Available online at www.tox.or.kr

Screening of Herbal Medicines for Recovery of Acetaminophen-induced Hepatotoxicity

Sung-Hwa Sohn[1], **Hyo-Eun Lee**[1], **Beom-Joon Lee**[1], **Sung-Hoon Kim**[2], **Minkyu Shin**[1], **Moochang Hong**[1], **Hyunsu Bae**[1] **& Yangseok Kim**[1]

[1]BK21 Oriental Medical Science Center, KyungHee University, Seoul 130-701, Korea
[2]Department of Oriental Pathology, College of Oriental Medicine, KyungHee University, Seoul 130-701, Korea
Correspondence and requests for materials should be addressed to H. S. Bae (hbae@khu.ac.kr), Y. S. Kim (yskim1158@khu.ac.kr)

Accepted 29 October 2008

Abstract

This study was conducted to quantitatively evaluate the recovery effects of herbal medicines on acetaminophen-induced hepatotoxicity. In the present study, the recovery effects of 251 herbal medicines on THLE-2 cells that had been damaged by acetaminophen were evaluated using an MTS assay. THLE-2 cells were cultured in 96-well plates and then pretreated with or without 60 μM acetaminophen (IC_{50} value: 35.84) for 1 hr. Next, different herbal medicines were added to the wells, after which the cells were reincubated at 37°C for 24 hr. After first round of screening, the candidate herbal medicines were selected based on a recovery rate of greater than 40% and their efficacy were then determined by dose response kinetic analysis. Among these extracts, 8 herbal medicines (*Terminalia chebula*, *Pueraria lobata*, *Acronychia laurifolia*, *Lopatherum gracile*, *Oroxylum indicum*, *Cynanchum atratum*, *Senecio scandens*, and *Sophora flavescens*) had a strong recovery effect on acetaminophen-induced damage in THLE-2 cells. Dose response non-linear regression analysis demonstrated that Senecio scandens showed the best recovery rate (98%), and that its EC_{50} was 19.54 ng/mL. Additional studies of these herbal medicines should be conducted to determine if they possess novel therapeutic agents for the prevention or treatment of liver disorders.

Keywords: Acetaminophen, Herb medicines, Hepatotoxicity, *Senecio scandens*, *Sophora flavescens*

Liver disease, including chronic viral hepatitis B and C, alcoholic steatosis, non-alcoholic fatty liver disease, fibrosis/cirrhosis, hepatocellular carcinoma or liver cancer, afflicts over 10% of the world's population[1,2]. Because of its unique metabolism and relationship to the gastrointestinal track, the liver is an important target of the toxicity of drugs, xenobiotics, and oxidative stress. In addition, reactive oxygen species derived from chemicals or drugs that are exposed to liver cells appear to mediate liver injury, although the mechanisms of free radical toxicity are not well understood. Therefore, it is important to understand the role played by antioxidants during drug mediated toxicity to determine if they can reduce the oxidative stress induced by reactive intermediates produced by various chemicals and drugs[3,4].

Acetaminophen is a widely used antipyretic and analgesic that is safely employed for a wide range of treatments[5]. However, acetaminophen is the most common pharmaceutical taken in overdose. Accurately predicting the risk of hepatotoxicity following acetaminophen overdose is essential for several reasons[6-8]. Cytochrome P450 oxidation of acetaminophen results in the production of a N-acetyl-p-benzoquinon imine (NAPQI), which then reacts with glutathione (GSH) to form an acetaminophen-GSH conjugate. At therapeutic doses NAPQI is removed by GSH. However, at overdoses of acetaminophen, the GSH is exhausted and the NAPQI then binds to cellular proteins, including a number of mitochondrial proteins, which leads to centrilobular necrosis in the liver[5,7,9,10].

