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Effect of the extracts from *Glycyrrhiza uralensis* Fisch on the growth characteristics of human cell lines: Anti-tumor and immune activation activities

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Abstract

Immune modulating activity of ethanol extracts from *Glycyrrhiza uralensis* Fisch was investigated by conserving growth characteristics of several human cell lines. All of the samples did not show severe cytotoxicity on normal human liver cell line, WRL-68, showing less than 25% inhibition of cell growth. The crude extract and its fractionized samples (F1 and F3) inhibited the growth of human hepatoma, Hep3B, down to ca. 70% of normal cell growth in adding 1.0 g l^{-1} of fraction F3. The result of anticancer experiments was well matched to the results of antimutagenicity using Chinese Hamster Lung cell lines (CHL V79). In adding 1.0 g l^{-1} of fraction F1, the growth of human B cell was enhanced, up to 60% of control growth. The secretion of two kinds of cytokines, Interleukin-6 and Tumor Necrosis Factor-α from human B cells was also enhanced in adding the crude extract, but not the standards such as Glycyrrhizin (GL) or 18,β-glycyrrhetinic acid (GM). It was found that both of the apoptosis and differentiation were more accelerated in supplementing the crude extract and fraction F1 than in adding the standards. A spot was found only in the crude extract and fractions, not standards by Thin Layer Chromatography (TLC) analysis. It tells that there must be another unknown component in crude and/or fraction F1 as a possible candidate of immune modulators. This component seems to be a derivative of a monomer, GM since its R_f was close to the monomer. It was also interesting that glycyrrhizin, a major component in *G. uralensis* Fisch was biologically activated by first being hydrolyzed by an enzyme.

Abbreviations: GL, Glycyrrizin; GM, 18,β-glycyrrhetinic acid; SRB, Sulforhodamine B; 6-TG, 6-thioguanine; 4-NQO, nitro-quinolin 1-oxide; E, crude ethanol extracts; F1, diethyl ether fraction; F2, chloroform fraction; F3, water fraction.

Introduction

Licorice, the dried root of *Glycyrrhiza uralensis* Fisch has been supplemented in many oriental medicines as a sweetener (1000 times higher than sucrose) and/or flavor enhancer for long period of time (Hongshan Yu et al., 1993). The most efficacious composition of the licorice is known to be a triterpenoids glycyrrhizin (6–14% in the licorice) (Bianxiezu and Huibian, 1992). Glycyrrhizin, extracted from licorice root, consists of a molecule of glycyrrhetic acid and two molecules of glucuronic acid (Ohtsuki et al., 1992). Recently the studies on biological activities of *G. uralensis* Fisch and/or glycyrrhizin have been focused as therapeutic

agents such as chronic active liver diseases, anti-inflammation, anti-ulcer (Doll et al., 1962) anti-viral (Pompei et al., 1979; Dargan et al., 1986; Hiravayashi et al., 1991) and interferon-inducing activity (Abe et al., 1982). However, most of the reports were carried out in utilizing *G. uralensis* Fisch or glycyrrhizin itself, no other possibly active components. There must be other promising components in *G. uralensis* Fisch to effectively treat cancer or cancer related diseases since plants have many complexes and most of them have not been thoroughly investigated so far. *G. uralensis* Fisch may have useful components other than glycyrrhizin. Therefore, in this work, cell growth and immune modulating activities of the extracts and consecutively partitioned fractions from *G. uralensis* Fisch are investigated. It will provide a chance of expanding the use of *G. uralensis* Fisch.

Material and methods

Sample preparation

Sun-dried roots of *G. uralensis* were extracted two times by ethanol at 80 °C for 12 h. The extracts were concentrated by a vacuum evaporator and freeze dried, and then the extract was fractionized by diethyl ether (F1), chloroform (F2) and water (F3) consecutively. The fractions were freeze-dried again and stored at –20 °C before use. Two standards, glycyrrhizin and 18,β-glycyrrhetinic acid were purchased from Sigma (USA). Glycyrrhizin was hydrolyzed by β-glucuronidase (Sigma, USA) at 37 °C for 6 h. The hydrolyzed solution was also freeze-dried. The prepared samples were denoted in all experiments as follows: Crude ethanol extract (E), its partitioned fractions (F1, F2 and F3), glycyrrhizin (GL) and its monomer, 18,β-glycyrrhetinic acid (GM).

