

# Analytical Research to Determine the effects of the Components of ONGABO on the Viability of HepG2 Cancer Cells by Using the Sovereign, Minister, Assistant and Courier Principle (君臣佐使論)

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## Key Words

assistant and courier principle, cell viability, HepG2, minister, ONGABO, sovereign

## Abstract

**Objectives:** This study used the basic principle of Oriental medicine, the sovereign, minister, assistant and courier principle (君臣佐使論) to investigate the effects of the component of ONGABO, which is composed of Ginseng Radix (Red Ginseng), Angelica Gigantis Radix, Schisandrae Fructus, Cuscuta Semen and Curcumae tuber on the viability of HepG2 cells.

**Methods:** Single and mixed extracts of the component of ONGABO were prepared by lyophilizing powder of Red Ginseng (6-year root from Kanghwa), Angelica Gigantis Radix, Schisandrae Fructus, Cuscuta Semen, Curcumae Tuber (from Omniherb Co., Ltd., Korea) at the laboratory of herbal medicine in Woosuk University and were eluted after being macerated with 100% ethanol for three days. The cell viability of HepG2 was determined by using an absorptiometric analysis with PrestoBlue (Invitrogen) reagent after the plate had been

incubated for 48 hours. All of the experiments were repeated three times to obtain the average value and standard deviation. The statistical analysis was done and the correlation factor was obtained by using Microsoft Office Excel 2007 and Origin 6.0 software.

**Results:** Although Ginseng Radix (Red Ginseng) and Schisandrae Fructus did not enhance the viability of HepG2 cells, they were shown to provide protection of those cells. On the other hand, Angelica Gigantis Radix decreased the viability of HepG2 cells significantly, Cuscuta Semen and Curcumae Tuber had a small or no effect on the viability of HepG2 cells.

**Conclusions:** In the sovereign, minister, assistant and courier principle (君臣佐使論), Ginseng Radix (Red Ginseng) corresponds to the sovereign component because it provides cell protection effects, Angelica Gigantis Radix corresponds to minister medicinal because it kills cells, Schisandrae Fructus corresponds to the assistant medicinal to help red ginseng having cell protect effects. Cuscuta Semen and Curcumae Tuber correspond to the courier medicinal having no effect in cell viability in HepG2. We hope this study provides motivation for advanced research on the sovereign, minister, assistant and courier principle.

Received: Aug 1, 2012 Accepted: Oct 10, 2012

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## 1. Introduction

The basic principle of oriental medicine formulation, “sovereign, minister, assistant and courier theory [1] (君臣佐使論, Jun Chen Zuo Shi Lun)” was created during an ancient monarchy, and it is the basic principle of herbal formulation [2]. It is a method, which had been developed based on phrases from plain questions (Huangdi’s Internal Classic “主病之謂君 佐君之謂臣 應臣之謂使”) to enhance the efficacy of herbs while reducing their side effects by using compositions involving various kinds of herbs. The theory indicates that sovereign medicines cure diseases, minister and assistant medicines support the sovereign, and courier medicines deliver the other herbs to the right places in the body [2].

ONGABO is a valuable medicine that warms the body because most of its herbs are warm, and it consists of Red Ginseng, *Angelica Gigantis Radix*, *Schisandrae Fructus*, *Cuscuta Semen* and *Curcumae Tuber*.

Red Ginseng is originally from regular ginseng, a perennial herb of *Panax* of *Araliaceae* [3], the skin color of which turns red because its starch becomes pre-gelatinized during a steaming process for two to three hours so that it can be stored for longer periods. Recent reports have shown that it has antidiabetes [4,5], antioxidant [6], anticancer [7], anti-hyperlipidemia [8], and skin moisturizing effects [9].

*Angelicae Gigantis Radix* is a rhizome of the perennial herb *Apiaceae*, and its properties are warm and non toxic in nature, and sweet and pungent in flavor. It is related to heart, liver and spleen meridians. The herb mainly cures any kind of blood deficiency and has the function of tonifying blood and moistening dryness [10]. It is also known that enhance antioxidant [11], anticancer [12], anti-thrombotic [13], anti-inflammatory [14], and immune activities [12].

*Schisandrae Fructus* is the dried ripe fruit of the perennial deciduous woody vine *Schizandra chinensis* (Turcz.) Bail. (Chinese magnolia vine), or *Schisandra sphenanthera* Rehd. Et Wils. (orange magnolia vine), of *Schisandraceae* [15]. The herb is sour in flavor and warm in nature; it constrains the lungs and the liver tonifies the kidneys, astringes seminal emission, prevents diarrhea, and engenders the production of body fluid [16]. Its medicinal effects are known to be liver protection, probably because of diphenyl-dimethyl-dicarboxylate (PCM), which is artificially synthesized by using extracts of *Schisandra*, and suppression of liver damage caused by  $CCl_4$  [17]. It is also reported to be known an efficient antioxidant [18,19], antianemic [20], and skin brightener [21].