Currently, studies are being conducted worldwide to identify protective molecules that can protect the liver and other organs with few or no side effects. A large number of herbs have traditionally been used to treat drug or toxin induced liver diseases. In addition, herbal medicines were the arsenal of therapies in the treatment of liver disease[2]. Therefore, this study was conducted to identify recovery herbal medicines in acetaminophen-induced hepatotoxicity. To accomplish this, acetaminophen was used to induce injury

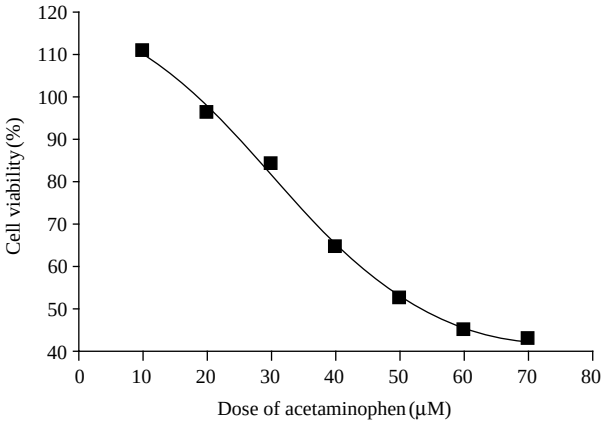

Figure 1. Effect of acetaminophen on cell viability. THLE-2 Cells were treated with various concentrations of acetaminophen were added for 24 hr. The values of cell viability were normalized in proportion to the value of control.

on liver cells which were then treated with various herbal medicines to determine if any of these medicines exerted a recovery effect.

Cytotoxicity of THLE-2 Cells

To evaluate the effect of acetaminophen on liver cell viability, THLE-2 cells were incubated with various concentrations of acetaminophen for 24 hr. The viability of THLE-2 cells was inhibited by acetaminophen in a dose-dependent manner (Figure 1). Next, the concentration of acetaminophen that resulted in 50% inhibition of cell viability (IC_{50}) was determined using non-linear regression analysis. The IC_{50} was found to be 35.84 μM in acetaminophen-induced damaged THLE-2 cells (Figure 1). However, THLE-2 cells were viability inhibited by over 50% after 60 μM acetaminophen treatment. We therefore used the 60 μM acetaminophen for selection of recovery herbal medicines in acetaminophen-induced hepatototoxicity.

Recovery Effect of Acetaminopnen-induced Damaged THLE-2 Cells

THLE-2 cells were treated with Acetaminophen for 1 hr, after 10 μg of 251 herb medicines were added to the cells. The cells were then incubated for an additional 24 hr and the best recovery effect of the herbal medicines was then evaluated using MTS assays. At 2-days, the cell viability of the herb-treated THLE-2 cells was higher than that of the non-treated control cells (acetaminophen-treated THLE-2 cells) with 8 herbal medicines (*Terminalia chebula*, *Pueraria lobata*, *Acronychia laurifolia*, *Lopatherum gracile*, *Oroxylum indicum*, *Cynanchum atratum*, *Senecio scandens*, and *Sophora flavescens*) showing greater than 40% recovery of acetaminophen-induced damaged in THLE-2 cells (Table 1). Therefore, these 8 herbal medicines were evaluated to determine the best effective dose for the treatment of damaged THLE-2 cells.

Effective Dose of 8 Herb Medicines in Damaged THLE-2 Cells

We also tested the dose-dependent protective effect of 8 herb medicines (*Terminalia chebula*, *Pueraria lobata*, *Acronychia laurifolia*, *Lopatherum gracile*, *Oroxylum indicum*, *Cynanchum atratum*, *Senecio scandens*, and *Sophora flavescens*) in damaged THLE-2 cells. THLE-2 cells were treated with acetaminophen (60 μM) for 1 hr, after different concentrations (0.0001, 0.001, 0.01, 0.1, 1, 10, and 100 μg/mL) of herb medicines were added. The samples were then incubated for an additional 24 hr and the optimal dose was then determined by evaluating cell viability using MTS assays. Treatment of the cells with the 8 herbal medicines increased cell viability in a dose-dependent manner (n=3) (Figure 2). The order of increased cell viability of the damaged cells occurred in the following order: *Senecio scandens* > *Sophora flavescens* > *Cynanchum atratum* > *Lopatherum gracile* > *Oroxylum indicum* > *Acronychia laurifolia* > *Terminalia chebula* > *Pueraria lobata*, with maximal recoveries of cell viability being 97.66, 86.65, 85.92, 85.22, 72.83, 57.27, 52.39, and 49.21%, respectively. In addition, the treated cells were found to have EC_{50} values of 19.54, 0.27, 1.11, 0.89, 9.58, 0.19, 21.77, and 52.94 ng/mL, respectively (Table 2).

