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Antitumor Effect of Mugwort (*Artemisia princeps* Pampan.) *in vivo*

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Abstract – This study was devised to observe the antitumor activity of mugwort (*Artemisia princeps* Pampan.) against ICR mice inoculated with sarcoma-180 cells. The antitumor compounds were partially purified from petroleum ether extract of mugwort by silicic acid column chromatography. The active fraction used in *in vivo* test was obtained under the elution with acetone in silicic acid column chromatography. When the acetone fraction was intraperitoneally injected to the mice which had been subcutaneously inoculated on the left groin with sarcoma-180, the growth rate of tumor (sarcoma-180 mass) was inhibited by 30%. In case the acetone fraction was injected to the mice which had been inoculated intraperitoneally with sarcoma-180, the average life span was prolonged by 20%. After the injection of the active fraction, the spleen index and γ-globulin ratio (%) were increased significantly ($p<0.05$). The administration of acetone fraction did not cause any abnormality in the body and the homeostasis of mice. Those observations suggest that the acetone fraction of mugwort extract has an antitumor effect *in vivo*.

Key words – *Artemisia princeps* Pampan., antitumor activity, acetone fraction.

Introduction

Medical cure of cancer is one of the most difficult problems with which the modern medical science face. Most of the medicines against cancer used recently in the clinical field are chemically synthetic compounds. These chemical compounds often exhibit serious side effects. Therefore, several attempts (Chichara *et al.*, 1970; Miyazaki *et al.*, 1981; Xu *et al.*, 1989) have been made to develop new anticancer and cancer prophylactic agents from naturally occurring compounds, which lack side effects. It has been studied and reported that the extracts of several herbs had potent cytotoxic activities against some cancer cells both *in vitro* and *in vivo* (Hwang *et al.*, 1988; Hwang, 1993; Lee *et al.*, 1998).

Mugwort has been extensively used as a food additive and a herb medicine in several oriental countries (Huang *et al.*, 1991; Liu *et al.*, 1992). It was reported that extract of *Artemisia lavendulaefolia* DC and volatile compounds of *Artemisia asiatica* Nakai showed the antimutagenic effects (Lee *et al.*, 1988; Kim *et al.*, 1992) and phenol compounds of some *Artemisia* spp. had antitumor activity (Ryakhovskaya *et al.*, 1989).

We reported that the anticancer compound was partially purified from petroleum ether extract of mugwort (*Artemisia princeps* Pampan.) by silicic acid column chromatography and the fraction which was eluted by acetone in chromatography showed potent cytotoxicity against mouse leukemia cell line (L1210), human colon cancer cell line (HCT-48) and human hepatoma cell line (HepG2), but was less effective with normal cell line (mouse embryo cell) (Hwang *et al.*, 1998).

As a part of on-going investigations, this study was performed to confirm the anticancer activity of mugwort extract *in vivo*. Effects of mugwort extract on the survival time of tumor bearing mice, the growth inhibition of solid tumor, weight of the immune organs, γ-globulin level and the biochemical components of serum were observed.

Experimental

Animals – ICR mice were purchased from Korea Experimental Animals Ltd. The weight was about 20-25 g. All mice used were female.

Mugwort extraction – The mugwort extract was prepared as reported previously (Hwang *et al.*, 1998). The mugworts were finely pulverized using mortar

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and 200 g powder were subjected to extraction with petroleum ether (2 L) at 40 °C for 3 days in shaking incubator. The crude extract prepared from the above was filtered by 0.22 μm membrane filter and thoroughly dried by vacuum evaporation using a rotary evaporator under nitrogen gas. The petroleum ether extract (0.5 g, dry weight) dissolved in 5 ml chroloform was loaded to Bio-Sil A (Bio-Rad Laboratories, Richmond, CA) silicic acid column (2.5×30 cm), and stepwise elution was processed with 175 ml chloroform, 700 ml acetone and 175 ml methanol. From each separated fraction, acetone fraction was selected for further studies, and the eluate was dried by vacuum evaporation. For the experiments, the dried acetone fractions were dissolved in small amount of absolute ethanol and diluted with saline to the desired concentration.

Growth inhibition test of solid tumor – Sixteen ICR mice (body weight, 20-25 g) were divided into two groups (each group contained 8 mice). At the beginning of the experiment, all mice were injected subcutaneously with 0.1 ml (1.0×10^6 cells/mouse) of sarcoma-180 cells on the left groin of mice on day 0. Experimental groups were injected intraperitoneally everyday with 2 mg/0.1 ml/mouse of acetone fraction for 10 days from day 1. Mice of control group were administered with saline (0.1 ml/mouse) during the same period. On the 12th day after the last treatment, all mice were sacrificed and their tumor mass were determined and photographed (Ha *et al.*, 1991).