*Cuscuta Fructus* is the dried ripe seed, of the closely related plants *Cuscuta chinensis* Lam., *Cuscuta japonica*

*Choisy*, and *Cuscuta australis* R. Brown, all of which are annual parasitic herbs, which belong to *Convolvulaceae* [22]. The herb is known to be effective in treating hepatic dysfunction [23], to be an antioxidant [24], and to have vasodilating effects [25].

*Curcumae Tuber*, the withered tuberous root of *Curcuma Longa Radix* of *Zingiberaceae*, is used after being steamed or boiling in the water. Its properties are cool and non-toxic in nature, and the flavor is pungent and bitter. It is related to the heart and liver, and it moves qi. It disperses accumulations by entering to the Lung and the Gallbladder meridians and Qi Aspect (氣分); it cools the blood and dispels blood stasis by entering to the Blood Aspect (血分), so it is called the medicinal herbs as the Qi treatment medicine in the Blood. A recent report of its medicinal effect demonstrated that it suppressed liver damage [26], and enhanced anti-inflammatory, antioxidant [27], and antibacterial [28] activities.

The purpose of this research was to examine the significance of the components of the Oriental medicine ONGABO on the basis of the sovereign, minister, assistant and courier principle. This was done by using single and mixed extracts of the components of ONGABO to observe their effects on the proliferation of cultured hepatoma (HepG2) cells.

## 2. Materials and methods

The extracts were prepared by lyophilizing powders of Red Ginseng (6 year-root from Kanghwa), *Angelica Gigantis Radix*, *Schisandrae Fructus*, *Cuscuta Semen*, *Curcumae Tuber* (from Omniherb Co., Ltd., Korea) at the laboratory of herbal medicine in Woosuk University, and were eluted after being macerated with 100% ethanol for three days.

The component ratios for the extracts used in this research are given in Table 1.

HepG2 was purchased from the National Cancer Center in Korea, and the culture medium was from the Lonza’s MEGM Bullet Kit. HepG2 was cultivated for several days in BPE, hEFG, hydrocortisone and insulin from the bullet kit, then 100 ng/ml of a mixture of cholera toxin, penicillin, and streptomycin were added to the MEGM medium.

HepG2 was treated with trypsin/EDTA solution, then it was incubated in the wells of a 96 well plate after having been aliquoted at  $10 \times 10^5$  cells per well. After 24 hours of incubation, the medium was removed from the plate, and HepG2 was cultured with each of the extracts, in various concentrations. The wells were also injected with MEGM medium to which, with the exception of supplements, only penicillin and streptomycin had been added. The cell

**Table 1** Component ratio of the ONGABO extract.

Name of Herb	Weight(g)	Ratio(%)
Ginseng Radix (Red Ginseng)	6.5	48
Angelica Gigantis Radix	2.5	19
Schisandrae Fructus	2.5	19
Cuscuta Semen	1.0	7
Curcumae Tuber	1.0	7
Total	13.5	100

viability of HepG2 was determined by using an absorptiometric analysis with PrestoBlue (Invitrogen) reagent after the plate had been incubated for 48 hours.

All of the experiments were repeated three times to obtain the average value and standard deviation. The statistical analysis and correlation factor was achieved by using Microsoft Office Excel 2007 and origin 6.0 software.

### 3. Results

#### 3.1. Effect of a single ethanol extract for the various components of ONGABO on the cell viability of HepG2

The Red Ginseng ethanol extract did not have any direct effect on the viability of HepG2 cells for various concentrations of the extract 5 mg/ml to 160 mg/ml. The cell viability of 71.5% at 160 mg/ml will be addressed in the next section (Fig. 1A).

The Angelica Gigantis Radix ethanol extract had a significant effect on the viability of HepG2 cells at low concentrations. A cell viabilities and 39.4% of 82.9% were observed at concentrations of 15.6 mg/ml and 31.3 mg/ml, respectively. Thus, the extract reduced cell viability of the hepatoma cell line (Fig. 1B).

The Schisandrae Fructus ethanol extract had a minor effect on the viability of HepG2 cells; cell viability was 78.2% at 62.5 mg/ml concentration, but a small increase 110.0% was observed at a concentration of 7.8 mg/ml (Fig. 1C).

Similar to Red Ginseng, the Cuscuta Semen ethanol extract did not have any direct effect on the viability of HepG2 cells in the concentration range of 0.8 ~ 25.0 mg/ml (Fig. 1D).

The experiment result for the Curcumae Tuber ethanol extract was similar to that for Schisandrae Fructus ethanol extract as cell viability was 83.7% at an 83.7 mg/ml, but increased slightly in the concentration range of 0.8 ~ 12.5 mg/ml (Fig. 1E).