Measurement of cell growth, cytokine secretion and apoptosis

Human normal liver cell (WRL-68, ATCC, USA), human B cell (Raji, ATCC, USA), human hepatoma carcinoma (Hep3B, ATCC, U.S.A.), human breast adenocarcinoma (MCF-7, ATCC, USA) and human stomach adenocarcinoma (AGS, ATCC, USA) were grown in DMEM/F12 basal medium with 10% fetal bovine serum (GIBCO, USA) in a CO_2 incubator at 37 °C. Cytotoxicity of human cell lines, which represents the inhibition ratio of the samples on the cell lines, was estimated by measuring viable and total cell densities using the trypan dye exclusion method with a haemacytometer (Freshney, 1987). Dead cell density was estimated by subtracting viable cell density from the total cell density. The percentage of inhibiting the cell growth was also calculated by the ratio of the maximum cell density without treating the sample to the maximum cell density with adding certain concentration of the sample at same culture interval (Doyle et al., 1993). The selectivity was estimated by dividing the inhibition ratio for the cancer cell by the inhibition ratio for normal cell, WRL 68 adding same concentration of the samples into a well plate. It represents that the samples can selectively inhibit the growth of cancer cells, not normal cells. Larger selectivity means better result. The cell line used for the basis of calculating selectivity was normal human lung cell, WRL 68.

Concentrations of Interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) secreted from human B cell were measured by an ELISA kit (Genzyme, USA). In detail, the cells were grown in DMEM medium with 10% FBS in a well plate for two days, and the sample was added in the range of 0.2, 0.4, 0.6, 0.8 and 1.0 g l^{-1}, respectively and then incubated for three more days. Culture medium was collected by a centrifuge at 1000 rpm, then measured O.D. at 450 nm by adding the substrates in the kit at 37 °C (Choi et al., 1998). The acidification kinetics of the cells responding to samples was observed by a microphysiometer (Molecular Device, New Brunswick, USA). In tests for chemotherapeutic efficacy and toxicity, acidification rate has been used as the cell activity and viability indicator (Wada et al., 1992). The microphysiometer is designed to rapidly monitor the cell growth and biological response by measuring pH changes within the cells, compared to the control which was not treated with samples as follows (Masanori et al., 1981; Longchuan et al., 1999): 3×10^5 cells ml^{-1} of WRL68 cells were put into a capsule cup in the microphysiometer and incubated for 24 h at 37 °C. Running buffer (PBS buffer) and culture medium (DMEM/F12 with 10% FBS) were continuously flown into the capsule. Then pH was measured and pH changes in the capsule according to time (Longchuan et al., 1999).

Apoptosis was observed by a fluorescent dye method as follows: 100 μl of cultured A549 cells was mixed with 4 μl of a dye solution which contains acridine orange and ethidium bromide (1:1 v/v) according to cultivation time. The size and numbers of the stained cells were counted through a fluorescent microscope and the pictures taken under a microscope

were also compared to those of normal cells. The ratio of apoptotic cells to total cells was estimated by counting the stained cells (Freshney, 1983). The soluble tetrazolium was reduced to formazan by generated superoxide ions from HL-60 during differentiation (Yen and Guernsey, 1986). Then, the formazan in HL-60 was measured by NBT (Nitroblue Tetrazolium) method as an indicator of differentiating the cells. The detail procedure was as follows (Whyte and Eisenman, 1992): 5×10^4 viable cells ml^{-1} with the sample was cultivated for two days. After that, 0.2 ml of cultured cells was collected from a flask every day and mixed with 1% nitro-blue tetrazolium chloride (NBT) and 1 μg ml^{-1} 12-O-Tetradecanoylphorbol 13-Acetate (TPA) for 30 min at 37 °C CO_2 incubator. The stained cells were observed by a fluorescent microscope. The differentiation ratio was calculated by the ratio of blue formazan formed cells grown in adding the samples to stained cells without adding the samples (control).

Measurement of antimutagenicity and Thin Layer Chromatography (TLC) analysis

To test antimutagenicity of the samples, Chinese Hamster Lung (CHL V79) cells were used as follows (Kuroda, 1996): Chinese Hamster Lung V-79 cells (generously donated by Dr. Kurodo) were grown in GHAT medium with 10% FBS to remove 6-thioguanine (6-TG) resistant cells. The cells were re-suspended in the medium, containing various concentrations of 4-nitro-quinolin 1-oxide (4-NQO) with or without the samples. The survival ratio was estimated by measuring the total cell density after four days cultivation for each case. The results are also used to explain the relationship between anticancer activity and antimutagenicity.

TLC analysis was carried out by the following method (Hongshan Yu et al., 1993): The plate used was pre-coated Silica gel plate (Kieselgel 60 F-254, 0.25 mm, Merk). Solvent system was butanol, acetic acid and water (6:1:1, v/v), which was spreading in the plate at room temperature. Spots were visualized by spraying with 10% sulfuric acid and heating at 110 °C for 10 min.

