Arch. Pharm. Res. **14**(1), 87-92 (1991)

Pharmacological Activities of Water Extracts of *Umbelliferae* Plants

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(Received November 30, 1990)

Abstract □ In order to evaluate the pharmacological activities of Chinese medicine, nine Umbelliferae plants were selected and their restoring activity against dexamethasone-induced disorders, liver protective activity, antimicrobial activity, anti-inflammatory activity and antimutagenic activity were tested and compared. *Angelica dahurica*, *Angelica acutiloba* and *Ostericum koreanum* showed various activities in these tests at the dose used in this study.

Keywords □ *Umbelliferae*, *Glehnia littoralis*, *Bupleurum longeradiatum*, *Angelica dahurica*, *Angelica gigas*, *Angelica polymorpha*, *Ostericum koreanum*, *Angelica acutiloba*, *Saposhinikovia divaricata* *Cnidium officinale*, pharmacological activity.

One of the different aspects between Chinese medicine and modern therapy is that water extracts from medicinal plants are orally administered to the patients in order to strengthen the patients' resistance against specific disease or to improve the various organ functions to circumvent the disease state, or both[1]. This kind of action is expected to be more desirable when the patients are using the plant extracts which are most frequently prescribed to the aged people or the chronic disease, having mild and broad pharmacological activities against disease or to the patients' own bodies[2]. However, it is strongly needed to clearly evaluate the plant /plant extracts depending on the pharmacological activities, because there are many allied plants or substitutes as a synonym without any identification of plant origin or the proven pharmacological activities.

In this study, *Glehnia littoralis* Schmid, *Bupleurum longeradiatum* Turez, *Angelica dahurica* Beuth. et Hook, *Angelica gigas* Nakai, *Angelica polymorpha* Max, *Ostericum koreanum* Kitakawa, *Angelica acutiloba* Kitakawa, *Saposhinikovia divaricata* Schischkin and *Cnidium officinale* Makino were selected based on the following aspects : a) These plants belong to the same family, *Umbelliferae*, and they are used quite frequently in the prescription of Chinese traditional medicines[3], b) Some of these plants have been used as allied drugs or substitutes since ancient time because their properties based on Chinese medicinal terms were known as similar one in spite of the different taxonomical origin[4], c) Their biological activities may be explained as enhancement of body resistance and/or improvement of organ functions[2], and d) There were few reports concerning the comparative pharmacological activities of these drugs even though the numerous studies were published related to each plant.

With these plant extracts, the pharmacological activities such as antimicrobial, anti-inflammatory and antimutagenic activities were compared. And the actions of affecting the vascular system and liver protection of the water extracts were also compared. Especially, the restoring activities against the adverse systemic effects induced by high dose of glucocorticoid were examined to evaluate the effects of the extracts on actions of the vascular system.

EXPERIMENTAL METHODS

Collection and extraction of medicinal plants

Glehnia littoralis, *Bupleurum longeradiatum* and *Ang-*

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Fig. 1. Treatment schedule of dexamethasone-induced disorders, dexamethsone diphosphate (↑), water extracts (⇑), sacrifice (▲)

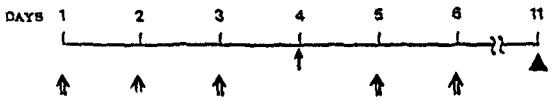

Fig. 2. Treatment schedule of rat cotton pellet granuloma inhibition,cotton pellet insertion (↑), water extracts (⇑), sacrifice (▲)

lica dahurica were collected from the area of Kangweon Province, Korea. *Angelica gigas, Angelica polymorpha, Saposhinikovia divaricata, Ostericum koreanum, Angelica acutiloba, Cnidium officinale* were purchased from commercial market and identified[5].

Each dried plant was cut into small pieces and 10 volumes of water was added. After hot extraction for 1 hr, the solution was filtered. To the residue, 5 volumes of water was added, recxtracted and filtered. The combined filtrate was evaporated *in vacuo*. For evaluation of the biological activities, the extracts were solubilized or emulsified with distilled water or appropriate vehicles at the proper concentrations as indicated below.

Animals and treatment

ICR mice and Sprague-Dawley rats were maintained with standard mouse pellet lab. chow (Sam Yang Co.) and tap water *ad libitum* in the animal room (College of Pharmacy, KNU) under conditions of 21~26℃, 40~60% humidity and 12 h/12 h (L/D) cycle. Fig. 1 represented the treatment schedule of dexamethasone diphosphate and the extracts. And Fig. 2 showed the treatment schedule for rat granuloma inhibition test.