#### 3.2. Effect of multiple ethanol extracts for the various

#### component of ONGABO on the cell viability of HepG2

The synergistic effects of ethanol extracts of various combinations of the components from ONGABO were measured using mixtures of Red Ginseng and Angelica Gigantis (A); Red Ginseng and Curcumae Tuber (B); Angelica Gigantis Radix and Curcumae Tuber (C); Red ginseng, Angelica Gigantis Radix, and Curcumae Tuber (D); and Angelica Gigantis Radix, Curcumae Tuber, and Cuscuta Semen (E).

The concentrations of the components were the same as in Fig. 1, and these were mixed and identified as follows; M1 (Mixture Level 1 ; control, all components at 0 mg/ml), M2 (Mixture Level 2 ; Red Ginseng at 5.1 mg/ml, Angelica Gigantis Radix at 2.0 mg/ml, Schisandrae Fructus at 2.0 mg/ml, Cuscuta Semen at 0.8 mg/ml, and Curcumae Tuber at 0.8 mg/ml), M3 (Mixture Level 3 ; Red Ginseng at 10.2 mg/ml, Angelica Gigantis Radix at 3.9 mg/ml, Schisandrae Fructus at 3.9 mg/ml, Cuscuta Semen at 1.6 mg/ml, and Curcumae Tuber at 1.6 mg/ml), M4 (Mixture Level 4 ; Red Ginseng at 20.3 