

원저

## Comparative study of Various Ginseng Herbal Acupuncture

-By measuring the increase of life span of mice and Expression of cytokine mRNA-

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### Abstract

**Objectives** : The purpose of this study was to investigate anti-cancer effects of various ginseng herbal acupuncture in mice and expression of cytokine mRNA.

**Methods** : Anti-cancer effects of various ginseng herbal acupuncture were tested by measuring the increase of life span of mice suffering from peritoneal cancer induced by Sarcoma-180, and expression of mRNA manifestation using RT-PCR. The results are as follows:

**Results** :

1. Increase of life span of mice suffering from peritoneal cancer induced by Sarcoma-180 was measured for anti-cancer effects. As a result, 115% increase was shown in the cultivated wild ginseng group, 11.1% increase in the red ginseng group, and no increase was detected in either white ginseng and fresh ginseng groups.
2. Measuring the expression of cytokine mRNA manifestation, expression of interferon- $\gamma$  was slightly increased in the cultivated wild ginseng group compared to the control group, but manifestation of interleukin-10 was slightly decreased.
3. For the red ginseng, white ginseng, and fresh ginseng experiment groups II, IL-2, IL-4, INF- $\gamma$ , and IL-10 all showed increase suggesting possible error occurring during the test process.

**Conclusion** :

From the results obtained in this study, we can reason that the ginseng we use may not match the ginseng cited in the texts of the past. Anti-cancer effects of cultivated wild ginseng can be more potent than those of white and fresh ginseng.

**Key words** : various ginseng, herbal acupuncture, cultivated wild ginseng, Sarcoma-180 cancer cell, cytokine mRNA

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## I. Introduction

Ginseng is a leading tonic herb in Oriental medicine. Despite its popular usage, ginseng in use nowadays differs from the one listed in the texts of the past<sup>1)</sup>. Ginseng used for the treatment purpose is cultivated and despite ginseng has been cultivated as a same species, changes in the environment and generations of cultivation brought suspicion as it may not yield same benefits listed in the old texts. This study was designed with this background.

Herbal acupuncture is an unique treatment method of Korean traditional medicine which combines the benefits of herb and acupuncture<sup>2)</sup>. Various herbs are developed for injection on the meridian points and most suitable herbal acupuncture points are selected depending on the patient's constitution and the state of disorder. Few of herbal acupuncture include meridian herbal acupuncture, eight principle herbal acupuncture, bee venom herbal acupuncture, and others. One of the primary benefits of herbal acupuncture is that optimal results can be gained from using small quantity of herb. Eight principle herbal acupuncture is a method trapping the steam generated during boiling and over 40 different herbs are in use clinically. Various types of ginseng, fresh, red, white, and cultivated wild ginseng which is planted from the seeds of natural wild ginseng were chosen and distilled as eight principle herbal acupuncture. Same amount of herbal acupuncture was injected into mice with sarcoma-180 induced peritoneal cancer and significant results were obtained in this study.

## II. Materials and Methods

### 1. Experiment Animals

4 weeks old Balb/c mice in the weight range of  $20 \pm 3g$  were fed with solid food and water for 2 weeks and adapted to the experiment surrounding before conducting the test. Experiment groups comprise of normal group, control group, white ginseng group, fresh ginseng group, red ginseng group, and cultivated wild ginseng group. Each group consisted of 10 animals.

### 2. Materials

#### 1) Preparation of herbal acupuncture<sup>2)</sup>

White ginseng aged 6 years and red ginseng used in the experiment were the products of Korea Tobacco and Ginseng Corporation. Fresh ginseng was diagnosed as 6 years of age by the expert and cultivated wild ginseng was estimated to be 10 years old and obtained from a mountain with the altitude of 600m in Kangwon province(Fig. 1-2).