Effects on glucocorticoid-induced alteration of the orgen weights and vascular system : To evaluate the effects of the extracts on the organ weights and vascular system, dexamethasone diphosphate (0.2 mg/kg/day) was subcutaneously injected to male SD rat (100~150g) for five consecutive days (A.M.) (Fig. 1) according to the procedure of Tani *et al*[6]. The plant extracts (200 mg/kg/day) were orally administered simultaneously for the same five days (P.M.). At the day of six, under the light anesthesia using ether, blood was collected by cardiac puncture. Thymus, spleen and adrenal gland were removed and weighed. Sera obtained by centrifugation of the whole blood were subjected to measuring concentrations of total lipid[7] and lipid peroxides[8]. Total cholesterol, GPT and GOT levels were measured using assay kit (Asan Pharm. Co., Korea) according to the procedures of test bulletin. As a separate experiment, the extracts were administered for five days without injection of dexamethasone and the organ weights were compared for examining the effects of the plant extracts on the immune-related organ weights.

Antimicrobial activity : The antimicrobial activity of the extracts was measured with the agar plate inhibition zone test[9] using *Bacillus subtilis* BD170 and *Escherichia coli* HB101 as test organisms.

Anti-inflammatory activity : Rat cotton pellet granuloma inhibition test and mouse ear edema inhibition test were used[10,11]. To the male SD rats (100~150g), the extracts were administered orally (200 mg/kg/day) for two consecutive days. On the 3rd day, cotton pellets (Richmond dental roll Co., USA) were implanted subcutaneously under each side of axilla. The extracts were administered again three consecutive days after the day of cotton pellet insertion (Fig. 2). The rats were sacrificed on the 7th day after the pellet insertion and the dried pellet weights were measured. The increased weights of pellet compared to the original weights (35± 1 mg) were considered as granuloma formation. Prednisolone (2.5 mg/pellet) was used as a positive control. For measuring the inhibitory acivity againt croton-oil induced ear edema, male ICR mice (20~26g) were used. The water extracts were administered orally (200 mg/kg/day) for 5 consecutive days. After 1 hr of the final treatment, 2.5% croton oil (50 μl) were applied to both ears of mice. After 5 hrs of croton oil treatment, the thickness of the ear were measured using microguage as edema formation. Prednisolone (0.01 mg/ear) was used as a positive control.

Prevention of CCl_4-induced liver toxicity : To evaluate the preventive effects of liver damage, the extracts (200 mg/kg/day) were administered orally to male ICR mice for 3 consecutive days and CCl_4 (0.05 mg/kg) in olive oil was injected intraperitoneally after 1 hr of the final administration of the extacts according to the procedure of Yamaraha *et al*[12].

Table I. Effects of water extracts on the dexamethasone-treated disorders in rats

Group[a]	Body wt. gain (g)	Thymus wt. (mg)	Thymus wt. (mg/100g BW)	Spleen wt. (mg)	Spleen wt. (mg/100g BW)	Adrenal wt. (mg)	Adrenal wt. (mg/100g BW)
Control	36.3± 7.3[b]	497.6± 61.4	308.9± 46.7	591.3± 115.8	365.2± 65.2	17.8± 2.3	11.0± 1.2
Dexamethasone	−3.0± 2.5[+++c]	47.3± 9.9[+++]	36.3± 9.4[+++]	296.8± 36.1[+++]	223.1± 16.6[+++]	9.3± 2.3	6.9± 1.5[+++]
C. officinale	−3.4± 5.8	60.1± 8.4	50.1± 6.5*[d]	304.2± 35.2	258.0± 49.9	9.4± 1.6	7.9± 1.6
A. polymorpha	0.8± 11.5	61.4± 8.5	40.4± 6.3	303.3± 41.7	200.0± 34.9	11.2± 1.9	7.5± 2.2
A. gigas	−1.8± 11.4	61.4± 20.0	51.3± 10.7	239.3± 51.4	201.5± 22.4	10.8± 1.1	9.9± 3.8
A. acutiloba	−5.3± 6.3	56.6± 10.1	52.5± 16.7	356.7± 25.9*	323.9± 58.7*	11.1± 2.4	10.1± 2.8
G. littoralis	−2.8± 4.7	59.5± 8.3	50.1± 8.1	320.1± 70.7	270.5± 64.8	10.4± 3.3	8.8± 3.1
S. divaricata	5.0± 6.2	62.6± 10.9	46.0± 11.0	315.3± 39.0	227.8± 31.0	10.5± 1.0	7.6± 1.2
B. longeradiatum	5.5± 4.4*	50.2± 5.8	31.6± 2.7	369.0± 48.0	236.9± 53.7	11.1± 1.6	7.1± 1.3
B. dahurica	6.8± 4.7**	64.0± 5.7*	45.3± 8.8	360.7± 37.2	251.3± 22.7	11.6± 1.8	8.1± 1.3
O. koreanum	−1.1± 6.7	47.3± 9.2	36.3± 4.0	304.8± 41.3	235.1± 22.9	11.4± 2.9	9.0± 2.4