Discussion

In vitro basal cytotoxicity tests have a contribution to make in several areas of toxicity testing. They can be used to screen large numbers of chemicals for their intrinsic toxic potentials and for their relative toxicities. Significant correlations between cytotoxicity *in vitro* and animal lethality have been demonstrated on numorous papers[12,13]. Therefore, during the screening of products produced by medicinal plants for new therapeutic agents for the treatment of liver diseases, the recovery activities of 251 spray-dried extracts of herbal medicines on acetaminophen-induced damaged THLE-2 cells were evaluated. Table 1 presents the list of the recovery effect of herbs on acetaminophen-induced damaged in THLE-2 cells. Among these extracts, 8 herb medicines (*Terminalia chebula* (family: *Combretaceae*), *Pueraria lobata* (family: *Fabaceae*), *Acronychia laurifolia* (family: *Rutaceae*), *Lopathe-*

Table 1. Recovery effect of herbal medicines on acetaminophen-induced damaged in THLE-2 cells.

Herbal medicines	CV (%)	Herbal medicines	CV (%)	Herbal medicines	CV (%)
Pueraria lobata	110	*Epimedium saggitatum*	11	*Eriocaulon buergerianum*	−3
Cynanchum atratum	91	*Dictamnus dasycarpus*	11	*Homalomena occulta*	−3
Terminalia chebula	91	*Lilium brownii*	11	*Cuscuta chinensis*	−3
Oroxylum indicum	80	*Areca catechu*	11	*Buddleia officinalis*	−3
Acronychia laurifolia	66	*Ginkgo biloba*	11	*Zingiber officinalis*	−3
Senecio scandens	53	*Alpinia oxyphylla*	10	*Oryza sativa*	−4
Lopatherum gracile	48	*Leonorus beterophyllus*	10	*Ocimum basilicum*	−4
Sophora flavescens	45	*Blechnum orientate*	10	*Sterculia staphigera*	−4
Dolomiaea souliei	39	*Scrophularia ningpoensis*	10	*Rosa cymosa*	−4
Rwuwolfia serpentina	39	*Solanum nigrum*	10	*Cudrania tricupidata*	−4
Curcuma zedoaria	38	*Chaenomeles sinensis*	10	*Areca catechu*	−4
Pulsatilla dahurica	37	*Bletilla striata*	10	*Cynanchum stauntoni*	−5
Paeonia suffruticosa	36	*Perilla frutescens*	10	*Poria cocos*	−5
Notopterygium incisum	36	*G. jasminoides*	10	*Polygonum multiflorum*	−5
Citrus reticulata	32	*Stemona japonia*	9	*Chrysanthemum*	−5
Arnebia euchroma	31	*Aster tataricus*	9	*Morus alba*	−5
Euryale ferox	31	*Sanguisorba officianlis*	9	*Benincasa hispida*	−5
Trichosanthes kirilowii	30	*Lonicera japonica*	9	*Glycine max*	−6
Taraxacum mongolicum	30	*Perilla frutescens*	9	*Lepidium apetalum*	−6
Dolichos lablab	28	*Aconitum carmichaeli*	9	*Sargassum fusiforme*	−6
Paederia scandens	28	*Coix lachrymajobi*	8	*Trichosanthes kirilowii*	−6
Pharbitis nil	27	*Angelica daburica*	8	*Vitex rotundifolia*	−6
