JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. **2010,** *58,* 5315–5319 **5315**
DOI:10.1021/jf9037979

Group B Oleanane Triterpenoid Extract Containing Soyasaponins I and III from Soy Flour Induces Apoptosis in Hep-G2 Cells

Wei Zhang and David G. Popovich*

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

Soyasaponins I and III are monodesmodic oleanane triterpenoids that are thought to be one of the main potentially bioactive saponins found in soy (*Glycine max*) and related products. An extract that contained a majority of the soyasaponins as I (62%) and III (29%) was studied in a hepatocarcinoma cell line (Hep-G2) to determine the potential cellular bioactivity. The extract was found to inhibit the growth of Hep-G2 cells measured by the MTT viability assay (LC50 0.389 ± 0.02 mg/mL) dose-dependently. Cell death was determined using three distinct flow cytometry assays for apoptosis (sub-G1 cell-cycle analysis and multi-caspase and TUNEL assays), and cellular morphological images were acquired by confocal laser scanning microscopy. All of the apoptotic detection assays were positive for apoptotic cell accumulation. The TUNEL detection assay after 72 h of treatment showed the greatest apoptotic cell accumulation (40.45 ± 4.95%), followed by the multi-caspase assay for 48 h treated cells (6.97 ± 0.14% mid-apoptotic and 12.87 ± 0.81% late apoptotic) and sub-G1 accumulation (17.67 ± 0.42%). The multi-caspase assay indicated that the cellular protein expression of the caspase family of enzymes was the main apoptotic pathway trigger. Confocal laser scanning microscopy further confirmed the induction of apoptosis in Hep-G2 cells. Soyasaponins I and III may contribute to the reported bioactive and chemopreventative properties of soy by the induction of apoptosis.

KEYWORDS: Soy; soyasaponin; oleanane triterpenoid; apoptosis

INTRODUCTION

Soyasaponins are oleanane triterpenoids that are amphiphilic molecules, with polar, water-soluble sugar moieties attached to a non-polar, water-insoluble pentacyclic ring structure. Soyasaponins are classified into two main groups, A and B, based on the chemical structure of their respective aglycones (*1*). Group B soyasaponins are the most abundant group of saponins found in soy (*2*) and are thought to contribute to the chemopreventative properties of soy (*3*). Soyasaponin I and structurally related compound soyasaponin III are monodesmodic triterpenoids. The soyasaponin I structure has an additional rhamnose moiety compared to soyasaponin III. In plants, saponins are secondary metabolites whose function is likely to thwart or discourage herbivore predators, but similar to many secondary plant metabolites, there has been a resurgent interest in the associated bioactivity of these molecules (*4*).

Much of the bioactive research on soyasaponins has used crude methanolic extracts prepared from soy flour (*5*,*6*). There are only a few reports in the literature relating the bioactivity to specific soyasaponins. This is likely due to the complex and diverse chemical structures rendering purification difficult and laborious (*5*, *6*). Individual soyasaponins, such as soyasaponin I, have been reported to interact with sialic acid on the cellular surface of cultured melanoma (B16F10) cells, potentially reducing the metastatic ability of cancer cells (*7*, *8*). Furthermore, suppression of cultured cancer cell (hepatocarcinoma and intestinal) growth by the soyasaponin aglycones soyasapogenol A and B has been reported (*5*, *9*). We have shown that the bioactivity of soyasaponin extracts depends upon group B soyasaponin composition, which varied by extraction conditions (*10*), and we have recently reviewed both the biological and chemical classification of soyasaponins (*1*).

Soyasaponins are found in low amounts in soy and are usually extracted together with isoflavones because of the overlapping polarity of the compounds, making specific determinates of groups or the bioactivity of individual soyasaponins difficult (*6*). We have previously reported a method to separate group B soyasaponins from group A, by removal of the competing isoflavones and the production of an extract that contained 62% soyasaponin I and 29% soyasaponin III using alkaline hydrolysis (*6*). However, the bioactivity has not been established. The objective of this current study was to determine the bioactivity of a purified soyasaponin extract that contains mainly soyasaponins I and III on hepatocarcinoma (Hep-G2) cell growth, cell-cycle analysis, and apoptosis. Hep-G2 cells were chosen as the model biological system to assess cytotoxicity and allow for a comparison to the recent literature (*5*, *10*).