Group	Total lipid (mg/dl)	Lipid peroxide (nmol/ml)	Total cholesterol (mg/dl)	GOT (karmen U./ml)	GPT (karmen U./ml)
Control	514.3± 88.5	2.3± 0.4	72.0± 14.4	230± 53	63± 11
Dexamethasone	1032.5± 474.7	3.2± 1.0	80.7± 16.8	385± 93[+]	107± 30[+]
C. officinale	823.7± 532.2	8.1± 5.7	65.2± 13.9	353± 79	82± 16
A. polymorpha	770.4± 100.4	5.5± 4.8	48.5± 11.3	650± 634	106± 54
A. gigas	1091.6± 762.2	2.7± 1.1	80.5± 19.7	235± 46*	84± 37
A. acutiloba	370.0± 112.0*	2.6± 0.3	83.8± 1.1	243± 54*	77± 15
G. littoralis	1328.1± 684.5	9.7± 7.7	73.9± 20.0	266± 37*	78± 19
S. divaricata	655.7± 65.5	2.8± 0.9	68.0± 11.3	463± 196	99± 33
B. longeradiatum	1732.0± 715.3	5.3± 4.2	71.1± 24.9	464± 185	141± 48
B. dahurica	956.2± 388.2	2.6± 1.1	64.9± 15.5	246± 43*	80± 11
O. koreanum	323.2± 90.6*	3.8± 3.4	40.5± 8.7*	285± 73	51± 8*

[a]. Sprague-Dawley rats (♂, 100-150 g, n=5) were used. [b]. Mean± SD. [c]. Significant compared with the control group ([+]; $p<0.05$, [++]; $p<0.01$, [+++]; $p<0.005$) [d]. Significant compared with the dexamthasone-treated group (*; $p<0.05$, **; $p<0.01$, ***; $p<0.005$)

Blood was withdrawn by cardiac puncture after 19 hrs and the serum was obtained by centrifugation. GPT levels were measured as described above.

Antimutagenic activity : According to the procedure of Schmid[13], cyclophosphamide (2.5 mg/kg) was injected intraperitoneally and the extracts (200 mg/kg) were simultaneously administered orally to male ICR mice (25~30g). After 30 hrs, bone marrow cells were obtained with 0.2 ml fetal calf serum (GIBCO) and blood cells were stained. The micronucleated polychromatic erythrocytes (MNPCEs) were calculated on the basis of 1,000 polychromatic erythrocytes as percent formation.

RESULTS AND DISCUSSION

Plant extracts having broad spectrum of improving activity have been used on the disease-caused vascular system and/or organ functions including immune-related organs to restore their normal functions in Chinese medicine. And the medicinal plants having broad and mild spectrum of activities are classified as the first class of drugs in Shen nung Pen t'ao King[2]. Therefore, it could be valuable for determining the pharmacological activity of Chinese medicine by evaluating the improving or restoring activities using the disease-induced experimental animals having impaired organ functions rather than evaluating the general pharmacological activities using normal experimental animals. Because present investigation was intended to compare the pharmacological activities of nine Umbelliferae plants as a primary screening procedure, various pharmacological activities such as antimicrobial ac-

Tabll II. Antimicrobial activity of water extracts against *B. subtilis* and *E. coli*.

Group	*B. subtilus*	*E. coli*
Penicillin[a]	+++[d]	NT
Chloramphenicol[b]	NT	+++
C. officinale[c]	++	+
A. polymorpha	±	−
A. gigas	−	−
A. acutiloba	−	−
G. littoralis	++	+
S. divaricata	±	±
B. longeradiatum	−	−
B. dahurica	++	+
O. koreanum	+	±

[a]. Penicillin G(1 μg), [b]. Chloramphenicol (3 μg/disk), [c]. All the extracts were applied at the dose of 3 mg /disk., [d]. Diameter of the inhibition zone (+++)>25 mm, (++)>20 mm, (+)>15 mm, (±) 15-9 mm, (−) no inhibition found, NT: not tested