Amomum cardamomum	24	*Atractylodes macrocephala*	8	*Leonorus heterophyllus*	−7
Imperata cylindrica	23	*Zizyphus jujuba*	8	*Eclipta prostata*	−7
Celosia cristata	23	*Raphanus sativus*	7	*hordeum vulgare*	−7
Cibotium barometz	22	*Viola yedoensis*	6	*Dioscorea hypoglauca*	−8
Zea mays	22	*Eugenia caryophyllata*	5	*Houttuynia cordata*	−8
Perilla frutescens	22	*Schisandra chinensis*	4	*Boswellia carterii*	−8
Patrinia scabiosaefolia	21	*Trichosanthes kirilowii*	4	*Oryza sativa*	−8
Gentiana macrophylla	20	*Curcuma longa*	3	*Angelica pubescens*	−8
Lycium chinense	18	*Paeonia lactiflora*	3	*Equisetum hiemale*	−8
Polygala tenuifolia	18	*Pheretima aspergillum*	3	*Cyperus rotundus*	−8
Elephantopus scaber	18	*Thuja orientalis*	2	*Prunus armeniaca*	−8
Gardenia jasminoides	17	*Trogopterus xanthipes*	2	*Senna acutifolia*	−8
Scutellaria barbata	17	*Corydalis yanhusuo*	1	*Rosa laevigata*	−9
Angelica sinensis	16	*Allium tuberosum*	1	*Punica granatum*	−9
Rheum palmatum	16	*Sesamum indicum*	1	*Atractylodes lancea*	−9
Ampelopsis japonica	16	*Lasiosphaera fenslii*	0	*Dimocarpus longan*	−9
Inula japonica	15	*Melia toosendan*	0	*Curcuma longa*	−9
Dichondra repens	15	*Panax ginseng*	0	*Citrus grandis*	−10
Caesalpinia sappan	14	*Foeniculum vulgare*	0	*Siegesbeckia pubescens*	−10
Homo sapiens	14	*Verbena officinalis*	−1	*Laminaria japonica*	−10
Ledebouriella divaricata	14	*Artemista argyi*	−2	*Rhaponticum uniflorum*	−10
Sinapis alba	14	*Mentha arvensis*	−2	*Salvia miltiorrhiza*	−10
Belamcanda chinensis	14	*Tussilago farfara*	−2	*Cirsium japonicum*	−10
Elsholtzia splendens	14	*Abrus precatorius*	−2	*Prunus persica*	−10
Aetemisia capillaris	13	*Ephedra sinica*	−2	*Juncus effusus*	−10
Centipeda minima	13	*Cartbamus tinctorius*	−3	*Hovenia dulcis*	−10
Clematis chinensis	11	*Ophiopogon japonicus*	−3	*Cinnamomum cassia*	−11
Drynaria fortunei	−11	*Litchi chinensis*	−14	*Prinsepia uniflora*	−19
Citrus reticulata	−11	*Allium macrostemon*	−14	*Santalum album*	−19
Lonicera japonica	−11	*Malva verticillata*	−15	*Paeonia lactiflora*	−19
Citrus reticulata	−11	*Sophora subprostata*	−15	*Astragalus complanatus*	−19
Gleditsia sinensis	−11	*Alpinia officinarum*	−15	*Sparganium simplex*	−19
Phyllostachys migra	−11	*Liquidambar*	−15	*Diospyros kaki*	−19
Prunella vulgaris	−12	*Phaselus calcaratus*	−15	*Arctium lappa*	−20
Ephedra sinica	−12	*Lycium chinense*	−15	*Plantago asiatica*	−20
Evodia rutaecarpa	−12	*Schizonepeta tenuifolia*	−15	*Rubia cordifolia*	−20
Ligusticum walichii	−12	*Eupatorium fortunei*	−15	*Acorus gramineus*	−20
Trichosanthes kirilowii	−12	*Polygonum aviculare*	−15	*Cnidium monnieri*	−20

Table 1. Continued.