mg/ml, Angelica Gigantis Radix at 7.8 mg/ml, Schisandrae Fructus at 7.8 mg/ml, Cuscuta Semen at 3.1 mg/ml, and Curcumae Tuber at 3.1 mg/ml), M5 (Mixture Level 5 ; Red Ginseng at 40.6 mg/ml, Angelica Gigantis Radix at 15.6 mg/ml, Schisandrae Fructus at 15.6 mg/ml, Cuscuta Semen at 6.3 mg/ml, and Curcumae Tuber at 6.3 mg/ml), M6 (Mixture Level 6 ; Red Ginseng at 81.3 mg/ml, Angelica Gigantis Radix at 31.3 mg/ml, Schisandrae Fructus at 31.3 mg/ml, Cuscuta Semen at 12.5 mg/ml, and Curcumae Tuber at 12.5 mg/ml), and M7 (Mixture Level 7 ; Red Ginseng at 162.5 mg/ml, Angelica Gigantis Radix at 62.5 mg/ml, Schisandrae Fructus at 62.5 mg/ml, Cuscuta Semen at 25.0 mg/ml, and Curcumae Tuber 25.0 mg/ml).

In the ethanol extracts of the mixture of Red Ginseng and Angelica Gigantis Radix, the cell viability initially dropped to 88.8% at the M5, 37.6% at the M6, and 17.9% at the M7 concentration levels where as the cell viability was 100% at the M1, and the control concentration levels (Fig. 2 A).

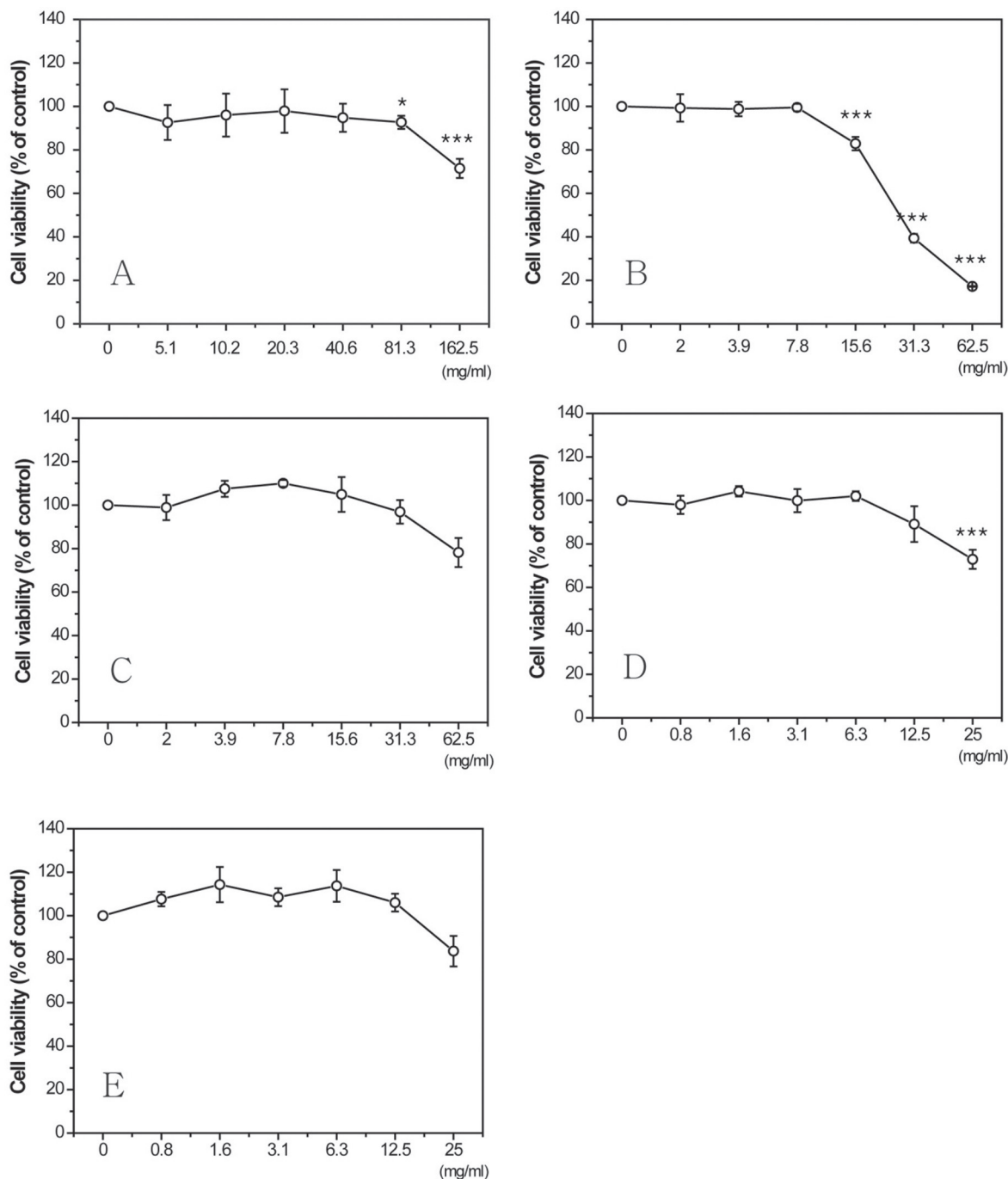
In the ethanol extracts of the mixture of Red Ginseng and