Table III. Effects of water extracts on rat cotton pellet granuloma formation

Group[a]	Granuloma wt. (mg)	Inhibition (%)
Control	61.3+ 7.7	−
Prednisolone	25.9+ 7.7*[b]	57.8
C. officinale	60.3+11.7	1.5
A. polymorpha	61.1+ 8.6	0.3
A. gigas	56.8+ 5.8	7.4
A. acutiloba	62.5+10.9	−2.0
G. littoralis	58.0+16.2	5.4
S. divaricata	58.6+10.8	4.3
B. longeradiatum	61.7+ 5.5	−0.7
B. dahurica	59.9+11.4	2.3
O. koreanum	67.4+ 7.5	−10.0

[a]. Male SD rats were used (n=5)., [b]. Significantly different compared with the control group (*: $p<0.005$).

tivity, anti-inflammatory activity, liver protective activity against CCl_4-induced toxicity, antimutagenic activity and restoring activities against glucocorticoid-induced impairments were examined. The well-established activity testing procedures were used as described in the experimental section. For producing the glucocorticoid-induced impairments, dexamethasone diphosphate was injected to rats, because long-term or massive dose of glucocorticoids has been reported to show various adverse systemic effects[14]. Tani *et al*[6]. injected high dose of betamethasone for 7 days to produce the weight loss of immune-related organs and the increased levels of serum lipids, lipid peroxides and hyperviscosity syndrome, and successfully employed glucocorticcoid-induced impairment model for evaluating the pharmacological activity of Chinese medicine. When we injected a high dose of dexamethasone phophate (0.2 mg/kg/day) to male rats (SD) for five days, it was found that various vascular parameters measured including weights of adrenal, thymus and spleen were significantly changed and these changes were coincided with the previous report[6]. When each plant extract (200 mg/Kg/day) was administered to dexamethasone-treated rats to find out the improving effects on these parameters induced by glucocorticoid, several plant extracts showed significant changes compared with the steroid-treated group (Table I). In immune-related organ weight, *A. acutiloba* and *A. dahurica* significantly recovered spleen and thymus weights. In vascular parameters, *A. acutiloba*, *A. dahurica* and *S. divaricata* significantly decreased GOT level compared to the dexamethasone-treated control. Especially *O. koreanum* reduced concentrations of GPT, total lipid and total cholesterol significantly whereas *A. acutiloba* reduced only total lipid concentrations. Although the results showed the various restoring activities of some of the plant extracts, it may be suggested that these results be obtained from only one dose level and there is a possibility that the extracts showing no activity in this experiment might be active at the other dose level. However, at the dose level tested, *A. acutiloba*, *A. dahurica* and *O. koreanum* were found to show restoring activities on various parameters against dexamethasone-induced disorders. When each extract was administered without injection of dexamethasone diphosphate to rats, no significant change of thymus, spleen and adrenal weights was observed (data not shown).

When nine plant extracts were tested for antimicrobial activity, *C. officinale*, *S. divaricata*, *A. dahurica* and *O. koreanum* showed significant activity against *B. subtilis* and *E. coli* (Table II).

In anti-inflammatory activity test, using rat cotton pellet granuloma test, all the extracts showed no significant activity at the dose level tested (200 mg /kg/day), whereas prednisolone (2.5 mg/pellet) showed more than 50% reduction of granuloma formation (Table III). When we employed croton oil-induced mouse ear edema test, all the extracts except

Table IV. Effects of water extracts on croton oil-induced edema in mice

Group[a]	Ear thickenss (μm)	Edema inhibition (%)
Control[b]	0.42± 0.03	0.0
Prednisolone[c]	0.25± 0.03*	81.0
C. officinale	0.33± 0.01*	42.9
A. polymorpha	0.33± 0.01*	42.9
A. gigas	0.34± 0.02*	38.1
A. acutiloba	0.31± 0.03*	52.4
G. littoralis	0.31± 0.00*	52.4
S. divaricata	0.34± 0.01*	38.1
B. longeradiatum	0.39± 0.01	14.3
B. dahurica	0.32± 0.03*	47.6
O. koreanum	0.33± 0.02*	42.9

[a]. Male ICR mice (20-25 g, n=5) were used., [b] only croton oil was applied., [c] 0.01 mg/ear, *: $p<0.001$ (compared to the control group)