Inhibition ratio was obtained by calculating as follows;

$$\text{Tumor growth inhibition ratio (\%)} = \frac{C_w - T_w}{C_w} \times 100$$

(C_w: tumor weight of control group, T_w: tumor weight of experimental group)

Effect on survival time of tumor-bearing mice – Twenty ICR mice (body weight, 20-25 g) were divided into two groups (each group contained 10 mice). All mice were intraperitoneally injected with 0.1 ml (1.0×10^6 cells/mouse) of sarcoma-180 cells on day 0, and survived mice were estimated daily. Experimental groups were intraperitoneally injected everyday with 2 mg/0.1 ml/mouse of acetone fraction for 10 days from day 1. Mice of control group were administered with saline (0.1 ml/mouse) during the same period. On the 7th and 14th day after injection of sarcoma-180 cells, the body weight of all mice was measured and the mean survival time was calculated from day 0 to day 30 (Lee *et al.*, 1992).

Prolongation ratio was calculated as follows ;

$$\text{Prolongation ratio (\%)} = \frac{T - C}{C} \times 100$$

(C: mean survival day of control group, T: mean survival day of experimental group)

Effect on immune organ weight – Thirty ICR mice (body weight, 20-25 g) were divided into three groups (each group contained 10 mice). Two experimental groups were intraperitoneally administered with 1 mg/0.1 ml /mouse and 2 mg/0.1 ml/mouse of acetone fraction respectively for 10 days daily. Mice of control group were administered with saline (0.1 ml/mouse) during the same period. On the 15th day after the last treatment, all of the mice were sacrificed and liver, spleen and thymus were dissected in order to determine their weights (Kim *et al.*, 1988).

Organ index was expressed as follows ;

$$\text{Organ index (\%)} = \frac{\text{organ weight}}{\text{body weight}} \times 100$$

Effect on γ-globulin level – Thirty ICR mice (body weight, 20-25 g) were divided into three groups (each group contained 10 mice). Two experimental groups were intraperitoneally administered with 1 mg/0.1 ml /mouse and 2 mg/0.1 ml/mouse of acetone fraction for 10 days daily, respectively. Mice of control group were administered with saline (0.1 ml/mouse) during the same period. On the 15th day after the last treatment, blood from all mice was obtained by cardiac puncture. Blood was kept in room temperature for 30 min and centrifuged at 3,000 rpm. The supernatant of all serum samples was subjected to cellulose acetate electrophoresis (Helena, USA) and albumin, α_1-globulin, α_2-globulin, β-globulin and γ-globulin levels were analyzed (Hwang *et al.*, 1996).

Effect on biochemical components of blood – In the all serum samples prepared from the above, the levels of total protein, albumin, bilirubin, alkaline phosphatase (ALP), creatinine and glutamate pyruvate transaminase (GPT) were determined by serum auto-analyzer (Hitachi 747, Japan). Clinical methods were shown in Table 1. In blood obtained by cardiac puncture, the numbers of hemoglobin, hematocrit and red blood cells (RBC) were measured. Mean corpuscular volume (MCV) and mean corpuscular

Table 1. Methods for clinical chemistry

Test	Method
Total protein	Biuret (Layne, 1957)
Albumin	Bromocresol green (Gustafsson, 1978)
Total bilirubin	Diazo reaction (Iwata, 1975)
ALP	*p*-Nitrophenyl phosphate (Tiez *et al.*, 1986)
Creatinine	Jaffe reaction (Jung, 1987)
GPT	Photometric (Hamada *et al.*, 1976)

hemoglobin (MCH) were calculated. From the differential count of white blood cells (WBC), total number of WBC was counted and the ratio of neutrophil, lymphocyte, monocyte and eosinophil was calculated.

Statistical analysis – All data represented means± standard error. A Student's t-test was used for measuring the significance of a difference of means between control and experimental groups (Huck *et al.*, 1974).

Results and Discussion

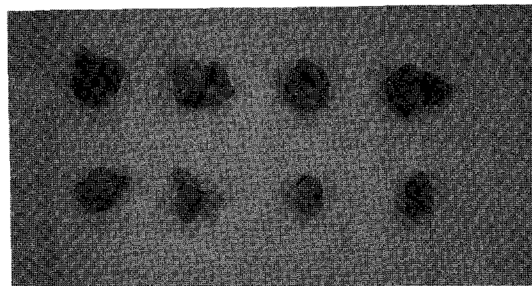
Growth inhibition of solid tumor – As shown in Table 2, The average tumor weight obtained in experimental group was 2.52±0.29 g and in control group 3.59±1.09 g. The acetone fraction inhibited the growth rate of solid tumor in experimental group by 29.8% compared to control group. Photographs in Fig. 1 show the decrease of solid tumor's size in experimental group. These results clearly indicated an anticancer effect of acetone fraction, evidenced by decreasing the size as well as weight of tumor mass. It is noted that due to death of one mouse by individual variation, seven samples of experimental group were shown in Fig. 1.