Curcumae Tuber, the cell viabilities were 106.9% at the M2, 108.1% at the M3, 106.2% at the M4, 109.1% at the M5, 99.7% at the M6, and 76.7% at the M7 concentration levels (Fig. 2 B).

In the ethanol extracts of the mixture of Angelica Gigantis Radix and Curcumae Tuber, the cell viabilities were 96.3%

at the M2, 101.0% at the M3, 94.9% at the M4, 80.0% at the M5, 41.7% at the M6, and 18.3% at the M7 concentration levels (Fig. 2 C).

In the ethanol extracts of the mixture of Red Ginseng, Angelica Gigantis Radix, and Curcumae Tuber, the cell viabilities were 105.5% at the M2, 100.5% at the M3, 98.0%

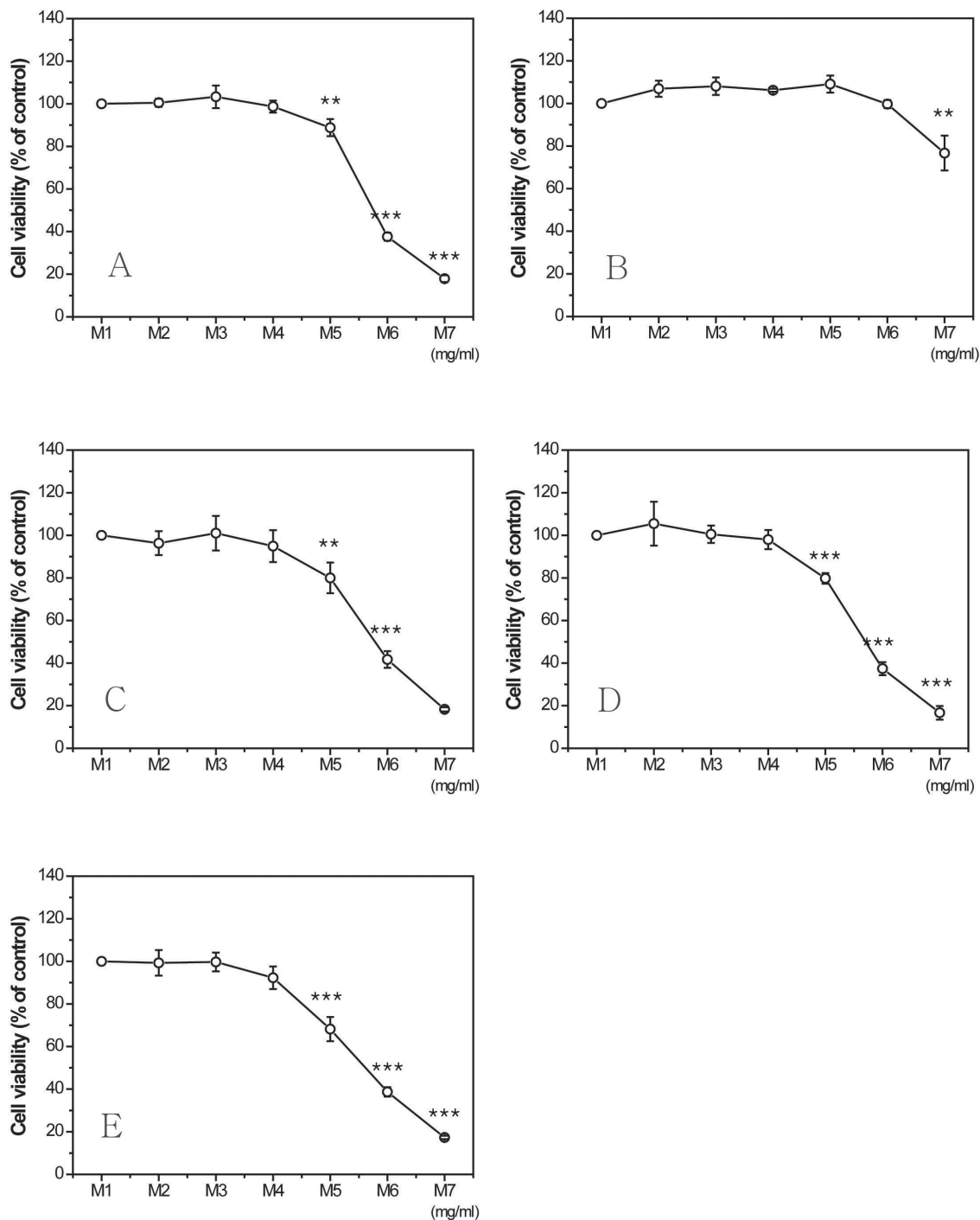


**Figure 1** Effects of the ethanol extracts of a single herb from among the herbs in ONGABO on the viability of HepG2 cells. A: Ginseng Radix (Red Ginseng), B: Angelica Gigantis Radix, C: Schisandra Fructus, D: Cuscuta Semen, and E: Curcumae Tuber effects. Data are means  $\pm$  SDs for triplicate experiment results. The *t*-test was performed by using origin 6.0 (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , vs. the control group).

at the M4, 79.8% at the M5, 37.4% at the M6, and 16.7% at the M7 concentration levels (Fig. 2 D).

In the ethanol extracts of the mixture of Angelica Gigantis Radix, Cuscuta Semen, and Curcumae Tuber, the cell

viabilities were 99.3% at the M2, 99.7% at the M3, 92.3% at the M4, 68.2% at the M5, 38.7% at the M6, and 17.3% at the M7 concentration levels (Fig. 2 E).



**Figure 2** Effects of ethanol extracts of mixture of the herbs in ONGABO on the viability of HepG2 cells.

A : Ginseng Radix (Red Ginseng) + Angelica Gigantis Radix, B : Ginseng Radix (Red Ginseng) + Schisandrae Fructus, C : Angelica Gigantis Radix + Curcumae Tuber, D : Ginseng Radix (Red Ginseng)+ Angelica Gigantis Radix + Curcumae Tuber and E : Angelica Gigantis Radix + Cuscuta Semen + Curcumae Tuber. Data are means  $\pm$  SDs for triplicate experiment results.

The *t*-test was performed by using by origin 6.0 (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , vs. control group).

### 3.3. Comparison of the effects of single and multiple ethanol extracts of ONGABO components on cell viability

The effect of the single and the multiple ethanol extracts on the viability of HepG2 cells were observed at a various concentration levels to compare and contrast the efficiency of the extracts based on data from previous experiments.