MATERIALS AND METHODS

The preparation and analysis of an extract containing group B soyasaponins I (62 ± 0.9%) and III (29 ± 2.9%), soyasaponins βg

*To whom correspondence should be addressed. Telephone: (65) 6516-4695. Fax: (65) 6775-7895. E-mail: chmpdg@nus.edu.sg.

Published on Web 03/26/2010

(3.2 ± 0.2%) and Be (2.6 ± 0.5%), and acetyl genistin (< 1%) extracted from defatted soy flour were previously described (*6*). Briefly, 100 g of defatted soy flour was obtained from Archer Daniels Midland Co. (Decatur, IL), extracted in methanol (500 mL) with stirring for 24 h at room temperature, filtered, and vacuum-evaporated. A 10 g C-18 Sep-Pak (Phenomenex, Torrance, CA) solid-phase extraction (SPE) cartridge was conditioned with water, and the soyasaponin sample was applied. The cartridge was washed with water (150 mL) to remove any traces of carbohydrates and washed using 45% methanol (200 mL) to remove any isoflavones (*11*), and group A soyasaponins were removed with a 50% methanol wash. Group B soyasaponins were eluted with methanol (80%). Group B soyasaponins in 80% methanol were diluted to 50%, re-applied to the SPE column, and eluted with 50 mL of absolute methanol. Sodium hydroxide (5%, v/v) was added to group B soyasaponins, and the mixture was hydrolyzed for 30 min at a temperature of 80 °C in sealed tubes. After hydrolysis, the solution was diluted with water until the concentration of methanol was 50% and applied to a Phenomenex 10 g C-18 Sep-Pak cartridge, which was pre-conditioned with deionized water. The cartridge was washed with water (200 mL), and the hydrolyzed solution was eluted with methanol, vacuum-evaporated, and lyophilized.

Cell Culture and MTT Viability. Hep-G2 cells were purchased from the American Type Culture Collection (Manassas, VA). The cells were maintained in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum (Sigma), 100 units of penicillin, and 100 μg/mL streptomycin (Gibco, Invitrogen, Burlington, Canada) in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were maintained at a concentration between 2×10^5 and 1×10^6 cells/mL. Cells were subcultured every 2−3 days by total media replacement using 0.25% (w/v) trypsin and 0.53 mM ethylenediaminetetraacetic acid (EDTA) solution (GIBCO). Viable cells were assessed by 0.04% trypan blue exclusion dye (MP Biomedicals, Solon, OH) using a hemocytometer and assessed in quadruplicate.

Hep-G2 cells were seeded in 96-well plates to a final concentration of 1×10^4 cells/mL. Controls contained test model cells, culture media, but no test compounds. Soyasaponin I and III extract was prepared with dimethylsulfoxide (DMSO) and culture media to achieve concentrations between 0.1 and 1.0 mg/mL. The DMSO concentration was less than 0.5%, with equal amounts added to the control samples. Cells were incubated for 3 days, to allow for a comparison to previous publications (*5*, *10*), before MTT was added at a concentration of 0.5 mg/mL, and measured as previously described (*5*).

Soyasaponin I and III extract stability during the experimental conditions was investigated and quantified by high-performance liquid chromatography (HPLC) before cell culture, after 72 h incubation with cells, and with culture media alone. Cells were seeded in 24-well plates at a cell concentration of 5×10^4 cells/mL and allowed to attach overnight. Soyasaponin I and III extract was added to media and media plus Hep-G2 cells and incubated for 72 h. Media was collected after incubation, and cells were removed by trypsinization using 0.25% (w/v) trypsin and 0.53 mM EDTA solution (GIBCO). The cells were pelleted by centrifugation (150*g*, 10 min), and the supernatant was combined with media, syringe-filtered (0.2 μm), and quantified by HPLC as previously reported (*6*).