Effect on survival time of tumor-bearing mice – As shown in Table 3, acetone fraction had a prolongation effect of 20.5% on the life-span of ICR mice inoculated with sarcoma-180 cells. In this experiment, there were no survived mice more than 30 days. The average life-span of experimental group was 20.60 ±1.34 days and that of control group was 17.10±1.35 days. Fig. 2 shows survival time of experimental group and control group. These results clearly suggest that the life-span of treated group has been prolonged against sarcoma-180 cells.

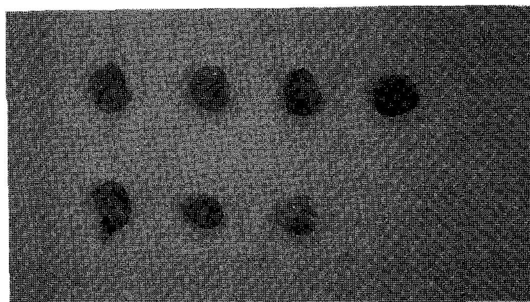
Table 2. Antitumor effects of acetone fraction of mugwort in ICR mice inoculated with sarcoma-180 cells

Group	Tumor weight[1] (g)	Inhibition ratio (%)
Control	3.59±1.09	0
Treatment (2 mg/mouse)	2.52±0.29*	29.81

Acetone fraction was intraperitoneally administered to treatment group. [1]All data are expressed as means±standard error. *p<0.05



Control group



Experimental group

Fig. 1. Photographs of S-180 mass of tumor bearing ICR mice injected with (2 mg/head) or without (control) acetone fraction of mugwort from silica gel column chromatography.

Table 3. Effect of acetone fraction of mugwort on life span of tumor bearing ICR mice inoculated with sarcoma-180 cells

Group	Survival time[1] (day)	Prolongation ratio (%)
Control	17.10±1.35	0
Teatment (2 mg/mouse)	20.60±1.34*	20.47

Acetone fraction was intraperitoneally administered to treatment group. [1]All values are expressed as means±standard error. *p<0.05

Effect on immune organ weight – Table 4 shows the effect by injection of acetone fraction on the immune organ weight of ICR mice. Liver and thymus index did not show any differences between control and experimental group. In two experimental groups of 1 mg/mouse and 2 mg/mouse injection,

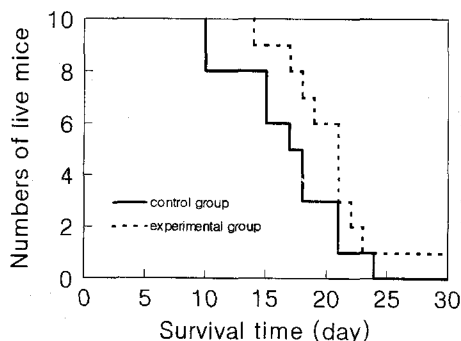


Fig. 2. Effects of acetone fraction (2 mg/mouse) of mugwort from silicic acid column chromatography on the life span of ICR mice inoculated with sarcoma-180 cells.

Table 4. Effect of acetone fraction of mugwort on the immunoorgan weight of ICR mice

Items	Dose (mg/mouse)		
	0 (control)	1	2
Liver index (%)	5.12±0.01	5.43±0.01	5.28±0.16
Spleen index (%)	0.51±0.02	0.65±0.05*	0.71±0.09*
Thymus index (%)	0.22±0.02	0.23±0.02	0.22±0.03

All data are expressed as means±standard error. *Significant enhancement of the spleen index in treated group compared to control group ($p<0.05$)

spleen index was increased (0.65±0.05 and 0.71±0.09, respectively) significantly compared to that of control group (0.51±0.02). Spleen is a peripheral lymphoid organ which produces antibodies and effecter cells against foreign materials (Roitt *et al.*, 1985). It was, therefore, thought that acetone fraction of mugwort extract might influence the activation of immune system.