Herbal acupuncture is manufactured under the following process: Ginseng is rinsed in a running water and then decocted for 2 hours in

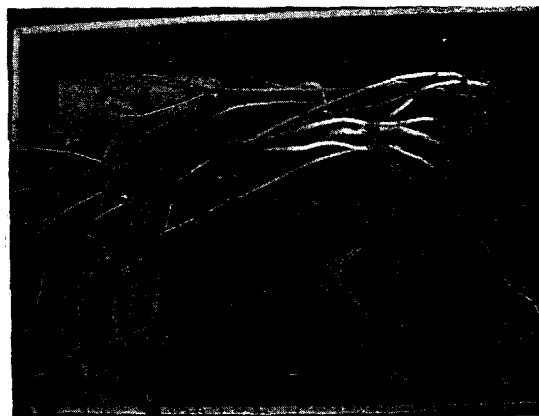


Fig. 1. Shape of cultivated wild ginseng

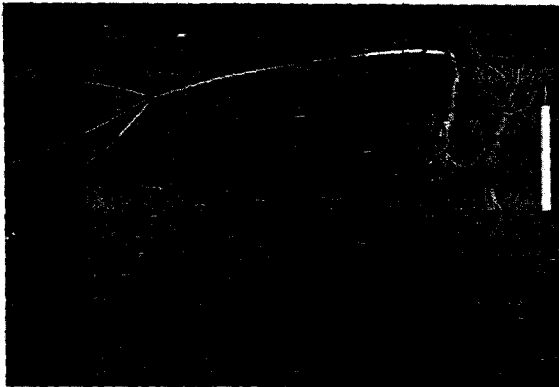


Fig. 2. Size of cultivated wild ginseng. About 8-16cm in length

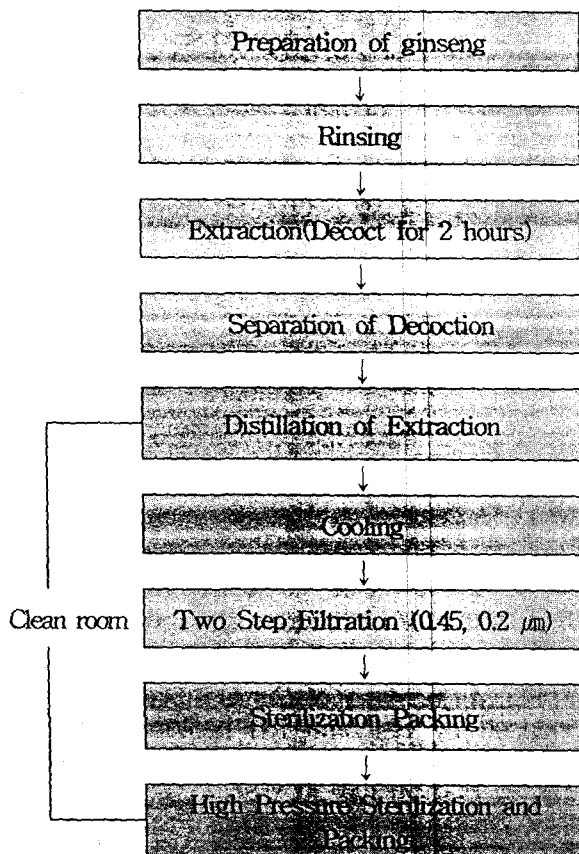


Fig. 3. Manufacturing process of Various Distilled Ginseng Herbal Acupuncture

distilled water. Remnants are then removed and decoction went through distillation before yielding the desired herbal acupuncture. Then the herbal acupuncture was filtered twice using 0.45 $\mu$ m, 0.2 $\mu$ m filtering paper and then kept in

the container. Finally, herbal acupuncture is sterilized before being used (Fig. 3).

## 2) Syringe

30 gauge 1 ml insulin syringe (Becton Dickinson, U.S.A.) was used for the experiment.

## III. Methods

### 1. Cultivation of Cancer Cells

To induce peritoneal cancer in the Balb/c mice, Sarcoma-180 cancer cell line was obtained from KCLB (Korean Cell Line Bank) in the frozen state.

### 2. Administration of Herbal Acupuncture

Sarcoma-180 cancer cells were injected peritoneally in Balb/c mice and after 2 days, intravenous injection was done twice a week for total of 8 times. For the control group, same procedure was operated using 0.1cc of saline solution.

### 3. Anti-cancer effects against Sarcoma-180

#### 1) Measurement of increase in survival rate

Presence of peritoneal cancer and survival rate were observed for 30 days. Subjects without occurrence of peritoneal cancer after 30 days were omitted from the calculation of survival rate. Using the mean survival time accounted by Geran<sup>3)</sup> and others, increase of life span was yielded.

$$\text{Median survival time} = \frac{X + Y}{2}$$

$$\text{increase of life span} = T - C / C \times 100$$

X : First time when the surviving animals are 1/2 of the total animals (day)

Y : First time when the surviving animals are 1/3 of the total animals-1 (day)

T : median survival time (days) of the experiment groups

C : median survival time(days) of the control group

## 2) Manifestation of Cytokine mRNA

### ① Total RNA isolation

Total RNA was extracted from the spleen of the mice using Tissue RNA PrepMate kit (Bioneer, Korea) and quantified with UV spectrophotometer (260/280nm).