Herbal medicines	CV (%)	Herbal medicines	CV (%)	Herbal medicines	CV (%)
Uncaria rhynchophylla	−12	*Ligusticum sinense*	−15	*Morus alba*	−20
Plantago Asiatica	−12	*Rubus chingii*	−16	*Bupleurum chinense*	−20
Fritillaria thunbergii	−12	*Pteris multifida*	−16	*Gastrodia elata*	−20
Cannabis sativa	−12	*Nelumbo nucifera*	−16	*Pogonatherum crinitum*	−21
Sophora japonica	−12	*Luffa cylindrica*	−16	*Alisma orientalis*	−21
Citrus aurantium	−12	*Arisaema consanguineum*	−16	*Albizzia julibrissin*	−22
Artemisia apiacea	−12	*Allium fistulosum*	−16	*Polygonatum odoratum*	−22
Crataegus pinnatifida	−12	*Tetrapanax papyrifera*	−16	*Gentiana scabra*	−22
Loranthus parasiticus	−12	*Zanthoxylum avicennae*	−16	*Typha latifolia*	−22
Ligustrum japonicum	−12	*Zizyphus spinosa*	−16	*Ailanthus altissima*	−22
Celosia argentea	−12	*Rehmannia glutinosa*	−16	*Smilax glabra*	−22
Isatis tinctoria	−12	*Commiphora myrrha*	−16	*Forsythia suspensa*	−23
Poria cocos	−13	*Citrus aurantium*	−16	*Fraxinus rhynchophyllae*	−24
Dioscorea opposita	−13	*Xanthium sibiricum*	−17	*Asiasarum sieboldi*	−24
Agrimonia pilosa	−13	*Rehmannia glutinosa*	−17	*Pinus tabulaeformis*	−24
Lindera strychnifolia	−13	*Psoralea corylifolia*	−17	*Ludwigia prostata*	−24
Dianthus superbus	−13	*Pogostemon cablin*	−18	*Magnolia liliflora*	−24
Sus scrofa domestica	−13	*Lobelia chinensis*	−18	*Asparagus cochinchinensis*	−25
Artemisia anomala	−13	*Triticum aestivum*	−18	*Curculigo orchioides*	−25
Anemarrhena asphodeloides	−13	*Polyporus umbellatus*	−18	*Morinda officinalis*	−25
Polygonum multiflorum	−14	*Spatholobus suberectus*	−18	*Tribulus terrestris*	−25
Peucedanum praeruptorum	−14	*Platycodon grandiflorum*	−18	*Dendrobium nobile*	−25
Eriobotrya	−14	*Citrus medica*	−19	*Lycopus lucidus*	−25
Cornum officinalis	−14	*Zingiber officinalis*	−19		

The results are the average of three independents with less than 10% standard deviations. The effects of various herbs (10 μg/mL, each) on acetaminophen (60 μM)-induced damaged THLE-2 cells show the cell viability. Cell viability was normalized by positive control (acetaminophen-induced damaged cells) becomes 0% and negative control (acetaminophen non-treated cells) becomes 100% for all data sets. Abbreviation: CV, cell viability.

rum gracile (family: *Gramineae*), *Oroxylum indicum* (family: *Bignoniaceae*), *Cynanchum atratum* (family: *Asclepiadaceae*), *Senecio scandens* (family: *Asteraceae*), and *Sophora flavescens* (family: *Fabaceae*)) showed the highest recovery activity on acetaminophen-induced damaged in THLE-2 cells, therefore this herb medicines was selected for this study. Unexpectedly, taxonomical classification reveals that the relative relationships between these herb medicines were not closely associated.

These herb medicines did not show cytotoxicity at the test concentration (data not shown). The EC_{50} values of these selected herb medicines, and their effective dose are presented in Table 2. *Terminalia chebula*, *Pueraria lobata*, and *Acronychia laurifolia* showed over 40% recovery of acetaminophen-induced damage in THLE-2 cells, compared to those of non-treated control cells (Figure 2). *Lopatherum gracile*, *Oroxylum indicum*, *Cynanchum atratum*, *Senecio scandens*, and *Sophora flavescens* showed over 70% recovery of acetaminophen-induced damage in THLE-2 cells (Figure 2). Among these 8 herb medicines, *Senecio scandens* and *Sophora flavescens*, which showed the highest recoveries of the tested compounds, have previously been shown to have

Table 2. Recovery effect of herbal medicines on acetaminophen (60 μM)-induced damaged in THLE-2 cells.