Cell-Cycle Analysis. Soyasaponin I and III extract was added to Hep-G2 cells (1×10^6 cells/mL) in 24-well plates at the LC50 concentration (0.389 ± 0.02 mg/mL described below). Cells were incubated at 37 °C in a 5% CO_2 humidified incubator for 24, 48, and 72 h to highlight time course changes in the cell cycle, with the untreated cells acting as a control. After treatment, cells in suspension were centrifuged for 10 min (150*g*) to collect detached cells and the supernatant was discarded. Adherent cells were trypsinized using 0.25% (w/v) trypsin and 0.53 mM EDTA solution (GIBCO), combined with detached cells, and collected by centrifugation (150*g*, 10 min). Cells were washed twice in phosphate-buffered saline (PBS), and PBS was removed by centrifugation. The pellet was fixed in 1 mL of ice-cold 70% ethanol and stored overnight at 4 °C. Ethanol was removed by centrifugation (750*g*, 5 min) and gently vortexed. Guava cell-cycle reagent was added, and cells were incubated at room temperature in the dark for 30 min and analyzed by the Guava PCA system with Cytosoft software (Guava Technologies, Inc., Hayward, CA) as previously described (*6*).

TUNEL Apoptosis Assay. Hep-G2 cells were seeded in 24-well plates at a concentration of 1×10^5 cells/mL for the deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Soyasaponin I and III extract was added at the LC50 concentration for 72 h to allow for quantification and visualization of late apoptotic events; untreated cells acted as a control. After treatment, culture media was removed and placed in 15 mL conical screw cap tubes. Cells were washed with PBS, detached with trypsin (described above), and combined with the media in the conical tubes. The mixture was centrifuged (300*g*, 7 min) to remove the supernatant, and the cells were fixed in methanol (70%) for 12 h. Methanol was removed by centrifugation (300*g*, 7 min), and cells were washed 3 times with 1 mL of the wash buffer (Guava Technologies, Inc.) according to the protocol of the manufacturer. Cells were stained with the rinsing buffer and suspended in 50 μL of the anti-BrdU staining mix (Guava Technologies, Inc.) for 30 min at room temperature in the dark. At the end of incubation, 150 μL of rinsing buffer was added to each tube and analyzed using a Guava PCA system flow cytometer.

Caspase Apoptotic Assay. The extract was added at the LC50 concentration for 72 h to 1×10^6 cells/mL in 24-well plates. Cells were treated for 48 h to allow for the determination of early to mid-apoptotic events. Cell media was removed and centrifuged (350*g*, 5 min), and cells were detached by typsinization, washed with PBS twice, and combined with the collected media. The cell concentration was adjusted to 5×10^5 cells/mL using 1× apoptotic wash buffer (Guava Technology, Inc.). Sulforhodamine-valyl-alanyl-fluoromethyl-ketone (20× SR-VAD-FMK) reagent was added according to the instructions of the manufacturer and incubated for 1 h at 37 °C in the dark. The cells were washed twice in washing buffer and resuspended in 1× apoptotic wash buffer (100 μL), and 5 μL of caspase 7-amino-actinomycin D (7-AAD) regent (Guava Technologies, Inc.) was added and incubated for 10 min at room temperature. After incubation, the sample volume was adjusted to 200 μL with 1× apoptosis wash buffer and analyzed on a Guava PCA flow cytometer.

Cell Morphology. Hep-G2 cells were seeded in a 4-well Lab-Tek chamber slide system (Electron Microscopy Science, Hatfield, PA) with 1×10^4 cells/mL, with untreated cells as controls. The extract was added at the LC50 concentration for 72 h. After treatment, culture media was removed and cells were washed with PBS. Cells were fixed with 4% paraformaldehyde in 0.1 M NaH_2PO_4 for 15 min and washed in PBS 3 times for 5 min. Cells were incubated with termination buffer [TB, 0.5% Tween-20 and 0.2% bovine serum albumin (BSA) in PBS] for 15 min at room temperature. After incubation, cells were washed with PBS for 2 min and incubated with 50 μL of terminal deoxynucleotidyl transferase (TdT) end-labeling cocktail (Guava Technologies, Inc.) for 60 min at room temperature. TdT end-labeling cocktail was removed, and the reaction was stopped by immersing the cells in TB buffer (1×) for 5 min at room temperature then washed with PBS 3 times for 2 min. A blocking buffer (50 μL) was added to the cells and incubated at room temperature for 20 min. The blocking buffer was removed by gentle tapping, and 50 μL of avidin-FITC solution (Guava Technologies, Inc.) was added to the sample cells and incubated in the dark for 30 min at room temperature. The cells were washed with PBS 3 times for 5 min in the dark, and propidium iodide (PI) was added as counter stain cells for 15 min. A coverslip was mounted, and the cell image was acquired by Olympus Fluoview FV500 confocal microscopy (Center Valley, PA).