All of the experiments were carried out with at least five duplications. The data points in Tables and Figures are the mean of five duplicated experiments and the bar is the standard mean deviation calculated by Statistical Analysis System (SAS, NC, USA).

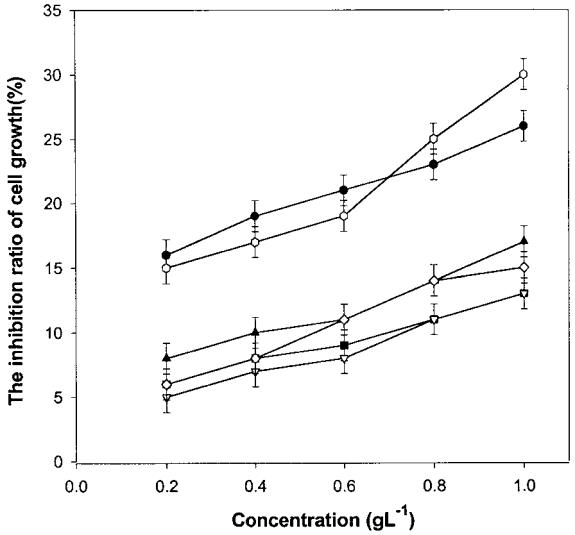

Figure 1. Cytotoxicity on human normal liver cell (WRL-68) by adding the standards and samples; ethanol extracts (E, ●), Diethyl ether fraction from ethanol extract (F1, ▲) Chloroform fraction from ethanol extract (F2 ▽), water fraction from ethanol fractions (F3, ◊), glycyrrhizin (GL, ■), 18,β-glycyrrhetinic acid (GM, ○).

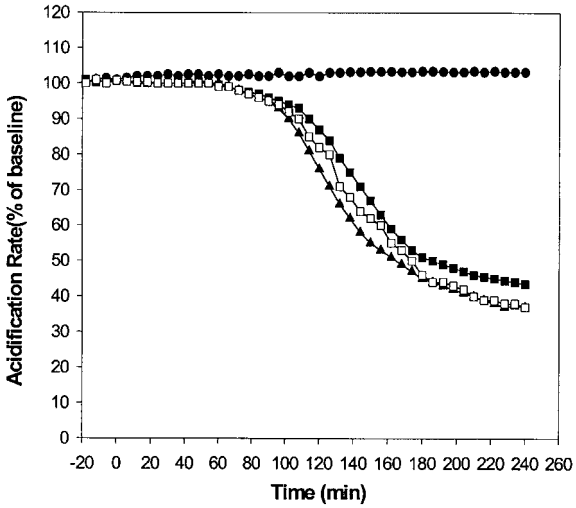

Figure 2. The results of measuring cellular activity of Hep3B by adding 0.5 g l^{-1} of the extracts from Gl. uralensis using a microphysiometer; control (●), etanol extract (E, ▲), glycyrrhizin, (GL, ■), 18,β-glycyrrhetinic acid, (GM, □).

Result and discussion

Figure 1 shows the effect of the samples extracted from *G. uralensis* Fisch on the growth of normal human cell line. The crude ethanol extract showed the highest cytotoxicity, inhibiting 25% of normal cell growth in adding 1.0 g l^{-1} of the highest supplementation. A standard, GL, which has been considered to

Table 1. Comparison of the ratio inhibiting the growth of three different cancer cell lines in adding ethanol extract or the fractions from *Glycyrrhiza uralensis* Fisch