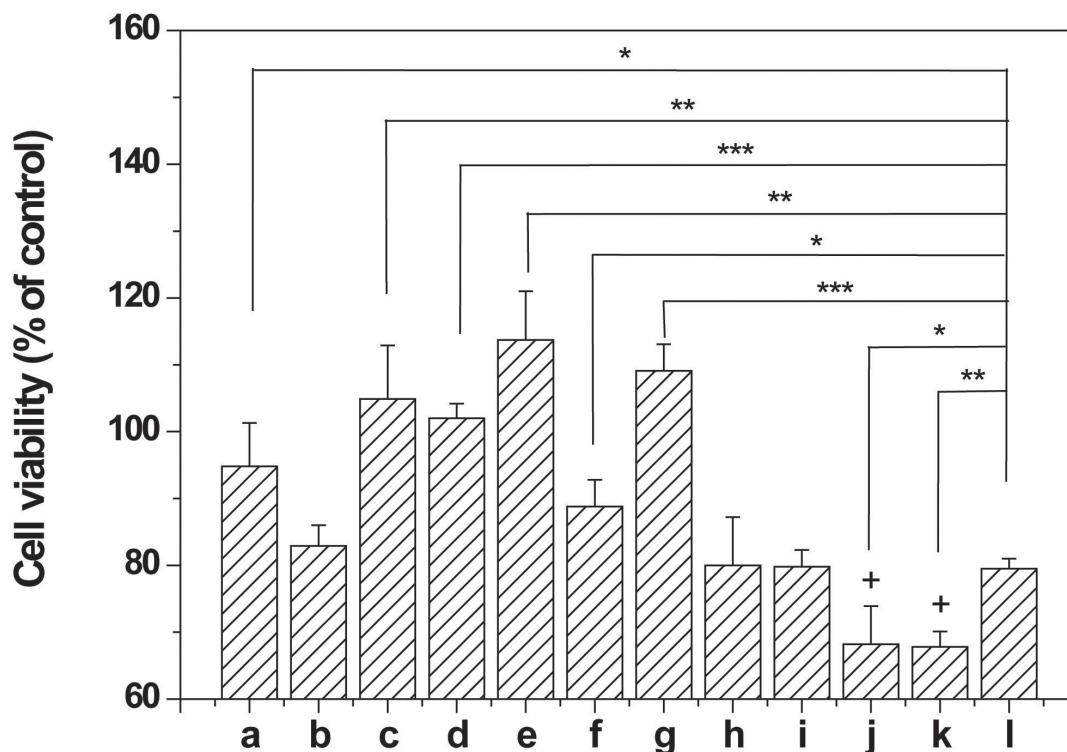
As Fig. 3 shows, for the single ethanol extracts, the viability of HepG2 cells was 94.8% at a 40.6 mg/ml Red Ginseng concentration (a), 82.9% at a 15.6 mg/ml Angelica Gigantis Radix concentration (b), 104.9% at a 15.6 mg/ml Schisandrae Fructus concentration (c), 102.0% at a 6.3 mg/ml Cuscuta Sement concentration (d), and 113.7% at a 6.3 mg/ml of Curcumae Tuber concentration (e).

For the multiple ethanol extracts at the same concentration of the each as in (a) to (e), the cell viability in Red Ginseng and Angelica Gigantis Radix extract was 88.8% (f); that in Red Ginseng and Curcumae Turber extract was 109.1% (g); that in Angelica Gigantis Radix and Curcumae

Turber was 80.0% (h); that in Red Ginseng, Angelica Gigantis Radix, and Curcumae Turber was 79.8% (i); that in Angelica Gigantis Radix, Cuscuta Semen, and Curcumae Turber was 68.2% (j); that in Red Ginseng, Angelica Gigantis Radix, Cuscuta Semen, and Curcumae Turber was 67.8% (k); and that in Red Ginseng, Angelica Gigantis Radix, Schisandrae Fructus, Cuscuta Semen, and Curcumae Tuber, all the component of ONGABO, was 79.5%.

## 4. Discussion

To treat diseases, various methods of enhancing the nature of drug and reducing their side effects have existed since ancient times; Processing of Herbal Medicinals Method (炮製, Pao Zhi; the preparation of Oriental medicinal herbs), Four Qi and Five Flavors Theory (four properties of drugs and five kinds of flavors), and Meridian Entry Theory and Tonification and Sedation Method



**Figure 3** Comparisons of the effects of single and the mixture ethanol extracts of the herbs in ONGABO on the viability of HepG2 cells.

a : Ginseng Radix (Red Ginseng), b : Angelica Gigantis Radix, c : Schisandrae Fructus, d : Cuscuta Semen, e : Curcumae Tuber, f : Ginseng Radix (Red Ginseng) + Angelica Gigantis Radix, g : Ginseng Radix (Red Ginseng) + Schisandrae Fructus, h : Angelica Gigantis Radix + Curcumae Tuber, i : Ginseng Radix (Red Ginseng) + Angelica Gigantis Radix + Curcumae Tuber, j : Angelica Gigantis Radix + Cuscuta Semen + Curcumae Tuber, k : Ginseng Radix (Red Ginseng) + Angelica Gigantis Radix + Cuscuta Semen + Curcumae Tuber, and l : Ginseng Radix (Red Ginseng) + Angelica Gigantis Radix + Schisandrae Fructus + Cuscuta Semen + Curcumae Tuber.

The concentrations of the various components in the experiment are Ginseng Radix (Red Ginseng) 40.6 mg/ml, Angelica Gigantis Radix 15.6 mg/ml, Schisandrae Fructus 15.6 mg/ml, Cuscuta Semen 6.3 mg/ml, and Curcumae Tuber 6.3 mg/ml. The *t*-test was performed by using origin 6.0 (\*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$  vs. the control group).

utilizing rising, falling, floating, or sinking for a single medicinal herb [1].

On the other hand, the basic principle of Oriental medicine for formulating drug, the sovereign, minister, assistant and courier principle, Jun Chen Zuo Shi Lun, is the method that reduces side effects and enhances the efficacy of a drug by combining many types of medicinal plants. The principle was developed, on the basis of the phrase, 'a sovereign medicine cures diseases, minister and assistant medicine support the sovereign, and a courier medicine delivers the other herbs to the right place in the body,' which are taken from The Great Treatise on what is Consummately True and Essential of The Inner Classic of the Yellow Emperor (Huangdi Neijing Suwen) [1].