Statistical Methods. A one-way analysis of variation (ANOVA) (SPSS 12.0) was used to analyze the experimental data. Significance was judged at $p < 0.05$ using the Tukey post-hoc multiple comparisons of observed means and a Student's paired *t* test, with significance also judged at $p < 0.05$. The MTT, extract stability, caspase, and TUNEL assays were preformed with three replicates in three separate experiments. TUNEL confocal image acquisition consisted of two replicates with two separate experiments.

RESULTS AND DISCUSSION

An oleanane triterpene soyasaponin extract derived from soy flour containing primarily group B soyasaponins I and III reduced the growth of Hep-G2 cells in a dose-dependent manner measured by the MTT viability assay. The MTT cell viability dose−response relationship of the soyasaponin I and III extract effect on Hep-G2 cell growth is shown in **Figure 1**. The LC50 was

calculated from a plot of viability (%) versus the log concentration (graph not shown), which yielded a linear equation of $y = -229.8x + 645.31$ ($R^2 = 0.9781$). The LC50 was determined from three separate experiments with three replicates per experiment and was found to be 0.389 ± 0.02 mg/mL. The stability of soyasaponin I and III extract was tested in culture conditions with and without Hep-G2 cells. The concentration of both soyasaponin I and III after 72 h of incubation with cells was not significantly different to the initial concentration or the control (same conditions without cells). Soyasaponin I incubated without cells was 98.6% of the concentration with cells. The concentration of soyasaponin III incubated without cells was lower compared to the incubation with cells (91.5%), but it was not found to be significantly different after 72 h of incubation. The soyasaponins used in this study do not appear to be metabolized to any significant extent during the course of testing.

Saponins from a variety of plants have been reported to reduce the growth of cancer cells. In general, saponins seem to have a characteristic effect on cultured cell growth that is dependent upon structural characteristics (*5*, *9*, *12*, *13*). Monodesmodic saponins, having one attachment site of a sugar glycoside, have been reported to be more biologically active compared to the bidesmodic saponins, possessing two sugar attachment sites (*4*). Bidesmodic saponins are likely storage form of saponins in plants and are deglycosolated when needed to increase biological activity (*4*) for plant defense. Specifically, soyasaponin bioactivity has largely been studied using crude extracts or semi-purified fractions. Little is known about the bioactivities of specific compounds or structurally related groups of compounds, such as the monodesmodic soyasaponins I and III. Representative DNA cell-cycle histograms of untreated control and soyasaponin I and III extract-treated Hep-G2 cells for 24, 48, and 72 h are shown in **Figure 2**, and the corresponding cell-cycle data of each phase of the cell cycle is shown in **Table 1**. Soyasaponin I and III extract produced a significantly ($p < 0.05$) greater percentage of cells in the G1 phase compared to untreated control cells after 72 h of treatment, which significantly ($p < 0.05$) decreased in S-phase cells after 24 h and cells in G2/M phase after 48 and 72 h of treatment. Sub-G1 apoptotic cells were significantly ($p < 0.05$) increased after 24 h (5.50 ± 0.56%), 48 h (17.67 ± 0.42%), and 72 h (8.90 ± 0.50%) compared to control cells after 24 h (0.43 ± 0.21%), 48 h (0.73 ± 0.12%), and 72 h (0.80 ± 0.17%), respectively. The maximum sub-G1 accumulation was observed at 48 h of treatment. **Figure 3** shows the different stages of apoptosis in Hep-G2 cells treated with soyasaponin I and III extract measured using dual fluorescent-labeled cells assessed flow cytometry analysis. This assay used SR-VAD-FMK inhibitor, which is a fluorochrome-conjugated inhibitor of caspases and can covalently bind to multiple caspases that have been activated during the apoptosis process (*14*). In this assay, cells with positive SR-VAD-FMK staining correspond to cells committed to apoptosis. 7-AAD is an intercalating fluorescent dye, which is excluded from live cells and early to mid-stage apoptotic cells but permeates in later stage apoptotic and dead cells. Four populations of cells can be distinguished in this assay; they include live and non-apoptotic committed cells SR-VAD-FMK (−) and