samples	concentration g l^{-1}	Cell line					
		MCF-7 (%)	selectivity	AGS (%)	selectivity	Hep 3B (%)	selectivity
Ethanol extract (E)	0.2	41 ± 0.05	2.5	27 ± 0.02	1.7	30 ± 0.03	1.9
	0.4	48 ± 0.01	2.5	35 ± 0.04	1.8	39 ± 0.02	2.0
	0.6	59 ± 0.02	2.8	46 ± 0.05	2.1	48 ± 0.05	2.3
	0.8	64 ± 0.02	2.7	55 ± 0.03	2.3	54 ± 0.01	2.4
	1.0	76 ± 0.05	2.9	62 ± 0.05	2.4	66 ± 0.03	2.5
Diethyl ether fraction from ethanol extract (F1)	0.2	39 ± 0.03	4.8	32 ± 0.04	4.0	37 ± 0.02	4.6
	0.4	46 ± 0.02	4.6	44 ± 0.02	4.4	46 ± 0.03	4.6
	0.6	57 ± 0.04	5.1	50 ± 0.05	4.5	53 ± 0.05	4.8
	0.8	64 ± 0.05	4.6	61 ± 0.03	4.4	61 ± 0.03	4.3
	1.0	73 ± 0.05	4.3	70 ± 0.03	4.1	69 ± 0.02	4.0
Chloroform fraction from ethanol extract (F2)	0.2	36 ± 0.01	7.2	35 ± 0.01	7.0	36 ± 0.01	7.2
	0.4	45 ± 0.01	6.4	47 ± 0.03	6.7	42 ± 0.01	6.0
	0.6	53 ± 0.05	6.6	59 ± 0.05	7.3	49 ± 0.05	6.1
	0.8	60 ± 0.03	5.4	67 ± 0.03	6.0	57 ± 0.04	5.1
	1.0	65 ± 0.02	5.0	75 ± 0.05	5.7	68 ± 0.02	5.2
Water fraction from ethanol extract (F3)	0.2	37 ± 0.01	6.1	31 ± 0.01	5.1	37 ± 0.03	6.1
	0.4	45 ± 0.01	5.6	42 ± 0.05	5.2	44 ± 0.05	5.5
	0.6	56 ± 0.02	5.1	48 ± 0.05	4.3	52 ± 0.03	4.7
	0.8	63 ± 0.05	4.5	54 ± 0.04	3.8	64 ± 0.01	4.5
	1.0	68 ± 0.03	4.5	60 ± 0.05	4.0	71 ± 0.02	4.7
18,β-glycyrrhetinic acid	0.2	28 ± 0.02	3.5	24 ± 0.03	3.0	27 ± 0.05	4.5
	0.4	32 ± 0.05	2.9	30 ± 0.05	2.7	38 ± 0.04	4.2
	0.6	39 ± 0.03	3.0	39 ± 0.01	3.0	42 ± 0.02	4.2
	0.8	47 ± 0.01	2.9	50 ± 0.04	3.1	46 ± 0.02	4.1
	1.0	55 ± 0.03	2.9	57 ± 0.05	3.0	51 ± 0.05	3.9
Glycyrrhizin (GL)	0.2	31 ± 0.04	5.1	27 ± 0.01	4.5	25 ± 0.05	4.1
	0.4	34 ± 0.03	4.2	37 ± 0.05	4.6	33 ± 0.04	4.1
	0.6	41 ± 0.04	4.6	40 ± 0.03	4.3	35 ± 0.05	3.8
	0.8	49 ± 0.05	4.4	48 ± 0.04	4.3	48 ± 0.05	4.3
	1.0	56 ± 0.02	4.3	59 ± 0.04	4.5	59 ± 0.01	4.5

be the most active substance in *G. uralensis* Fisch so far (Mitsuhike et al., 1998), showed very low cytotoxicity such as ca. 10%. Other fractions (F1-F3) also showed low cytotoxicity on normal human cell line. Based on Figure 1, it can be assumed that the extracts from *G. uralensis* Fisch had relatively low cytotoxicity (< 15%). This cytotoxicity on normal human cell line seemed to have low value, compared to them observed in other medicinal herbs maintaining in the range of 15–25% (Kim et al., 2000).

Table 1 compares the inhibition of the samples on the growth of several human cancer cell lines as well as the selectivity. The growth of cancer cell lines was gradually inhibited as the supplementation of the samples increased. Among them, partially purified fractions showed better results in inhibiting cancer cell growth than the crude extract (E) and standards (GL

Table 2. Antimutagenicity of the ethanol extract and its fractions from *Glycyrrhiza uralensis* Fisch by using CHL V79 cells

Samples	concentration g l^{-1}	Antimutagenicity (%)*
Ethanol extract (E)	0.2	28 ± 0.02
	0.4	33 ± 0.04
	0.6	38 ± 0.01
	0.8	41 ± 0.04
	1.0	44 ± 0.05
Diethyl ether fraction	0.2	35 ± 0.02
from ethanol extract (F1)	0.4	42 ± 0.04
	0.6	46 ± 0.03
	0.8	52 ± 0.05
	1.0	57 ± 0.05
Chloroform fraction	0.2	35 ± 0.01
from ethanol	0.4	43 ± 0.03
extract (F2)	0.6	49 ± 0.05
	0.8	54 ± 0.03
	1.0	58 ± 0.05
Water fraction from	0.2	34 ± 0.02
ethanol extract (F3)	0.4	41 ± 0.04
	0.6	46 ± 0.03
	0.8	50 ± 0.05
	1.0	53 ± 0.02
Enzymatically	0.2	23 ± 0.03
hydrolyzed	0.4	25 ± 0.02
glycyrrhizin (HGL)	0.6	30 ± 0.04
	0.8	33 ± 0.01
	1.0	37 ± 0.02
Glycyrrhizin (GL)	0.2	25 ± 0.01
	0.4	27 ± 0.01
	0.6	32 ± 0.04
	0.8	36 ± 0.02
	1.0	40 ± 0.03