Herbal medicines	Effective dose (ng/mL)	
	EC_{50}	V_{max}
Terminalia chebula	21.77	52.39
Pueraria lobata	52.94	49.21
Acronychia laurifolia	0.19	57.27
Lopatherum gracile	0.89	85.22
Oroxylum indicum	9.58	72.83
Cynanchum atratum	1.11	85.92
Senecio scandens	19.54	97.66
Sophora flavescens	0.27	86.65

Effective doses were normalized by viability of acetaminophen (60 μM)-induced damaged THLE-2 cells. The data were normalized with % of acetaminophen-treated THLE2 cells. EC_{50} and V_{max} values were determined as described in materials and methods.
Abbreviation: EC_{50}, effective concentration for half-maximum response.

therapeutic uses. For example, *Senecio scandens*, and *Sophora flavescens* have antiinflammatory, antipyretic, antiseptic, antidotic, diuretic, and antioxidant activities[14]; however, *Senecio scandens* is used for the treatment of inflammatory, bacterial diarrhea, enteritis, conjunctivitis, respiratory tract infection, arthtitis

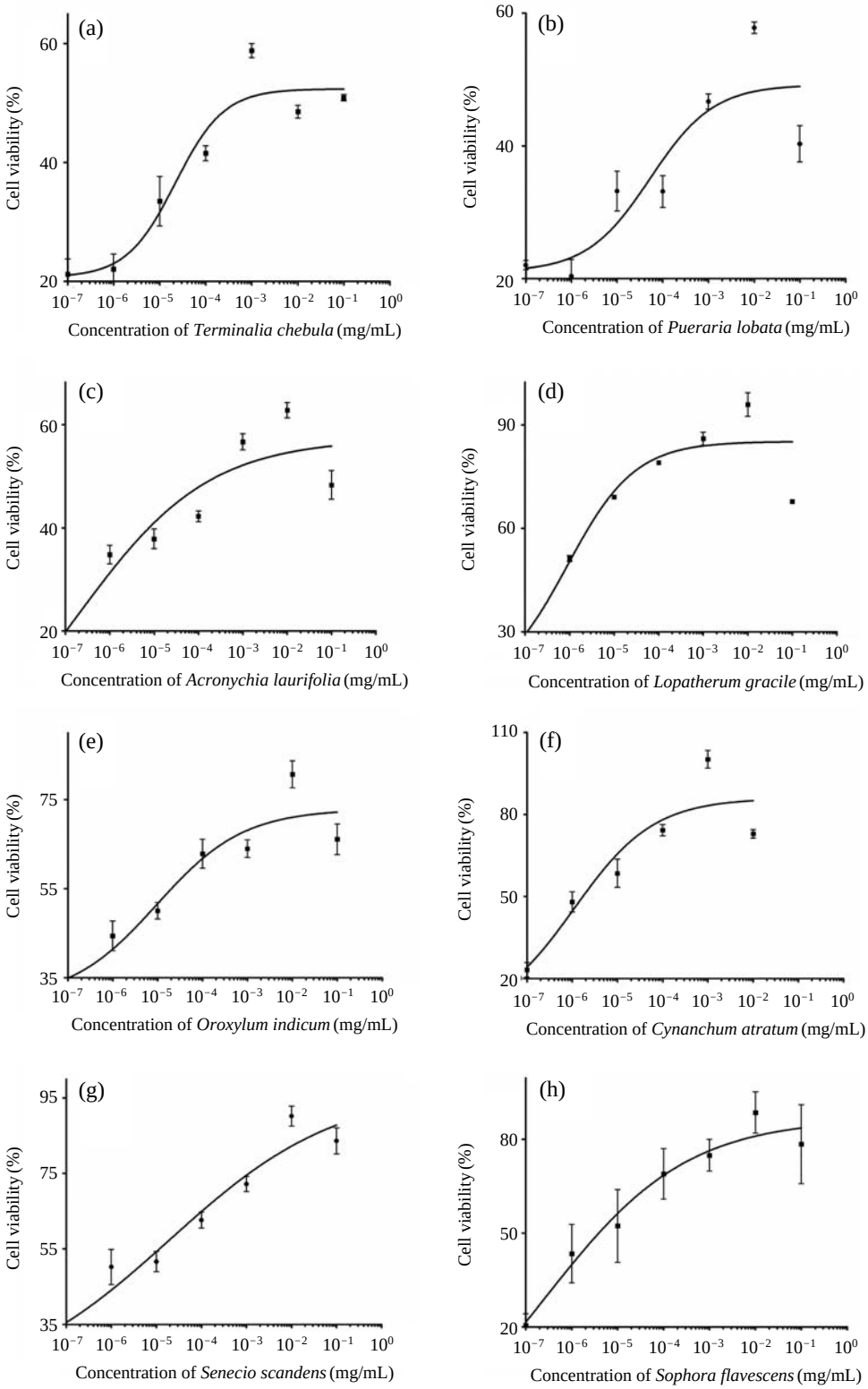

Figure 2. Effective dose of herb medicines on cell damaged THLE-2 cells. THLE-2 cells were cultured with 60 μM acetaminophen for 1 hr. After, THLE-2 Cells were treated with various concentrations of 8 herb medicines (a: *Terminalia chebula*, b: *Pueraria lobata*, c: *Acronychia laurifolia*, d: *Lopatherum gracile*, e: *Oroxylum indicum*, f: *Cynanchum atratum*, g: *Senecio scandens*, and h: *Sophora flavescens*) were added for 24 hr. The values of cell viability were normalized in proportion to the value of control.

and rheumatic disease[15,16]. In addition, *Sophora flavescens* is a well-known traditional medicine with the function of reliving heat, depriving the evil wetness, puring fire for removing toxin and killing parasites to relieve itching. It has also been used eczema, antitumor, anthelmintic, and hepatoprotection[17-19]. In conclusion, *Senecio scandens* and *Sophora flavescens* showed significant activities in this assay system, and thus further study for their mechanism of action can be encouraged for the discovery of new therapeutic agents for the treatment of liver diseases.

Materials & Methods

Preparation of Herbs