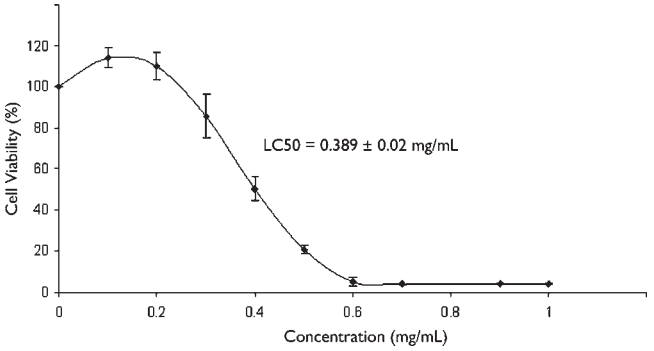

Figure 1. MTT dose−response relationship viability curve of soyasaponin I and III extract after a 72 h treatment with Hep-G2 cells. Values are expressed as mean ± standard deviation (SD) of three replicates in three separate experiments and expressed as a percentage of the untreated cell control.

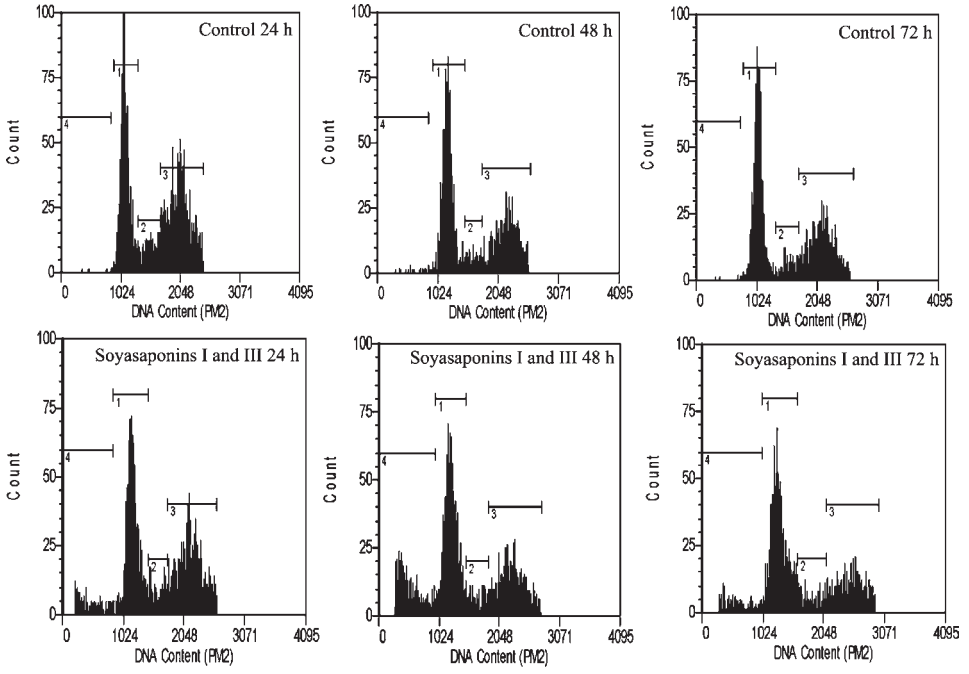

Figure 2. Representative cell-cycle histograms and corresponding table of analysis is shown in **Table 1** of three replicates repeated in three separate experiments tested at the LC50 concentration. Regions labeled 1−3 correspond to G0/G1, S, and G2/M phases of the cell cycle, respectively, and the region labeled 4 corresponds to the sub-G1 accumulation.