Table V. Effects of water extracts on CCl_4-induced liver toxicity in mice

Group[a]	GPT (Karmen U./ml)
Control	62± 12
CCl_4	8463± 1672[+++b]
C. officinale	5852± 2508*[c]
A. polymorpha	5853± 2876
A. gigas	7248± 1516
A. acutiloba	7109± 1922
G. littoralis	6594± 821*
S. divaricata	8782± 1123
B. longeradiatum	9223± 658
B. dahurica	6202± 756**
O. koreanum	7003± 1306

[a] Male ICR mice (20-30 g) were used., (n=8) [b] Significant compared with the control group ([+++]; $p<0.001$), [c]. Significant compared with the CCl_4-treated group (*; $p<0.05$, **; $p<0.01$)

B. longeradiatum showed 38~52% inhibition of edema formation, in which prednisolone (0.01 mg/ear) showed 81% inhibition (Table IV). Discrepancies between the results of granuloma inhibition and edema inhibition by the plant extracts might be at least, partly due to the different inflammatory mechanisms, edema vs granuloma. It may be suggested that these plant extracts could improve acute inflammatory disorders (edema), whereas they could not affect subchronic or chronic granulomatous inflammatory disorders[15] and the more intensive research is required to clearly evaluate the anti-inflammatory activities.

To evaluate the preventive effects of each extract on CCl_4-induced acute liver damage, high dose of CCl_4 was used to induce severe toxic effects to mice liver and GPT activities were checked. As shown in Table V, CCl_4 treatment produced high levels of GPT activity in serum (8463 U/ml). Among the extracts tested, *A. dahurica, S. divaricata* and *C. officinale* showed significant reduction of CCl_4-induced increase of GPT activity, while *A. polymorpha* showed a reduction pattern, but not statistically significant. However, *B. longeradiatum* did not show any significant activity in contrast to the wellknown reported liver protective activities by Arichi *et al*[16] and Abe *et al*[16], who showed that saiko saponins from *B. logeradiatum* exerted improving effects including GOT and GPT levels of hepatitis patients and *D*-galactosamine induced liver toxicity.

In order to measure the antimutagenic effects *in vivo*, each extract was administered to cyclophosphamide (CPA)-treated mice. As shown in Table VI, CPA-treated group showed about 0.53% micronucleated polychromatic erythrocytes (MNPCEs) which was the same level as reported earlier[18]. When the extracts were administered orally to CPA-treated mice, only *A. dahurica* showed reduction of MNPCEs. Because cyclophosphamide is a secondary mutagen, which converts to ultimate mutagen *via* liver metabolism, the antimutagenic acivity shown by *A. dahurica* might be due to the metabolic alteration of CPA or interference of DNA-CPA metabolites adduct formation. We also observed that the extract of *A. dahurica* exerted about 40% reduction of MNPCEs induced by direct mutagen, ethylmethane sulfonate (EMS).

In conclusion, *A. dahurica*, *A. acutiloba* and *O. koreanum* showed restoring activities against glucocorticoid-induced disorders and anti-inflammatory activity. *A. dahurica* also showed antimicrobial activity, preventive activity against CCl_4-induced liver toxicity and antimutagenic activity. *O. koreanum* was found to have antimicrobial activity. *C. officinale* was shown to be more active than *A. polymorpha* in recovering effects against glucocorticoid-induced disorders. *S. divaricata* showed liver protective activity while *G. littoralis* did not show liver protective activity. And *A. acutiloba* was found to exert higher

Table VI. Antimutagenic effects of water extract against cyclophosphamide-induced MNPCEs

Group[a]	MNPCE (%)
Control	0.20± 0.10
Cyclophosphamide	0.53± 0.12[+b]
C. officinale	0.63± 0.05
A. polymorpha	0.63± 0.12
A. gigas	0.53± 0.12
A. acutiloba	0.30± 0.08
G. littoralis	0.46± 0.12
S. divaricata	0.50± 0.00
B. longeradiatum	0.37± 0.12
B. dahurica	0.23± 0.05*[c]
O. koreanum	0.63± 0.05

[a]. Male ICR mice (20-30 g) were used (n=3). [b] Significant with the control group (+; $p<0.05$). [c]. Significant compared with the CPA-treated group (*; $p<0.05$)

activity in restoring spleen weight and decreasing total lipid concentration against glucocorticoid-induced impairment than *A. gigas*. We are now under investigation to isolate active principles from *A. dahurica* and *O. koreanum*.

ACKNOWLEDGEMENT

This research was supported by the Korea Science and Engineering Foundation (1989~1991). The technical assistances by Mr. Kee Sun Yu, Mr. Young Soo Kwon and Mr. Hong Pil Choi were greatly acknowledged.

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