanocarpa* [Michx] Elliott) and mulberry (*Morus alba* L.) leaves against sensitive and multidrug resistant HL60 cells. Phytother Res 2008; **22:** 689−94.
13. Shi H, Hiramatsu M, Komatsu M, Kayama T. Antioxidant property of Fructus Momordicae extract. Biochem Mol Biol Int 1996; **40:** 1111–21.
14. Lee-Huang S, Huang PL, Chen HC, Bourinbaiar A, Huang HI, Kung HF. Anti-HIV and anti-tumor activities of recombinant MAP30 from bitter melon. Gene 1995; **161:** 151–6.
15. Cunnick JE, Sakamoto K, Chapes SK, Fortner GW, Takemoto DJ. Induction of tumor cytotoxic immune cells using a protein from the bitter melon (*Momordica charantia*). Cell Immunol 1990; **126:** 278–89.
16. Limtrakul P, Khantamat O, Pintha K. Inhibition of P-glycoprotein activity and reversal of cancer multidrug resistance by *Momordica charantia* extract. Cancer Chemother Pharmacol 2004; **54:** 525−30.
17. Aoki S, Chen ZS, Higasiyama K, Setiawan A, Akiyama A, Kobayashi M. Reversing effect of agosterol A, a spongean sterol acetate, on multidrug resistance in human carcinoma cells. Jpn J Cancer Res 2001; **92:** 886−95.
18. Shi Z, Tiwari AK, Shukla S, Robey RW, Kim IW, Parmar S et al. Inhibiting the function of ABCB1 and ABCG2 by the EGFR tyrosine kinase inhibitor AG1478. Biochem Pharmacol 2009; **77:** 781−93.
19. O'Driscoll L, Daly C, Saleh M, Clynes M. The use of reverse transcriptase-polymerase chain reaction (RT-PCR) to investigate specific gene expression in multidrug-resistant cells. Cytotechnology 1993; **12:** 289−314.

20. Bosch S, Siavoshian S, Jacquot C, Tomasoni C, Dabouis G, Elanbaloussi Y et al. Correlation between multidrug resistance and the degree of differentiation of non-small-cell bronchopulmonary carcinoma (NSCLC) in vitro and in vivo. Anticancer Res 1997; **17:** 4595−8.
21. Chadderton T, Wilson C, Bewick M, Gluck S. Evaluation of three rapid RNA extraction reagents: relevance for use in RT-PCR's and measurement of low level gene expression in clinical samples. Cell Mol Biol (Noisy-le-grand) 1997; **43:** 1227−34.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; **193:** 265–75.
23. Chu TM, Lin TH, Kawinski E. Detection of soluble P-glycoprotein in culture media and extracellular fluids. Biochem Biophys Res Commun 1994; **203:** 506−12.
24. Ejendal KF, Hrycyna CA. Multidrug resistance and cancer: the role of the human ABC transporter ABCG2. Curr Protein Peptide Sci 2002; **3:** 503−11.
25. Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM. P-glycoprotein: from genomics to mechanism. Oncogene 2003; **22:** 7468–85.
26. Abraham I, Jain S, Wu CP, Khanfar MA, Kuang Y, Dai CL et al. Marine sponge-derived sipholane triterpenoids reverse P-glycoprotein (ABCB1)-mediated multidrug resistance in cancer cells. Biochem Pharmacol 2010; **80:** 1497−506.
27. Limtrakul P, Khantamat O, Pintha K. Inhibition of P-glycoprotein function and expression by kaempferol and quercetin. J Chemotherapy 2005; **17:** 86−95.
28. Sampson KE, Wolf CL, Abraham I. Staurosporine reduces P-glycoprotein expression and modulates multidrug resistance. Cancer Lett 1993; **68:** 7−14.
29. Muller C, Bailly JD, Goubin F, Laredo J, Jaffrezou JP, Bordier C et al. Verapamil decreased P-glycoprotein expression in multidrug-resistant human leukemic cell lines. Int J Cancer 1994; **56:** 749−54.
30. Andjelkovic T, Pesic M, Bankovic J, Tanic N, Markovic ID, Ruzdijic S. Synergistic effects of the purine analog sulfinosine and curcumin on the multidrug resistant human non-small cell lung carcinoma cell line (NCI-H460/R). Cancer Biol Ther 2008; **7:** 1024−32.
31. Chavez ML, Jordan MA, Chavez PI. Evidence-based drug-herbal interactions. Life Sci 2006; **78:** 2146−57.
32. Zhou SF, Zhou ZW, Li CG, Chen X, Yu X, Xue CC et al. Identification of drugs that interact with herbs in drug development. Drug Discov Today 2007; **12:** 664−73.

Submitted 17 Jun 2014
Accepted 30 Jun 2014
Published 24 Oct 2014
Online: http://myspot.mona.uwi.edu/wimjopen/article/1599