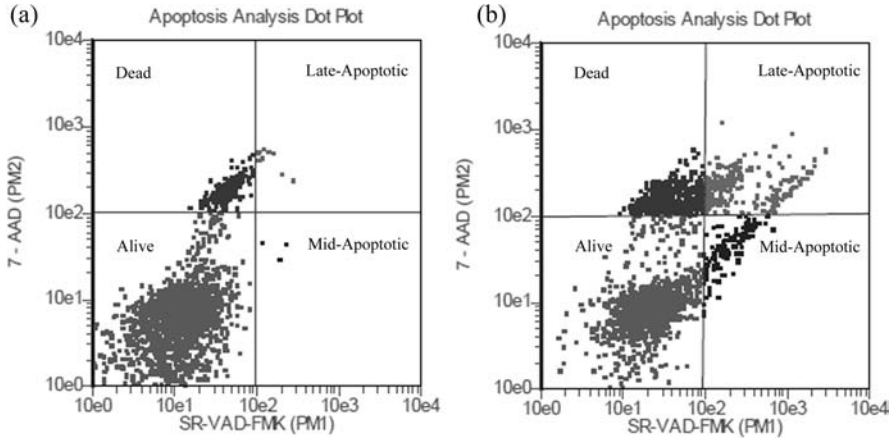

Figure 3. Representative flow cytometer analysis of caspase activity after soyasaponin I and III extract treatment. Hep-G2 cells were treated by soyasaponin I and III extract at the LC50 concentration determined by the MTT analysis for 48 h (panel b). Untreated cells acted as controls (panel a). Data are expressed as mean ± SD. Quadrant analysis of control cells resulted in 88.17 ± 2.93% (control) alive compared to 57.00 ± 1.45%* (soyasaponins I and III); dead cells were 11.35 ± 3.17% (control) and 23.17 ± 0.68%* (soyasaponins I and III); mid-apoptotic cellls were 0.08 ± 0.08% (control) and 6.97 ± 0.14%* (soyasaponins I and III); and late apoptosis cells were 0.40 ± 0.18 (control) and 12.87 ± 0.81%* (soyasaponins I and III). SR-VAD-FMK is labeled on the *x* axis, and 7-AAD is labeled on the *y* axis. Three replicates in three separate experiments were preformed. An asterisk represents a significant difference ($p < 0.05$) compared to the corresponding control value.

Table 1. Hep-G2 Cell-Cycle Distribution[a]

time (h)	control (%)	soyasaponins I and III (%)
	Sub-G1	
24	0.43 ± 0.21	5.50 ± 0.56*
48	0.73 ± 0.12	17.67 ± 0.42*
72	0.80 ± 0.17	8.90 ± 0.50*
	G0/G1	
24	33.83 ± 2.04	39.73 ± 0.67
48	41.30 ± 0.61	40.77 ± 1.07
72	46.90 ± 0.78	51.57 ± 1.01*
	S	
24	8.20 ± 0.36	6.77 ± 0.15*
48	9.30 ± 1.05	7.60 ± 0.7
72	10.37 ± 0.76	10.07 ± 0.57
	G2/M	
24	40.90 ± 1.10	38.53 ± 1.20
48	48.70 ± 0.52	30.83 ± 0.87*
72	41.87 ± 1.31	29.03 ± 0.12*

[a] Values are expressed as mean ± SD. Values in the same period with an asterisk are significantly different ($p < 0.05$) from corresponding control values.

7-AAD (−), early to mid-stage apoptotic cells SR-VAD-FMK (+) and 7-AAD (−), late stage apoptotic and dying cells SR-VAD-FMK (+) and 7-AAD (+), and dead cells SR-VAD-FMK (−) and 7-AAD (+). After 48 h of treatment, soyasaponin I and III extract-treated cells showed a significant ($p < 0.05$) reduction in the percentage of viable cells, which corresponded to a reduction of 31.2% compared to control cells. Cells undergoing mid-apoptotic events showed a significant ($p < 0.05$) increase for treated cells compared to a control (6.89%) and a significant ($p < 0.05$) increase (12.47%) for cells in late apoptotic stage. Dead cells were also significantly ($p < 0.05$) increased compared to control cells (11.82%). **Figure 4** shows the results of the TUNEL assay assessed using flow cytometry. The analysis showed a large significant ($p < 0.05$) increase in apoptotic cells after soyasaponin I and III extract treatment and a corresponding decrease in non-apoptotic cells compared to untreated control cells. This increase

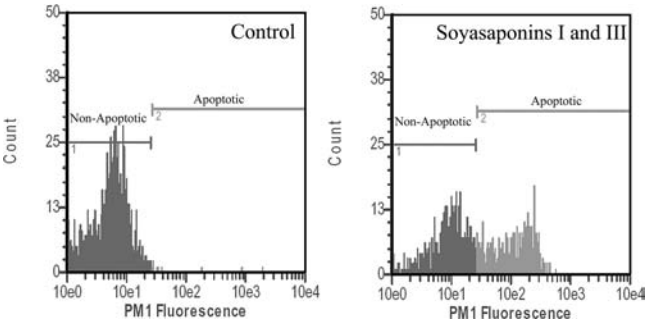

Figure 4. TUNEL apoptotic analyses of soyasaponin I and III extract-treated Hep-G2 cells for 72 h at the LC50 concentration. Data are expressed as mean ± SD of three replicates with three separate experiments. Analysis of control cells resulted in 98.7 ± 0.3% found to be non-apoptotic, and 1.3 ± 0.3% were apoptotic. Soyasaponin I and III extract-treated cells resulted in 59.5 ± 4.9%* found to be non-apoptotic, and 40.5 ± 4.9%* were apoptotic. An asterisk represents a significant difference ($p < 0.05$) compared to the corresponding control value.