Herbs were purchased from the Sun Ten Phamaceutical Co., Ltd. (Taipei, Taiwan), powdered to 0.1 g and then extracted by stirring in 10 mL DW (distilled water) using stirrer for overnight at room temperature. The sample was then centrifuged for 10 min at 3,000 rpm, after which the supernatant was removed and sterilized by passing it through a 0.22 μm syringe filter and then used for the experiments. A voucher specimen was deposited at the Herbarium of College of Oriental Medicine, KyungHee University, Korea. Dr. Minkyu Shin, the director of herbarium, was identified the plants and assigned the herbarium sheet number (No. PMP0081).

Cell Culture

THLE-2 cells, an SV40 large T antigen-immortalized human liver cell line, were obtained from the American Type Culture Collection (ATCC, Rockville, MD). THLE-2 was plated into culture plates precoated for 1 hr at 37˚C with BEBM (bronchial epithelial basal medium) containing human fibronectine (0.01 mg/mL), bovine collagen type I (0.03 mg/mL) and bovine serum albumin (0.01 mg/mL). Cells were cultured at 37˚C in a 5% CO_2 humidified incubator and maintained in BEGM supplemented with 10% fetal bovine serum (FBS), human epidermal growth factor (5 ng/mL), and phosphoethanolamine (70 ng/mL). THLE-2 cells were then plated onto tissue culture flasks (T-75 cm^2) at a density of 1×10^7/mL in hormonally defined DMEM media as described previously. The medium was changed every 3 days until the cells become 80-90% confluent at which point they were used for experiments.

Cytotoxicity Assay

Cell growth was measured by an MTS assay using the Cell Titer 96® Aqueous One Solution Cell Proliferation Assay Kit (Promega, Madison, WI, U. S. A.). The MTS assay was performed as described by Chung *et al.*[11] with some modifications. Briefly, THLE-2 cells were first cultured in 96-well plates (2×10^4/mL) for 24 hr, and pretreated with 60 μM acetaminophen. After 1 hr incubation, 10 μg/mL of herb was added to the wells, and the cells were reincubated for 24 hr. Control cells have not herb added. On the day of the proliferation assay, media were removed, 100 μL of fresh media were added followed by 20 μL of the MTS solution was added to each of the 96 wells and incubated at 37˚C for 1 hr in a humidified (5% CO_2) environment. The absorbance was read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, U. S. A.). The percentage inhibition was expressed as [viability level of test samples/viability level of acetaminophen-treated control)] × 100. The IC_{50} value, the sample concentration resulting in 50 % inhibition of cell viability, was determined using non-linear regression analysis (% inhibition versus concentration).