* The ratio of the growth in no addition to the growth in adding the sample.

and GM) such as 70–75% vs 55–60% in adding 1.0 g l^{-1}, respectively. The crude extract (E) showed higher cytotoxicity on cancer cell lines than two standards. The result of estimating the selectivity also showed similar pattern with the inhibition of cell growth. It may suggest that a monomer, GM or smaller components can play a certain role in inhibiting and/or controlling the growth of cancer cells since fraction F1 showed the highest inhibition for all cases. Among three cell lines, hepatoma carcinoma cell seemed to be more sensitively affected by the samples than other cell lines as shown in Table 1. Measuring simple endpoints in adding the samples may impose significant limitations on the assay system if the prediction of irritancy is the object. Therefore, it is also necessary to examine how quickly the samples response to the target cells. Kinetics of cells responding to the samples will better illustrate the effectiveness of the samples affecting on the cancer cell growth. To do so, the acidification kinetics of the samples were monitored

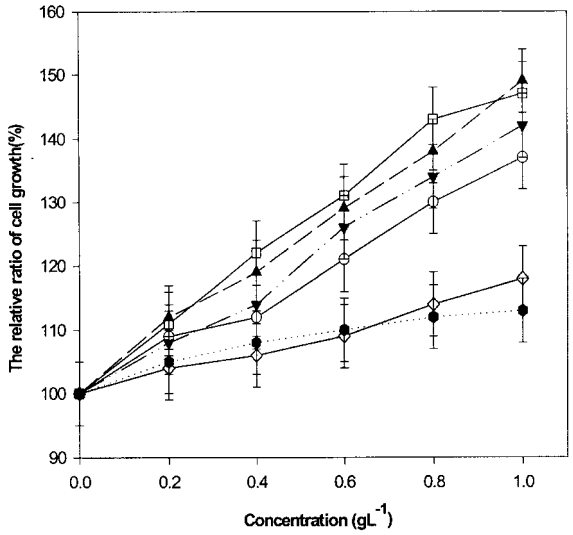

Figure 3. Enhancement of human immune B cell growth in adding the extracts from Gl. uralensis Fisch; ethanol extract (E, ○), Diethyl ether fraction from ethanol extract (F1, □), Chloroform fraction from ethanol extract (F2, ▲), water fraction from ethanol fractions (F3, ▼), glycyrrhizin standards (GL, ◊), 18,β-glycyrrhetinic acid standard (GM, ●).

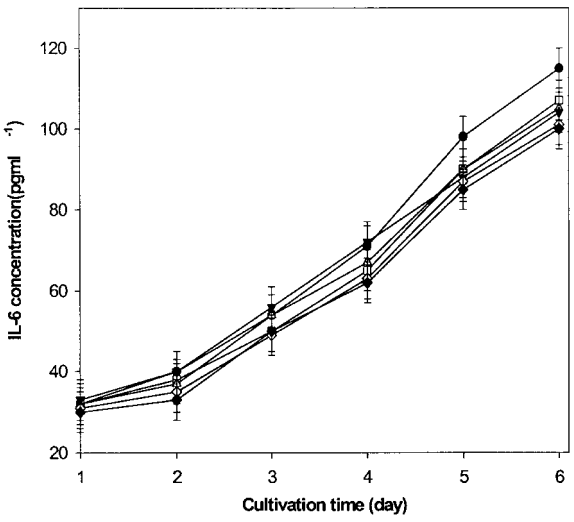

Figure 4. The secretion of IL-6 from human B cells in adding 1.0 (g l^{-1}) of the samples according to cultivation time; ethanol extracts (E, ●), hydrolyzed glycyrrhizin (HGL, □), diethyl ether fraction from ethanol extract (F1, △), chloroform fraction from ethanol extract (F2, ▼), glycyrrhizin (GL, ◊), 18,β-glycyrrhetinic acid (GM, ♦).

