ORIGINAL ARTICLE

Safety of the aqueous extract of *Portulaca grandiflora* Hook in healthy volunteers

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Abstract

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A phase 1 clinical trial was performed in 16 healthy volunteers to primarily investigate safety of *Portulaca grandiflora* (*P. grandiflora*) as well as to preliminarily assess its efficacy on the immune system. The volunteers received 500 mg/day of *P. grandiflora* aqueous extract (250 mg capsule twice daily) for 2 months. No major side effects were reported from any of the subjects throughout the study. It was found that some significant changes in biochemical parameters were within normal limits. Hematological and immunological parameters were not altered after oral administration of *P. grandiflora*. Our results indicated that the *P. grandiflora* aqueous extract at the dose of 500 mg/day given to normal volunteers for 2 months was safe.

Key words : *Portulaca grandiflora*, clinical trials, safety

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การศึกษาความปลอดภัยของสารสกัดแพรเซี่ยงไฮ้ (*Portulaca grandiflora*) ในอาสาสมัคร

ว. สงขลานครินทร์ วทท. มีนาคม 2550 29(ฉบับพิเศษ 1) : 95-100

ได้ทดสอบความปลอดภัยและประสิทธิผลเบื้องต้นต่อระบบภูมิคุ้มกันของแพรเซี่ยงไฮ้ในอาสาสมัครจำนวน 16 ราย โดยให้อาสาสมัครรับประทานแคปซูลที่บรรจุผงสารสกัดแพรเซี่ยงไฮ้ที่ได้จากการสกัดด้วยน้ำครั้งละ 1 แคปซูล (250 มก./แคปซูล) วันละ 2 ครั้ง ในเวลาเช้า-เย็น เป็นเวลา 2 เดือน พบว่าอาสาสมัครทั้ง 16 รายไม่มีอาการข้างเคียงใดระหว่างรับประทานสารสกัด ค่าทางชีวเคมีบางค่าที่เปลี่ยนแปลงจากก่อนได้รับสารสกัดนั้นพบว่าอยู่ในช่วงของค่าปกติ ค่าทางโลหิตวิทยาและค่าที่เกี่ยวข้องกับระบบภูมิคุ้มกันไม่เปลี่ยนแปลงในขณะที่รับประทานสารสกัดแพรเซี่ยงไฮ้ จากการศึกษานี้แสดงว่าสารสกัดแพรเซี่ยงไฮ้ที่ขนาด 500 มก./วัน มีความปลอดภัยเมื่อรับประทานติดต่อกันนาน 2 เดือนในอาสาสมัคร

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It has apparently been noted that an increasing number of people prefer to take natural medicines rather than chemical synthetics to strengthen their health. Little, however, has been studied on toxic effects during taking over-the-counter natural medicines/products. Therefore, research into safety of the natural (herb) extract should be performed to support its use in humans.

Portulaca grandiflora (*P. grandiflora*) Hook. (Family Portulacaceae) (Backer & Bakhuizen Van Den Brink, 1963; Liu & Chen, 1976) is a succulent plant. This plant grows throughout Thailand and is usually cultivated as an ornamental annual herb. In oriental traditional medicine, *P. grandiflora* is used for the relief of sore throat, skin rash and detoxification. *P. grandiflora* was reported as an effective anti-HBsAg herb by ELISA technique for the recognition of anti-HBsAg capability (Zheng & Zhang, 1990). In addition, antimutagenic effect of *P. grandiflora* on the mutation induced by alfatoxin B1 and cyclophosphamide in mice was demonstrated (Liu *et al*.,1990). Studies performed in our laboratory suggested that an aqueous extract of *P. grandiflora* showed an *in vitro* enhancement on normal human lymphocyte proliferative response at concentrations ranging from 1 ng/ml to 100 μg/ml.

The aqueous extract of *P. grandiflora* was further tested for its chronic toxicity in rats by orally given the extract at doses of 10, 100 and 1,000 mg/kg/day for 6 months. The aqueous extract did not demonstrate any relevant serious signs or significant changes in hematological, biochemical and histopathological parameters even at the dose of 1,000 mg/kg/day (Chavalittumrong *et al*., 2004).

Owing to the pharmacological and toxicological profiles of the *P. grandiflora* extract, in this study, a phase I clinical trial was conducted at the clinic of the Department of Medical Sciences in healthy volunteers to determine its safety in humans as well as its effects on immunological parameters.

Materials and methods

Selection of subjects

Volunteers were informed that the *P. grandiflora* extract was a herbal product. A summary of all laboratory tests was explained to them in simple non-technical language. The volunteers were encouraged to ask questions which they need further clarification. They were informed that they could withdraw at anytime during the trial while the clinical investigators would advise any

volunteers to withdraw from the trial if he/she developed adverse reactions to the *P. grandiflora* extract.