Statistical Analysis

Statistical analysis of the data was carried out using the Prism 3.02 software (GraphicPad Software Inc., CA, USA). V_{max} is maximal viability. EC_{50} is the concentration of herbs required to increase cell viability by 50%. All values are presented as means ± S.E.M. The differences between means of control and treatment data were determined using the two-way ANOVA. A value of $P < 0.05$ was considered statistically significant.

Acknowledgements

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (No. R13-2007-019-00000-0).

References

1. Luk, J. M. *et al*. Traditional chinese herbal medicines for treatment of liver fibrosis and cancer: From laboratory discovery to clinical evaluation. *Liver Int* **27**: 879-890 (2007).
2. Stickel, F. & Schuppan, D. Herbal medicine in the treatment of liver diseases. *Dig Liver Dis* **39**:293-304 (2007).
3. Sohn, J. H., Han, K. L., Choo, J. H. & Hwang, J. K. Macelignan protects hepg2 cells against tert-butylhydroperoxide-induced oxidative damage. *Biofactors* **29**:1-10 (2007).
4. Wu, Y., Li, L., Wen, T. & Li, Y. Q. Protective effects

of echinacoside on carbon tetrachloride-induced hepatotoxicity in rats. *Toxicology* **232**:50-56 (2007).
5. Yapar, K. *et al*. Hepatoprotective effect of l-carnitine against acute acetaminophen toxicity in mice. *Exp Toxicol Pathol* **59**(2):121-128 (2007).
6. Sivilotti, M. L., Yarema, M. C., Juurlink, D. N., Good, A. M. & Johnson, D.W. A risk quantification instrument for acute acetaminophen overdose patients treated with n-acetylcysteine. *Ann Emerg Med* **46**:263-271 (2005).
7. Kon, K., Kim, J. S., Jaeschke, H. & Lemasters, J. J. Mitochondrial permeability transition in acetaminophen-induced necrosis and apoptosis of cultured mouse hepatocytes. *Hepatology* **40**:1170-1179 (2004).
8. Devi, K. P., Sreepriya, M., Balakrishna, K. & Devaki, T. Protective effect of premna tomentosa extract (l. Verbanacae) on acetaminophen-induced mitochondrial dysfunction in rats. *Mol Cell Biochem* **272**:171-177 (2005).
9. James, L. P., Mayeux, P. R. & Hinson, J. A. Acetaminophen-induced hepatotoxicity. *Drug Metab Dispos* **31**:1499-1506 (2003).
10. Jaeschke, H., Knight, T. R. & Bajt, M. L. The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. *Toxicol Lett* **144**:279-288 (2003).
11. Chung, H. S. *et al*. Inhibition of nitric oxide and tumor necrosis factor-alpha by moutan cortex in activated mouse peritoneal macrophages. *Biol Pharm Bull* **30**:912-916 (2007).
12. Garle, M. J., Fentem, J. H. & Fry, J. R. In vitro cytotoxicity tests for the prediction of acute toxicity in vivo. *Toxicology in Vitro* **8**:1303-1312 (1994).
13. Zhang, L., Mu, X., Fu, J. & Zhou, Z. In vitro cytotoxicity assay with selected chemicals using human cells to predict target-organ toxicity of liver and kidney. *Toxicol In Vitro* **21**:734-740 (2007).
14. Liu, F. & Ng, T. B. Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sci* **66**:725-735 (2000).
15. Tian, X. Y. *et al*. Jacaranone glycosides from senecio scandens. *J Asian Nat Prod Res* **8**:125-132 (2006).
16. Li, S. L. *et al*. Identification of five hepatotoxic pyrrolizidine alkaloids in a commonly used traditional chinese medicinal herb, herba senecionis scandentis (qianliguang). *Rapid Commun Mass Spectrom* **22**: 591-602 (2008).
17. Jung, H. A. *et al*. Re-evaluation of the antioxidant prenylated flavonoids from the roots of sophora flavescens. *Biol Pharm Bull* **31**:908-915 (2008).
18. Liu, Z. *et al*. A mannose-binding lectin from sophora flavescens induces apoptosis in hela cells. *Phytomedicine* **15**(10):867-875 (2008).
19. Wang, Y. *et al*. Purification and preparation of compounds from an extract of scutellaria barbata d. Don using preparative parallel high performance liquid chromatography. *J Sep Sci* **31**:1669-1676 (2008).