1. Introduction

*B*rhizoma Smilacis Glabrae* (RSG) is a commonly used Chinese herbal medicine for detoxification and diuretic. *Rhizoma Smilacis Chinense* (RSC) is a herb with similar effects of RSG that was also embodied in Chinese Pharmacopoeia 2010 edition. These two plants are all belong to *Smilacaceae* family, *smilax* genera. The anti-cancer effect of RSG extract have also been confirmed in some vitro and vivo studies [1-4]. Previously, we found that the chemical constitutes of RSG and RSC were quite similar as detected by HPLC-DAD-MS/MS, and dihydroflavonol glycosides including astilbin and its enantiomers were the main bioactive constitutes [5,6]. In this present study, the anti-proliferative and pro-apoptotic effect of RSG and RSC extracts on human cancer line HepG2 were reported.

2. Experimental

HepG2 was cultivated in DMEM-F12 medium supplemented with 4% (v/v) FBS, 1.2 g/l sodium bicarbonate powder, 100 μg/ml streptomycin and 100 unit/ml penicillin. The Cell viability was assessed by MTT assay. The proportion of cells at different phases of the cell cycle was monitored by flow cytometer after staining with propidium iodide.

3. Results and Discussion

3.1 Cytotoxicity assay

\[\text{Concentration (mg/ml)} \times \text{cell death (percent)}\]

As shown in Fig. 1 and 2. Methanol extract (ME) and its ethyl acetate fraction (EF) of RSG, as well as astilbin all showed cytotoxicity to HepG2. The cytotoxicity of astilbin was weaker than that of EF, but stronger than ME. Water extract (WE) of RSG didn’t show any effect at all. Although constitutes in RSC were quite similar with RSG, the cytotoxicity of its extracts to HepG2 cells was about twenty times stronger than that of RSG.

3.2 Acridine orange/ethidium bromide (AO/EB) staining

\[\text{Concentration (μg/ml)} \times \text{cell death (percent)}\]

From Fig. 3, control cells exhibited higher density of growth and showed uniformly green after staining. After treatment with 0.4 mg/ml of RSG EF for 24h, cells with different color and morphology can be found. Chromatin condensation and nuclear fragmentation, the morphological hallmarks of an apoptotic cell, can be found in the picture after treatment.

3.3 Cell cycle analysis

\[\text{Concentration (mg/ml)} \times \text{cell death (percent)}\]

From Fig. 4, RSG EF treatment resulted in a decrease of cells percentage in the G1 phase and an increase in sub-G1 phase with a concentration-dependent manner. However, G2/M phase has no significant change. Distribution of the G1 phase cells increased from 62.3% to 45.2% while the percent of apoptotic cells was from 2.7% to 16.3% when RSG EF concentration rise from 0 to 1mg/ml.

![Fig. 4](image-url)  (A) Cell cycle phase distribution of HepG2 cells assayed by flow cytometry after treatment by different concentrations of RSG EF for 24 h.  (B) sub-G1 phase, G1 and G2/M phase percentage of HepG2 cells after treatment.

Treatment time also have significant effect. In Fig. 5, percent of apoptotic cells steadily increase from 2.78% to 16.2% with treatment time from 2 h to 24 h at 1mg/ml RSG EF. However, similar to concentration-dependent manner, no G2/M phase arrest was found.

![Fig. 5](image-url)  Phase distribution of HepG2 cells after treatment by 1 mg/ml of RSG EF for different time.

In contrast to RSG, RSC ME treatment resulted in a concentration and time-dependent arrest of cells at G2/M phase with concomitant losses from G1 phase. The percent of apoptotic cells was rose from 2.7% to 10.02% and 40.25% when treated with 25 μg/ml and 50 μg/ml of RSC ME for 24 h, respectively (Fig. 6). In Fig. 7, distribution of the G2/M phase cells in the population increased from 14.9% to 44.5%, and then it slowly decreased, while the percent of apoptotic cells was steady increased from 1.9% to 42.3%. These findings indicate that the effect of RSC on HepG2 cells could be primarily attributed to the induction of G2/M arrest and then cause the apoptosis, indicative of disturbance in cell division rather than DNA synthesis.

4. Conclusion

Both RSG and RSC extracts show anti-proliferative and pro-apoptotic effect to HepG2 cell. The cytotoxicity of its extracts to HepG2 cells is about twenty times stronger than that of RSG. AO/EB staining reveals that treating with RSG extract could cause the morphological change of HepG2 cells, with chromatin condensation and nuclear fragmentation. Cycle analysis shows that treating with ME of RSC will cause G2/M arrest and then apoptosis of HepG2 cells. However, the pro-apoptotic effect of RSG is concentration- and time-dependent and no phase arrest involved.

References