The sovereign, minister, assistant and courier principle has been shown to enhance the remedial value of medicinal herbs as follows.

The sovereign medicine significantly enhances the therapeutic action on the main causes or symptoms of disease, the minister medicine aids the sovereign to reinforce the effect of the medicine, and the assistant medicine can be explained in three ways. First, it treats coexisting symptoms or additional symptoms, suppresses the toxicity or moderates the harsh properties of the herbs, and assists the main herb by using properties opposite those of the sovereign medicine. For example, hot and cold properties can be moderated to reach the goal of treatment without any resistance by adding small amount of herbs with cool or cold properties to herbs with warm or hot properties and vice versa [1]. The courier medicine guides the properties and actions of the medicine to a specific meridian channel and harmonizes all the ingredients of the formula.

According to the principle of herb combination theory, the following are used to treat diseases; two or more herbs with similar functions to enhance the remedial value of herbs, called Mutual Reinforcement (相須, Xiang Xu); two or more herbs with different functions, one the main and the other assistant, to enhance the therapeutic goal called Mutual Assistance (相使, Xiang Shi); two herbs where one reduce the toxicity or side effects of the other herb, called Mutual Restraint (相畏 Xiang Wei); two herbs where one reduces the undesirable side effects of the other, called Mutual Suppression (相殺, Xiang Sha); two herbs that may minimize, reduce, or destroy, in extreme conditions, each other's original properties or therapeutic effects called Mutual inhibition (相惡, Xiang Wu); two substances which when used together causes side effects produced by neither alone called Antagonism (相反, Xiang Fan) [1].

Oriental medicinal herbs are used to treat various diseases, but medicinal herb processing methods have not been developed as much as the principle of formulation

which has been developed significantly from the unaccompanied ginseng decoction (獨蔘湯, Du Shen Tang) to multi-herbal formulations. Thus, the influence of the processing method on the development of Oriental medicine is quite important. Our ancestors were aware of diverse methods for controlling the human body; they found that utilizing many kinds of medicines to control various factors in the human body was more beneficial than simply suppressing or activating the main factor by using only one medicine for diseases treatment or pain control.

Actually, in this study, the viabilities of A549, ACHN, AGS, DU145, PC-3, Panc-1 and HepG2 cells were observed based on the sovereign, minister, assistant and courier principle (君臣佐使論, Jun Chen Zuo Shi Lun). In our study, the viability of HepG2 cells was decreased most significantly affected by the specificity of the drugs, explaining the significance of the basic Oriental formulation principle was easier with the results of the HepG2 cells.

Thus, the significance of this study regarding the efficacy of Red Ginseng, Angelica Gigantis Radix, Schisandrae Fructus, Cuscuta Semen, and Curcumae Tuber, ONGABO's composition, on the viability of HepG2 cells was to analyze the meaning of the basic principle of Oriental medicine formulation, the sovereign, minister, assistant and courier principle (君臣佐使論, Jun Chen Zuo Shi Lun) based on the experiments using various extracts of the components of ONGABO at various concentrations.

According to the results of the experiment, Red Ginseng, Schisandrae Fructus, and Curcumae Tuber did not have significant effects on the viability of HepG2 cells, but Angelica Gigantis Radix did (Fig. 1). A study with Red Ginseng had reported that it enhanced the anti-proliferative effect of 5-fluorouracil on the human colorectal cancer cell line, especially concentrations of 0.2 to 0.3 mg/ml induced cell cycle arrest in the G1 phase rather than affecting cancer cell apoptotic induction activity [7]. We assumed that result to be very similar to our experimental result at a relatively high concentration of Red Ginseng extract of 170 mg/ml at which the viability of HepG2 cells started to drop significantly. As a result, Red Ginseng, which can be said the sovereign medicine due to its having the largest concentration in ONGABO, is thought to have the function of suppressing and maintaining the cells instead of destroying them. The same concentration of Schisandrae Fructus and Angelica Gigantis Radix did not suppress the cell viability, which was similar to an existing report on the liver protection effect [17]. Decrusin, a component of Angelica Gigantis Radix, has been reported to have anti-cancer effect activating PKC [29]. In fact, the optimal cell viability

suppression was measured at a 15 mg/ml concentration, a comparatively small amount. Thus, we conclude that *Angelica Gigantis Radix*, the second largest concentration in ONGABO, had the opposite pharmacological action to that of Red Ginseng. *Cuscuta Semen* has been reported to have an anti-cancer effect at a relatively low concentration of 0.1 mg/ml [30], which was not observed in this study and might be because of differences in the kinds of cells or the harvesting times. *Curcumae Tuber* has been reported to inhibit liver damage, an effect similar to that observed in this study [26].

The suppression of cell viability in the multiple ethanol extract of Red Ginseng, *Angelica Gigantis Radix*, *Schisandrae Fructus*, *Cuscuta Semen*, and *Curcumae Tuber* was more significant than it was in the multiple extract of Red Ginseng, *Schisandrae Fructus*, *Cuscuta Semen*, *Curcumae Tuber*. However, its suppression was insignificant compared to that of the single ethanol of *Angelica Gigantis Radix*.