② Reverse transcription-polymerase chain reaction

Total RNA 2μg extracted from the spleen of the mice was used to measure β-actin, IFN-γ and IL-2 via oligo-(dT)15 primer (Promega, USA) 1μl, and IL-4 and IL-10 were pre-incubated by gene specific antisense primer 1μM from Bioneer. Then inactivation was carried out for AMV reverse transcriptase. cDNA 2μl obtained from reverse transcription was mixed with dNTP mixture 200μM, gene specific primer 300nM, MgCl2 2mM, reaction buffer(10mM Tris-HCl [pH9.0 at 25°C], 50mM KCl, 0.1% Triton X-100), and Taq polymerase 2U to conduct

PCR. Then RT-PCR product was confirmed with 2% agarose gel electrophoresis.

Specific primers for cytokines and β-actin are as follow: (Table 1).

## 4. Statistical analysis

SPSS (Release 10.9.7) was the program used for statistical analysis and P-value of less than 0.05 under the student's T-test was considered as significant.

## IV. Results

### 1. Increased Survival Rate of mice induced by Sarcoma-180 peritoneal cancer

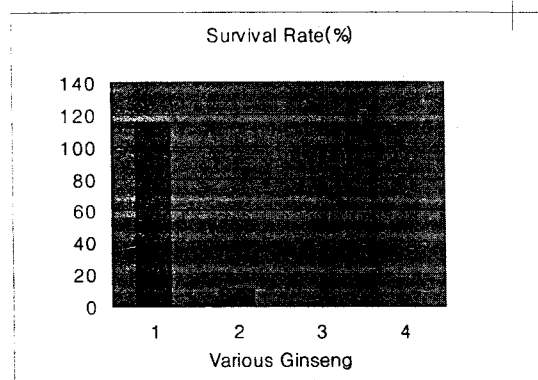


Fig. 4. Increase of Survival Rate of mice induced by Sarcoma-180 peritoneal cancer and treated with various Distilled Ginseng Herbal Acupuncture in vivo.

Table 1. Specific primers for cytokines and β-actin.

Name(Product size)		primer
β-actin (349 bp)	sense	5'-TGGAATCCTGTGGCATCCATGAAAC-3'
	anti-sense	5'-TAAAACGCAGCTCAGTAACAGTCCG-3'
IL-2 (168 bp)	sense	5'-TGATGGACCTACAGGAGCTCCTGAG-3'
	anti-sense	5'-GAGTCAAATCCAGAACATGCCGCAG-3'
IL-4 (132 bp)	sense	5'-ACGCCATGCACGGAGATGGAT-3'
	anti-sense	5'-CAAGCATGGAGTTTTCC-3'
IL-10 (421 bp)	sense	5'-AGACTTTCTTTCAAACAAAGGACCAGCTGGA-3'
	anti-sense	5'-CCTGGAGTCCAGCAGACTCAATACACACTGC-3'
IFN-γ (247 bp)	sense	5'-AGCGGCTGACTGAACTCAGATTGTAG-3'
	anti-sense	5'-GTCACAGTTTCAGCTGTATAGGG-3'

Increase of survival rate was 115% in the cultivated wild ginseng group compared to the control group and 11.1% increase was measured in the red ginseng group. No increase was detected in either white ginseng and fresh ginseng groups. (Fig. 4).

Group 1 was treated with Cultivated wild Ginseng Herbal Acupuncture, group 2 was treated with Red Ginseng Herbal Acupuncture, group 3 was treated with white Ginseng Herbal Acupuncture, and group 4 was treated with fresh Ginseng Herbal Acupuncture.