in apoptotic cells was confirmed by confocal laser scanning microscopy TUNEL images of the treated cells (**Figure 5**). Cell fragments that are stained green (FITC) are positive for apoptosis events, which are not visible for the untreated control cells.

The aglycones of the two main groups of soyasaponins, groups A and B, have been reported to induce apoptosis in cultured Hep-G2 cells (*5*), and fractioned soyasaponin extract induced apoptosis in Hela cells (*15*). Concentrated group B soyasaponin extracts prepared by reflux containing mainly soyasaponins I and III and the group B aglycone reportedly increased apoptotic cells, while an extract containing an abundance of 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)-conjugated soyasaponin βg induced cellular differentiation in Hep-G2 cells (*10*). A group B soyasaponin extract was reported to induce programmed micro-autophagic cell death in colon cancer cells (HCT-15) (*16*).

The results contained herein suggest that group B soyasaponin extract that contained a majority of structurally related soyasaponins I and III induced apoptosis in cultured Hep-G2 cells. This is evident by the use of three distinct assays used to measure apoptosis. The accumulation of sub-G1 cells measured during

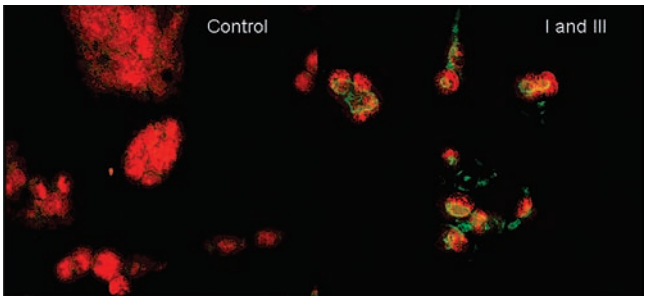

Figure 5. TUNEL apoptosis confocal laser scanning representative image of Hep-G2 cells treated with soyasaponin I and III extract at the LC50 concentration for 72 h. Cells were stained by both PI (red) and FITC (green). Control cells (left panel) are untreated cells and show no evidence of apoptosis compared to soyasaponin I and III extract-treated cells (right panel) that show evidence of apoptotic fragments (green, FITC stain). The assay was preformed with two replicates repeated twice in separate experiments.

cell-cycle analysis suggest apoptotic cell death, while the caspase and TUNEL measurement confirm apoptotic cell death. Using the TUNEL assay, which is specific for apoptotic fragments, showed that nearly 41% of the cells were positive for apoptosis after 72 h of treatment at the LC50 concentration. This indicates that a vast majority of treated cells was apoptotic. The dual-labeled multi-caspase assay was positive for apoptosis, which indicates that the caspase serine proteases were expressed after treatment and treatment triggered this cell death pathway leading to apoptosis. More studies are needed to determine the specific caspase that was activated. Soyasaponin I has been reported to interact with sialic acid on the surface-cultured melanoma cells and breast cancer cells, possibly reducing the metastatic potential of cancer cells (*7*, *8*). Monodesmodic saponins in generally may interact with membrane function (*12*, *13*), possibly through an association with cholesterol (*4*). Saponins from ginseng have been shown to exert a cytotoxic effect on Ehrlich ascite carcinoma cells, which was reported to be inversely proportional to the membrane cholesterol content (*17*). Cytotoxicity decreased as cultured cells were supplemented with additional cholesterol (*17*). Specific mechanistic studies are needed to definitely assess these effects; however, the final outcome for cultured cancer cells, shown in this study, is apoptotic cell death. This is an important finding because it lends credence to the multifaceted nature of soyasaponins as potential chemopreventative agents.

LITERATURE CITED

(1) Zhang, W.; Popovich, D. G. Chemical and biological characterization of oleanane triterpenoids from soy. *Molecules* **2009**, *14*, 2959–2975.