This result indicated that a certain concentration of multiple ethanol extract had better effect on cell viability than single extract. Moreover, the cell viability or the multiple ethanol extract of Red Ginseng, *Angelica Gigantis Radix*, *Cuscuta Semen*, and *Curcumae Tuber* was lower than that for Red Ginseng, *Angelica Gigantis Radix*, *Schisandrae Fructus*, *Cuscuta Semen*, and *Curcumae Tuber* ( $p < 0.01$ ), which means that *Schisandrae Fructus*, the second largest concentration in Ongabo, along with *Angelica Gigantis Radix* has a cell protection effect and has an effect similar to Red Ginseng (Fig. 3). The cell viability of the multiple ethanol extract of Red Ginseng with *Angelica Gigantis Radix* (appx. 88.8%) was in the middle between the results for single ethanol extracts of Red Ginseng (appx. 94.8%) and of *Angelica Gigantis Radix* (appx. 15.6%), so Red Ginseng is thought to protect cells.

If sovereign, minister, assistant and courier principle (君臣佐使論, Jun Chen Zuo Shi Lun) is applied to the experimental results and if Red Ginseng is the Sovereign medicine in ONGABO, then regarding the viability of HepG2 cells, the sovereign has cell protection function, *Schisandrae Fructus* is the minister due to its having a function similar to that of Red Ginseng, and *Angelica Gigantis Radix* is the assistant due to its significant effect on hepatoma cell apoptotic induction activity. The functions of *Cuscuta Semen* and *Curcumae Tuber* are insignificant, but their effects depend on the other ingredients; thus, they should be the courier based on the principle. As a result, ONGABO is composed of Red Ginseng and *Schisandrae Fructus* to preserve the Essential Qi (精氣, Jing Qi), *Angelica Gigantis Radix* to enhance the remedial value, and *Cuscuta Semen* and *Curcumae Tuber* to harmonize the pharmacological properties.

Many researchers participate in developing new drugs by using medicinal herbs and natural substances, as well as various analytic techniques, and this study links the experimental result to a major classic theory of Oriental medicine, the sovereign, minister, assistant and courier principle (君臣佐使論, Jun Chen Zuo Shi Lun).

This attempt reestablishes the classic theory of Oriental medicine based on the result of experiment, and tries to develop new prescriptions of herbs; however, the observation of cell viability in such a simple experiment has a limit, so more research in many different ways is needed.

## 5. Conclusion

The herbal composition of Red Ginseng, *Angelica Gigantis Radix*, *Schisandrae Fructus*, *Cuscuta Semen*, and *Curcumae Tuber*, named ONGABO, and its effect on the viability of HepG2 cells were measured to analyze how its composition can be explained using the theory of prescription design called the sovereign, minister, assistant and courier principle (君臣佐使論, Jun Chen Zuo Shi Lun), which is a basic principle of Oriental medicine. The experiment showed that Red Ginseng and *Schisandrae Fructus* enhanced HepG2 cell viability, whereas *Angelica Gigantis Radix* significantly suppressed HepG2 cell viability, *Cuscuta Semen* and *Curcumae Tuber* had no effect on HepG2 cell viability.

The experimental results showed that the components of ONGABO did not have the same medicinal effects, but those effects could be categorized based on the cell viability by using the sovereign, minister, assistant and courier principle (君臣佐使論, Jun Chen Zuo Shi Lun).

Red Ginseng corresponded to the sovereign due to its having the largest concentration in ONGABO, and *Schisandrae Fructus* was in the minister position because its pharmacological action was similar to Red Ginseng's. *Angelica Gigantis Radix* had sizable effect on the apoptotic induction activity of the hepatoma cells, so it should be the assistant that supports the sovereign. *Cuscuta Semen* and *Curcumae Tuber* were the couriers because they had an effect on the medicinal action of the other components even though their individual effects were marginal.

Thus, the interpretation of ONGABO's prescription design is that ONGABO is composed of Red Ginseng and *Schisandrae Fructus* to preserve the essential energy (Jing Qi), *Angelica Gigantis Radix* to enhance the remedial value, and *Cuscuta Semen* and *Curcumae Tuber* to harmonize the pharmacological properties.

This study was an attempt to consider and understand the meaning of a classical herbal medicine design principle so that it could be applied to developing new medicines.



However, the interpretations may vary in many ways depending on the composition ratios and the concentrations of the herbs and the experimental conditions.

We hope this study motivates research on various kinds of experimental conditions and subjects observed in many different ways to establish a contemporary interpretation of the sovereign, minister, assistant and courier principle (君臣佐使論, Jun Chen Zuo Shi Lun).

## Acknowledgments

This research was supported by Wonkwang University in 2012.

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