## 2. Expression of cytokine mRNA

To analyze the immune response of sarcoma-180 induced mice by various treatments, manifestation of cytokine mRNA was verified. Experiment groups comprise of normal group, control group induced with Sarcoma-180(no treatment), administered group I with 0.1cc injection and administered group II with 0.2cc injection. Total RNA was extracted from the spleen and investigated using RT-PCR method. As a result, comparing  $\beta$ -actin and each of cytokine used as internal control, manifestation of interferon- $\gamma$  was slightly increased in the cultivated wild ginseng group compared to the control group and interleukin-10 was slightly decreased(Fig. 5). Red ginseng group II showed increase of

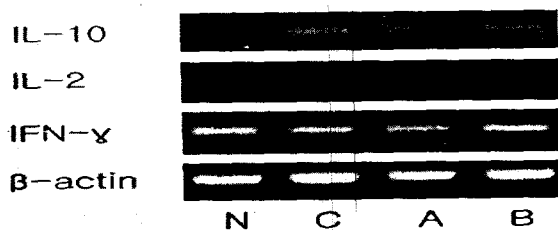


Fig. 5. Expression of cytokine mRNA extracted from the spleen by reverse transcription-polymerase chain reaction analysis. After a mouse was inoculated with Sarcoma-180, it was treated with in variable contents.

IL-2, IL-4, INF- $\gamma$ , and IL-10(Fig. 6), and IL-2 and INF- $\gamma$  were increased in both the experiment groups for red ginseng(Fig. 7).

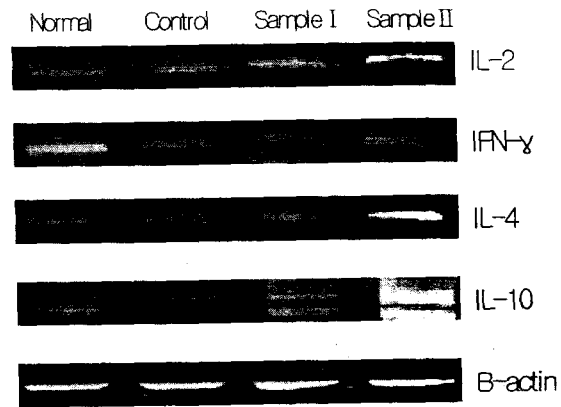


Fig. 6. Expression of cytokine mRNA extracted from the spleen using reverse transcription-polymerase chain reaction analysis. After a mouse was inoculated with Sarcoma-180, it was treated with distilled red-ginseng Herbal acupuncture in variable contents.

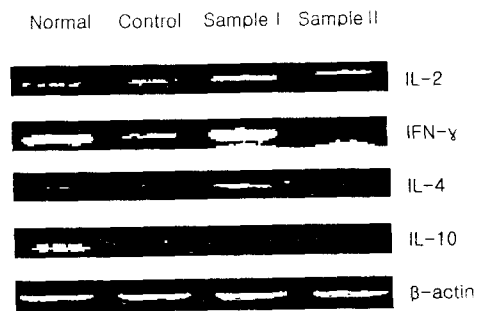


Fig. 7. Expression of cytokine mRNA extracted from the spleen by reverse transcription-polymerase chain reaction analysis. After a mouse was inoculated with Sarcoma-180, it was treated with distilled white-ginseng Herbal Acupuncture in variable contents.

For fresh ginseng herbal acupuncture, both group I and II showed increase of IL-2, IL-4, and INF- $\gamma$ (Fig. 8).

N refers to normal group, Normal is non-treated group, C refers to control group (only infected), A means treated with Cultivated wild Ginseng Herbal

Acupuncture(0.1ml), and B means treated with Cultivated wild Ginseng Herbal Acupuncture (0.2ml)

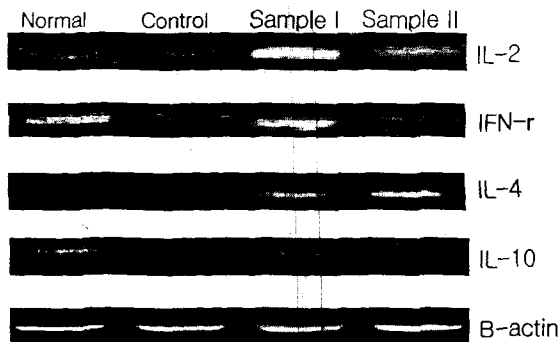


Fig. 8 Expression of cytokines mRNA from spleens by reverse transcription-polymerase chain reaction analysis. After a mouse was inoculated with Sarcoma-180, it was treated with distilled fresh ginseng Herbal acupuncture in variable contents.