(2) Shiraiwa, M.; Harada, K.; Okubo, K. Composition and structure of group-B saponin in soybean seed. *Agric. Biol. Chem.* **1991**, *55* (4), 911–917.

(3) Rao, A. V.; Sung, M. K. Saponins as anticarcinogens. *J. Nutr.* **1995**, *125* (supplement 3), 717S–724S.

(4) Wink, M. Evolutionary advantage and molecular modes of action of multi-component mixtures used in phytomedicine. *Curr. Drug Metab.* **2008**, *9* (10), 996–1009.

(5) Zhang, W.; Popovich, D. G. Effect of soyasapogenol A and soyasapogenol B concentrated extracts on Hep-G2 cell proliferation and apoptosis. *J. Agric. Food Chem.* **2008**, *56* (8), 2603–2608.

(6) Zhang, W.; Teng, S. P.; Popovich, D. G. Generation of group B soyasaponins I and III by hydrolysis. *J. Agric. Food Chem.* **2009**, *57* (9), 3620–3625.

(7) Chang, W. W.; Yu, C. Y.; Lin, T. W.; Wang, P. H.; Tsai, Y. C. Soyasaponin I decreases the expression of α 2,3-linked sialic acid on the cell surface and suppresses the metastatic potential of B16F10 melanoma cells. *Biochem. Biophys. Res. Commun.* **2006**, *341* (2), 614–619.

(8) Hsu, C. C.; Lin, T. W.; Chang, W. W.; Wu, C. Y.; Lo, W. H.; Wang, P. H.; Tsai, Y. C. Soyasaponin-I-modified invasive behavior of cancer by changing cell surface sialic acids. *Gynecol. Oncol.* **2005**, *96* (2), 415–422.

(9) Gurfinkel, D. M.; Rao, A. V. Soyasaponins: The relationship between chemical structure and colon anticarcinogenic activity. *Nutr. Cancer* **2003**, *47* (1), 24–33.

(10) Zhang, W.; Yeo, M. C.; Tang, F. Y.; Popovich, D. G. Bioactive responses of Hep-G2 cells to soyasaponin extracts differs with respect to extraction conditions. *Food Chem. Toxicol.* **2009**, *47* (9), 2202–2208.

(11) Gurfinkel, D. M.; Reynolds, W. F.; Rao, A. V. The isolation of soyasaponins by fractional precipitation, solid phase extraction, and low pressure liquid chromatography. *Int. J. Food Sci. Nutr.* **2005**, *56* (7), 501–519.

(12) Popovich, D. G.; Kitts, D. D. Structure−function relationship exists for ginsenosides in reducing cell proliferation and inducing apoptosis in the human leukemia (THP-1) cell line. *Arch. Biochem. Biophys.* **2002**, *406* (1), 1–8.

(13) Popovich, D. G.; Kitts, D. D. Ginsenosides 20(*S*)-protopanaxadiol and Rh2 reduce cell proliferation and increase sub-G1 cells in two cultured intestinal cell lines, Int-407 and Caco-2. *Can. J. Physiol. Pharmacol.* **2004**, *82* (3), 183–190.

(14) Ekert, P. G.; Silke, J.; Vaux, D. L. Caspase inhibitors. *Cell Death Differ.* **1999**, *6* (11), 1081–1086.

(15) Xiao, J. X.; Huang, G. Q.; Zhang, S. H. Soyasaponins inhibit the proliferation of Hela cells by inducing apoptosis. *Exp. Toxicol. Pathol.* **2007**, *59* (1), 35–42.

(16) Ellington, A. A.; Berhow, M.; Singletary, K. W. Induction of macroautophagy in human colon cancer cells by soybean B-group triterpenoid saponins. *Carcinogenesis* **2005**, *26* (1), 159–167.

(17) Popov, A. M. A comparative study of the hemolytic and cytotoxic activities of triterpenoids isolated from ginseng and sea cucumbers. *Biol. Bull.* **2002**, *29* (2), 120–128.

Received for review October 29, 2009. Revised manuscript received March 12, 2010. Accepted March 13, 2010. The authors acknowledge the National University of Singapore (NUS) and the Singapore Ministry of Education (Grants R-143-050-287-133/101) for financial support and graduate scholarship (to W.Z.).