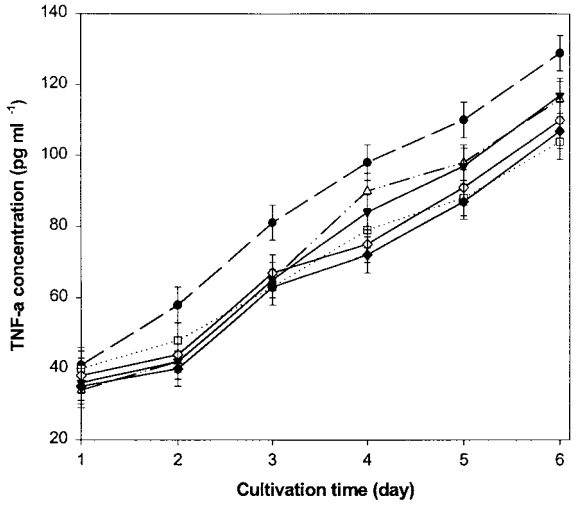

Figure 5. The secretion of TNF-a from human B cells in adding 1.0 (g l^{-1}) of the samples according to cultivation time; ethanol extracts (E, ●), hydrolyzed glycyrrhizin (HGL, □), diethyl ether fraction from ethanol extract (F1, △), chloroform fraction from ethanol extract (F2, ▼), glycyrrhizin, (GL, ◊), 18,β-glycyrrhetinic acid, GM, ♦).

to confirm the inhibition effect on the growth of cancer cells as shown in Figure 2. Most of the samples started to inhibit the cell growth after about 100 min. of addition, and then the cell growth (acidification rate in Figure 2) was rapidly dropped to a certain level, compared to the control where no samples were added. The crude extract inhibited the cell growth relatively faster than two standards did. It can be corresponded to the cytotoxicity of samples by measuring the endpoints shown in Table 1. There was not much difference in response time for three samples, E, GL and GM; however, sample E seemed to inhibit the cancer cell relatively faster than others by 20 min. It will also be supported by the result in Table 1 that the crude extract most effectively inhibited the cell growth than the standard extract.

Table 2 is the result of measuring antimutagenicity of the samples on a strong mutagen, 4-NQO because antimutagenicity may be strongly correlated to antitumor activity. Table 2 shows that the crude extract also had higher antimutagenicity than both standards. These results were well matched to the data of antitumor activity in Table 1 and Figure 2. Fractions (F1 and F3) showed higher antimutagenicity than the crude extract. This pattern is also similar to the result of cytotoxicity on cancer cells. It also explains that more than 50% of the normal cells can be alive when they were supplemented with fractionized sample F1