Eligible male or female subjects with ages ranging from 20 to 40 years, no detection of HBsAg, HCV and HIV-1 in sera, no medications for immunosuppressive or immunostimulant drugs and no history of diabetes, cancer, allergy, heart, lung and hematological disorders, were recruited into this study. Subjects who had liver or renal abnormalities were excluded from the study. Female subjects who were pregnant or in lactation periods were also excluded. No dietary supplements were allowed during the study. Sixteen volunteers, consisting of 7 males and 9 females, were recruited in this trial. Written informed consent was obtained from each of the sixteen persons who met those criteria.

A study protocol was approved by the Ethics Committee for Research in Human Subjects in the Fields of Thai Traditional and Alternative Medicine, Ministry of Public Health, on May 6, 2004.

Treatment of the subjects

The *P. grandiflora* preparations for the study were prepared from a single lot of *P. grandiflora* aerial parts, collected from Nonthaburi province, central Thailand. The botanical identification was determined (Backer & Bakhuizen Van Den Brink, 1963; and Liu & Chen, 1976) and compared with the authentic specimen at the Forest Herbarium of the Royal Forest Department, where a voucher specimen was deposited (RFD 99057). The aerial parts were extracted with distilled water using a reflux method and its quality was controlled by *in vitro* determining immunostimulating activities (Sriwanthana and Chavalittumrong, 2001). The extract was also examined for microbial, heavy metals (arsenic, lead, and cadmium) and pesticides contaminations. After examination, the extract was then formulated into standardized capsule dosage form. One capsule contained 250 mg of dried *P. grandiflora* aqueous extract. All 16 subjects were given capsules of *P. grandiflora* (b.i.d.) for 2 months.

Assessment of compliance

A known number of capsules was provided to each subject at every visit. Compliance was assessed through the measurement of the capsules returned at each visit.

Clinical assessment

At baseline and at biweekly visits, a physical examination, and a review of adverse events, concurrent medication and compliance, were performed. Adverse events were all disorders of wellbeing, subjective and objective symptoms, significant laboratory changes, concomitant illnesses occurring during the course of the study.

Laboratory assessment

Blood was taken from each volunteer on the first day and at the ends of weeks 2, 4, 6, 8 and 12 of the trial for hematological, biochemical and immunological assessments according to manufacturers for each test and instrument.

Hematological analysis was performed using an automatic hematological analyser (Cell dyne 3500, Abbott). Hematological parameters measured were white blood cell (WBC), % neutrophil, % lymphocyte, % monocyte, % eosinophil, % basophil, red blood cell (RBC), hemoglobin, hematocrit (Hct), and platelet.

Biochemical analysis of serum samples was performed using an automatic chemistry analyser (Hitachi model 912, Roche). Biochemical parameters measured were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, creatinine, blood urea nitrogen (BUN), cholesterol, triglycerides, total protein, albumin, uric acid and glucose.

Immunological assessment was quantitated for $CD3^+$, $CD4^+$ and $CD8^+$ cells by a flow cytometer (EPICS-XL, Becton Dickinson, USA). The levels of serum IL-2, IL-4 and IFN-γ were examined using ELISA kits

Chemicals

Reagents for hematological, biochemical and immunological parameters were from Abbott

Laboratories, Roche Diagnostics and Becton Dickinson, respectively. The ELISA kits were from ENDOGEN, Inc., USA.

Statistical analysis

Data were analyzed by a SPSS program version 13.0. Statistical comparisons of means of laboratory measurements were performed using repeated measured ANOVA. The data were firstly tested for homogeneity of variance by the Levene test. The Benferroni test was used for analysis of means when the variance was homogenous, whereas the Tamhane test was used for comparisons of the means when there was no homogeneity of variance. A difference was considered significant at $p<0.05$.

Results

The study was designed to primarily determine the safety of the *P. grandiflora* extract in normal volunteers. Additionally, preliminary assessment of its efficacy on immunity was examined. Sixteen volunteers, consisting of 7 males and 9 females, were recruited in this trial. Safety assessment was performed at the baseline and every 2 week of the trial period. Each person served as his/her own control.

1. Adverse effects

There was no anaphylactic reaction reported from any volunteers. Compliance during the study revealed that more than 85 percent of the subjects took 100 percent of the capsules for the whole 2 months. At the 2nd week of the trial, one of the volunteers reported having headache, getting diarrhea was reported from one subject and an increase in food consumption was observed in 3 persons. The subject who got headache reported that he normally got migraine. According to a physician's opinion, the one who got diarrhea did relate to his stress. One subject received medication for upper respiratory infection at week 4. It was investigated and found that the infection did not result from taking the extract. No other sign of side effects resulting in clinically significant symptoms was noticed during the duration of the trial.