Normal is non-treated group, Control is treated with normal saline(0.1 cc), Sample I is treated with distilled red-ginseng Herbal acupuncture(0.1 cc), and Sample II is treated with distilled red-ginseng Herbal acupuncture(0.2 cc)

Normal is non-treated group, Control is treated with normal saline(0.1 cc), Sample I is treated with distilled white-ginseng Herbal acupuncture(0.1 cc), and Sample II is treated with distilled white-ginseng Herbal acupuncture(0.2 cc)

Normal is non-treated group, Control is treated with normal saline(0.1 cc), Sample I is treated with distilled fresh-ginseng Herbal acupuncture(0.1 cc), and Sample II is treated with distilled fresh-ginseng Herbal acupuncture (0.2 cc)

## V. Discussion

Cancer refers to a new biological form

produced from genetic alterations and prolonged exposure to abnormal conditions. Cancer metastasizes into neighboring tissues and organs and threatens not only health but eventually leading into death.<sup>4)</sup> Ginseng is the root of the plant in the class of Araliaceae and is the leading tonic and stimulant in Oriental medicine.<sup>5)</sup> Ginseng has been known to be used for more than 2,000 years.<sup>6)</sup> Scientific research on ginseng only dates back to 1854 when Garriques<sup>7-8)</sup> named saponin composite retrieved from American ginseng (*Panax quinquefolium* L.) as Panaxin. Extensive study on ginseng was initiated by the Russian pharmacologist Brekhman<sup>9-10)</sup> in 1957 when he reported saponin as the responsible constituent of reactive agent in the CNS. Various components of ginseng play different pharmacological actions. Efficacies reported for saponin includes sedative function for CNS<sup>11-12)</sup>, facilitating protein synthesis<sup>13-14)</sup>, hormone secretion from adrenal cortex<sup>15-16)</sup>, detoxification<sup>17)</sup>, anti-inflammatory, inhibition of arteriosclerosis<sup>18)</sup>, diuresis, anti-cancer<sup>19)</sup>, recovery from fatigue<sup>20)</sup>, and others. Each ginsenoside is known to have specific pharmacological action.

From literary contemplation, ginseng used prior to the 15th century was natural wild ginseng not the cultivated ginseng of today<sup>1)</sup>. Same amount of ginseng is prescribed in modern days and this study was initiated from the suspicion whether current ginseng can yield the benefits of the past.

But natural wild ginseng is not only hard to find but its high expense hindered feasibility as an experiment subject as well as verification. This study substituted natural wild ginseng with cultivated wild ginseng. Cultivated wild ginseng refers to ginseng germinated in the high altitude region from the seeds of natural wild ginseng. Growth

environment is similar to that of natural wild ginseng and because of low cost in respect to natural wild ginseng, cultivated wild ginseng estimated to be 10 years old was chosen for the experiment.

Peritoneal cancer was induced by injecting Sarcoma-180 in mice and survival rate was measured. Increase of survival rate was 115% in the cultivated wild ginseng group compared to the control group and 11.1% increase was measured in the red ginseng group. No increase was detected in either white ginseng and fresh ginseng groups.

To analyze the immune response of sarcoma-180 induced mice by various treatments, manifestation of cytokine mRNA was verified. As a result, manifestation of interferon- $\gamma$  was slightly increased in the cultivated wild ginseng group compared to the control group and interleukin-10 was slightly decreased (Fig. 5). This brings about presumption that cultivated wild ginseng herbal acupuncture stimulates the production of INF- $\gamma$ , IL-12, and tumor necrosis- $\beta$  (TNF- $\beta$ ) leading into the production of Th1 cells which govern cell mediated immune response. In addition, it suppresses the production of Th2 cells which mediate humoral immune response by generating interleukin-4, interleukin-5, interleukin-10, and interleukin-13.

Red ginseng group II showed increase of IL-2, IL-4, INF- $\gamma$ , and IL-10 which doesn't comply with mutual restraining action of Th1 and Th2 suggesting possible error occurring during the test process (Fig. 6).

Identical results were obtained in both white and fresh ginseng groups where IL-2, IL-4, and INF- $\gamma$  all showed increase (Fig. 7, 8).

From the results obtained in this study, we can reason that the ginseng we use may not match the ginseng cited in the texts of the past. But this study was not aimed to

measure price/performance ratio, and further studies must be followed to build concrete outcome.

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