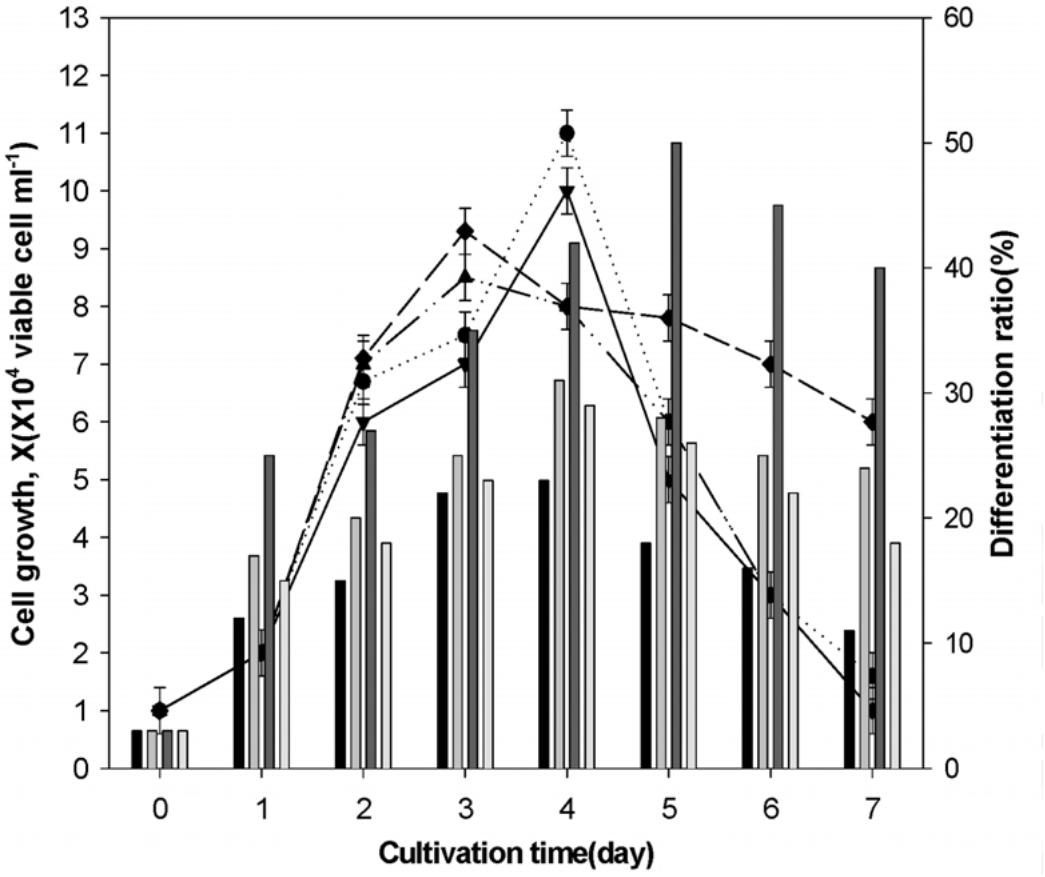

Figure 6. The effect of the extracts and standards on differentiation of HL-60 cells in adding 0.1 (g l^{-1}) of the samples; cell growth – ethanol extracts (E, ●), diethyl ether fraction from ethanol extract (F1, ▲) glycyrrhizin (GL, ♦), 18,β-glycyrrhetinic acid (GM, ▼), differentiation ratio – ethanol extracts (E, ■), diethyl ether fraction from ethanol extract (F1, ■), glycyrrhizin (GL, ■), 18,β-glycyrrhetinic acid (GM, ■).

or F3 in adding a strong mutagen, 4-NQO. That is, the samples may play a role in limiting the initiation of carcinogenesis and result in effectively inhibiting the growth of cancer cells.

Figure 3 demonstrates the effect of the samples on the enhancement of human immune cell growth. It may be well correlated to the result that most of the samples showed high anti-tumor activity. The growth of human B cell was increased in adding the fraction F1 up to ca. 50% of cell growth compared to the control. It is interesting that both standards could not enhance human B cell growth while other samples definitely improved the cell growth. Partially purified samples showed better effect on B cell growth than the crude extracts. Quantitative experiments may be necessary for the relation between cell growth and increased secretion of cytokines in controlling cancer cell growth. Figures 4 and 5 show the enhancement of secreting cytokines, IL-6 and TNF-α in adding the samples. On the contrary, two standards revealed relatively small increase. The enhancement of B cell growth was closely related to the increase of the secretion of IL-6 and TNF-α (see Figures 3 and 4); however, the crude extract did not much increase only both B cell growth and IL-6 secretion (Figure 4), but also TNF-α secretion (Figure 5). Interestingly enough, fraction F1 improved the growth of B cells and the secretion of cytokines. It may result in effectively enhancing anti-tumor activity of the fractionized samples.

Figures 6 and 7 are also to support this hypothesis by showing the acceleration of differentiation of HL-60 cell and apoptosis of A549 cell. Figure 6 shows how fraction F1 can enhance the differentiation of HL-60 cells by observing the amounts of blue formazan due to the formation of superoxide within the cell. It was found that more than 50% of cells was differentiated after five days cultivation in adding 1.0 g l^{-1} of