Table 5. Effect of acetone fraction of mugwort on the relative distribution of various serum proteins in mice

Items	Dose (mg/mouse)		
	0 (control)	1	2
Albumin (%)	49.66±2.30	46.64±2.67	47.98±0.61
α_1-globulin (%)	11.82±0.97	10.14±0.13	10.70±0.33
α_2-globulin (%)	4.020±0.55	5.220±0.18*	4.450±0.09
β-globulin (%)	21.94±1.12	20.92±1.27	21.30±0.19
γ-globulin (%)	12.58±1.13	17.06±1.84*	15.58±0.69*

All values are expressed as means±standard error. *Significant enhancement of γ-globulin in treated group compared to control group ($p<0.05$)

Effect on γ-globulin level – Antibodies or immunoglobulins were found in the γ-globulin fractions of plasma proteins which can be identified by electrophoresis. We conducted electrophoresis of serum samples to evaluate γ-globulin level. As shown in Table 5, the amount of albumin, α_1-globulin, α_2-globulin, β-globulin in experimental groups were not much different from control group. In two experimental groups of 1 mg/mouse and 2 mg/mouse injection, γ-globulin level has been increased (17.06±1.84 and 15.58±0.69, respectively) significantly compared to that of control group (12.58±1.13). It is likely that acetone fraction of mugwort extract influenced the immune system by increasing γ-globulin level. Therefore, it was estimated that acetone fraction caused immune reinforcement.

Effect on blood components – To observe the effect of acetone fraction on biochemical components of blood, the levels of total protein, albumin, bilirubin, ALP, creatinine and GPT were analyzed in serum of mice administered with acetone fraction. As shown in Table 6, the amount of total protein, albumin and globulin and the ratio of albumin/globulin in experimental groups were not significantly different from those of control group. The amount of bilirubin increases in liver disease (i.e. jaundice) and that of ALP does in bone disorder and obstructive liver disease (Murray *et al.*, 1990). The amount of creatinine increases in chronic renal failure and that of GPT does in hepatotoxicity (Scott *et al.*, 1988). But, the amount of bilirubin, ALP, creatinine and GPT of experimental group were not significantly different from those of control group. These results mean that

Table 6. Effect of acetone fraction of mugwort on the serum proteins and clinico-chemical values of ICR mice

Items	Dose (mg/mouse)		
	0 (control)	1	2
Total protein(g/dl)	6.380±0.070[1)]	6.600±0.202	6.400±0.091
Albumin (g/dl)	3.240±0.093	3.200±0.045	3.175±0.048
Globulin (g/dl)	3.140±0.068	3.400±0.243	3.225±0.048
A/G ratio	1.000±0.093	0.892±0.091	0.923±0.023
Bilirubin (mg/dl)	0.260±0.040	0.375±0.048	0.350±0.029
ALP (IU/L)	26.68±5.285	39.60±1.503	43.25±3.172
Creatinine (mg/dl)	0.260±0.024	0.300±0.000	0.275±0.048
GPT (IU/L)	44.40±1.806	40.00±13.36	45.00±2.000

All data are expressed as means±standard error

Table 7. Effects of acetone fraction of mugwort on hematologic values and white blood cells of ICR mice

Items	Dose (mg/mouse)		
	0 (control)	1	2
RBC (10^6/l)	6.742±0.419	7.149±0.176	6.816±0.122
Hemoglobin (g/dl)	10.82±0.648	11.40±0.270	10.99±0.264
Hematocrit (%)	31.98±2.111	32.02±0.747	31.63±0.770
MCV (fL)	47.28±0.459	46.07±0.670	46.33±0.507
MCH (pg)	16.06±0.191	15.94±0.237	16.13±0.141
WBC (10^3/l)	10.08±4.562	6.320±0.526	6.356±0.678
Neutrophil (%)	4.491±3.426	1.612±0.180	2.112±0.447
Lymphocyte (%)	5.536±1.232	4.565±0.465	4.140±0.581
Monocyte (%)	0.000±0.000	0.300±0.153	0.000±0.000
Eosinophil (%)	0.600±0.600	1.700±0.803	1.700±0.396

All values are expressed as means±standard error

the acetone fraction of mugwort extract had no hepatotoxicity and perturbation in homeostasis of normal mice. To compare hematologic change of experimental groups with those of control group, the values of RBC, hemoglobin, hematocrit, MCV and MCH were measured and calculated. As all of these had no significant difference (Table 7), administration of acetone fraction had no effect on hematologic values and did not represent the symptom of anemia. The contents of neutrophil, lymphocyte and eosinophil in experimental groups were not much different from those of control group (Table 7). There was no monocyte or a very small amount in mice, so the difference of monocyte was disregarded. Therfore, it was thought that administration of acetone fraction did not cause the abnormality in mice.

In conclusion, we have observed that by adminstration of acetone fraction of mugwort extract, the survival time of tumor-bearing mice were prolonged and the solid tumor growth was inhibited. The spleen weight and γ-globulin level were increased. The administration of acetone fraction did not cause any abnormality in the body and the homeostasis of mice. Therefore, we suggest that acetone fraction of mugwort extract appears to have antitumor effect *in vivo* by immune reinforcement. Further studies to observe the effect of acetone fraction on metastasis and to confirm the activation of immune system (i.e. measurement of natural killer cell's activity) by acetone fraction of mugwort extract will be carried out.

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