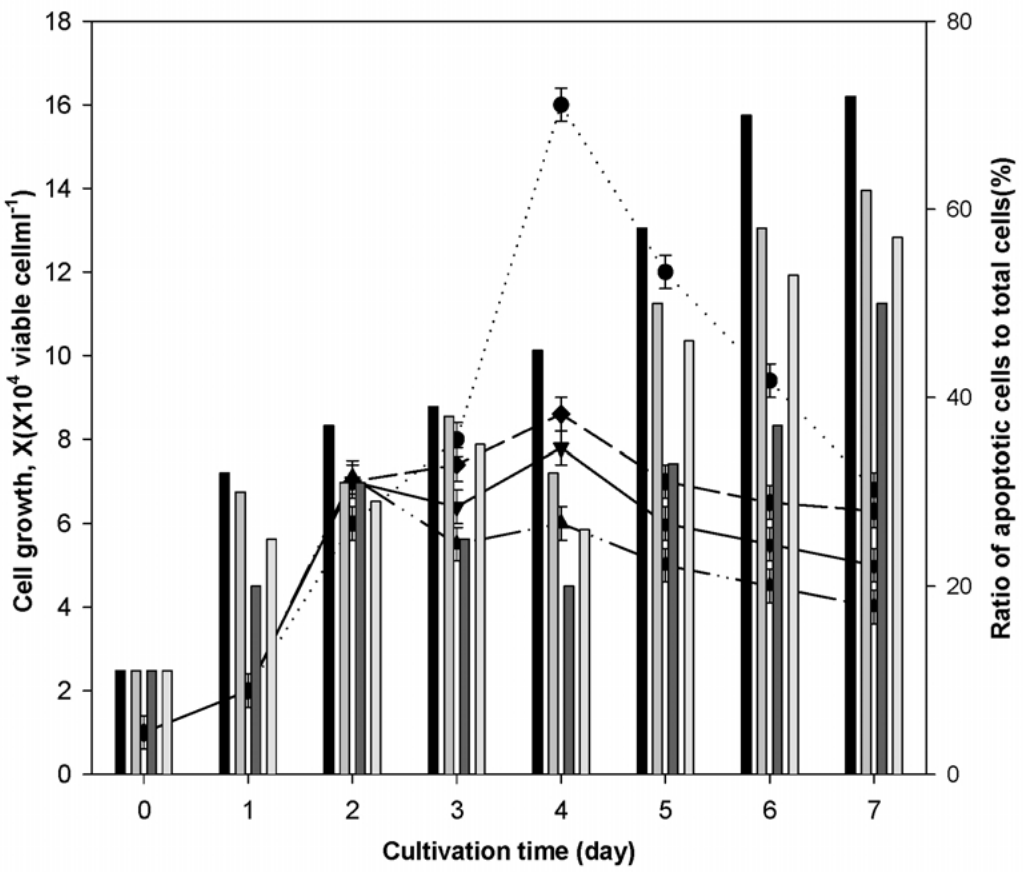

Figure 7. The cell growth and apoptosis of A549 cells in adding 1.0 mg l^{-1} of the standards and samples from Glycyrrhiza uralensis Fisch; cell growth – ethanol extracts (E, ●), Diethyl ether fraction from ethanol extract (F1, ▲), glycyrrhizin (GL, ♦), 18,β-glycyrrhetinic acid (GM, ▼), differentiation ratio – ethanol extracts (E, ■), Diethyl ether fraction from ethanol extract (F1, ■), glycyrrhizin (GL, ■), 18,β-glycyrrhetinic acid (GM, ■).

the samples. On the contrary, crude extract had limited differentiation activity. Two standards could not much improve the differentiation of HL-60 compared to F1. It can be explained that the standards may not play a significant role in modulating immune system and also the differentiation of HL-60, but other components in *G. uralensis* Fisch extracts do activate them. It is interesting that GL maintained the growth of HL-60 cell even at latter period of the cultivation, compared to other samples. Figure 7 shows that crude extract can also accelerate apoptosis of A549 cells possibly due to other unknown components in the crude extract, not pure standards. Two standards also increased the ratio of apoptotic cell death during the cultivation while fraction F1 yielded the lowest ratio of apoptosis. It reverses the result that fraction F1 most differentiated HL-60 cells in Figure 6, which implies that the components in *G. uralensis* Fisch may have complex biochemical mechanisms in controlling cancer cell growth. The crude extract maintained relatively high cell density during exponential phase and rapidly dropped as well as high ratio of apoptosis during the cultivation.

In general, crude extract and its fractions (F1 and F3) seemed to have better immune modulating activities based on data shown in this work. It is evident that a monomer of glycyrrhizin or other small molecules in *G. uralensis* Fisch has immune related biological functions rather than glycyrrhizin itself. It may be a controversial result since glycyrrhizin has been considered to be most active components from *G. uralensis* Fisch in many reports (Mitsuhiko Nose, 1998; Kim et al., 1998, Shiota et al., 1999; Suzuki et al., 1983). The components in the samples were compared with two standards, GL and GM through TLC analysis as shown in Figure 8. Crude ethanol ex-

Figure 8. The result of TLC Analysis of the samples and a monomer 18,β-glycyrrhetinic acid: glycyrrhizin (GL, standard)(A), enzymatically hydrolyzed GL (B), crude extract (C), Fraction 1 (D), 18,β-glycyrrhetinic acid (GM, standard) (E).

tract showed several spots, and two of them were well matched to two standards, respectively. Enzymatically hydrolyzed, GL also showed two spots, and one of them corresponded to a monomer, GM. The other spot may be a derivative of GM. This derivative was also found in partitioned fraction F1 and the crude extract, not in both standards GL and GM. Among several samples, crude extract and fraction F1 showed relatively good biological activities in many categories in our data. There is a possibility that this spot (possibly a derivative of the monomer, GM due to similar R_f and size of the spot) can play a role in affecting biological functions of *G. uralensis* Fisch. The spots were also re-confirmed by HPLC with standards and found to be almost same peak with a monomer, GM. Further, crude extract showed better immune modulating activity than standard glycyrrhizin (which has been pointed out to be the best active component in *G. uralensis* Fisch). The fraction F1 and/or F3 also seemed to have better activities than the crude samples. The active components existed in them may be another form or a derivative of a monomer, GM, not monomer itself, based on TLC analysis. The identification and biochemical mechanisms of the active component(s) will be further investigated to discover the biological functions of *G. uralensis* Fisch.

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