2. Laboratory results

2.1 Effect on hematological parameters

No significant changes in the number of white blood cell, % neutrophil, % lymphocyte, % monocyte, % eosinophil, the number of red blood cell, hemoglobin, hematocrit or platelet were observed at any weeks during the trial, as compared with the baseline (week 0) measurements (Table 1).

2.2 Effect on blood chemistry

To assess effects of the extract on metabolism, liver and renal functions, the levels of the biochemical profiles at baseline were compared with those at biweekly visits. There was no significant alteration in the levels of bilirubin, cholesterol, triglycerides, albumin and BUN (Table 2). The levels of AST were significantly decreased at week 6, 8 and 12. The levels of ALT and ALP were significantly lower than the baseline levels at week 6 and 4, respectively. Significant decrease in blood sugar and uric acid levels was found at week 4 and 6, respectively. The levels of creatinine were significantly lower than the baseline level at week 2, 6 and 12. Total protein level was significantly elevated at week 12.

2.3 Effect on immunological parameters

There were no significant changes in the numbers of $CD3^+$, $CD4^+$ and $CD8^+$ cells during the trial (Table 3). No detectable levels of serum IL-2, IL-4 and IFN-γ in any of the 16 volunteers were found either at the onset of the study or during the treatment (data not shown).

Discussion

We conducted the phase 1 clinical trial to determine safety of the aqueous extract of *P. grandiflora* given to 16 normal volunteers at the dose of 500 mg/day for 2 months. Few pharmacokinetic studies have been performed on herbal extracts, including the *P. grandiflora* aqueous extract. According to our *in vitro* study, the expected effective level in systemic circulation

Table 1. Hematological results of normal volunteers orally given *P. grandiflora* extract for 8 weeks (n = 16)

	Time after *P. grandiflora* administration (weeks)					
	0 week	**2 week**	**4 week**	**6 week**	**8 week**	**12 week**
WBC (10^3/μL)	5.75±1.44	6.20±1.48	5.61±1.33	5.87±1.13	5.39±1.03	5.88±1.17
Neutrophil (%)	47.7±6.2	50.0±8.3	46.8±6.3	48.8±7.3	46.0±5.9	48.5±8.7
Lymphocyte (%)	40.1±5.8	37.8±7.0	41.3±6.9	38.9±6.5	40.7±7.7	39.1±7.4
Monocyte (%)	7.18±1.88	7.63±2.29	7.39±1.56	7.34±1.97	8.00±1.97	7.13±1.50
Eosinophil (%)	3.83±2.06	3.35±2.00	3.49±2.16	3.82±2.38	4.11±2.72	4.15±3.95
RBC (x10^6/μL)	4.78±0.47	4.85±0.411	4.81±0.42	4.79±0.49	4.74±0.50	4.89±0.44
Hemoglobin (g/dL)	13.99±1.61	14.19±1.57	13.99±1.46	13.90±1.63	13.87±1.76	14.08±1.68
Hematocrit (%)	40.22±4.32	40.89±4.23	40.44±3.98	40.22±4.41	39.93±4.89	40.97±4.51
Platelet (10^3/μL)	263±62	284±55	276±59	278±56	270±59	269±65

Each value represents mean ± SD

Table 2. Blood chemistry results of normal volunteers orally given *P. grandiflora* extract for 8 weeks (n = 16)

	Time after *P. grandiflora* administration (weeks)					
	0 week	**2 week**	**4 week**	**6 week**	**8 week**	**12 week**
AST (U/l)	22.25±3.96	21.31±3.44	20.25±4.37	17.44±3.08*	20.44±3.10*	18.94±5.53*
ALT (U/l)	17.75±7.88	15.94±7.58	18.00±7.88	14.19±63.53*	15.94±7.16	17.69±7.72
ALP (U/l)	60.31±13.19	57.50±15.29	57.69±13.33*	57.69±15.53	57.88±14.46	58.56±14.94
Total bilirubin (mg/dL)	0.701±0.298	0.685±0.302	0.619±0.240	0.655±0.239	0.697±0.347	0.598±0.248
Glucose (mg/dL)	83.95±4.53	81.97±5.64	79.69±3.22*	83.15±5.05	82.21±4.33	83.53±8.46
Cholesterol (mg/dL)	174±18	177±21	174±21	181±19	180±22	181±19
Triglyceride (mg/dL)	76.08±32.66	80.76±33.80	75.62±36.29	79.27±36.45	82.61±42.92	82.99±29.79
Total protein (g/dL)	7.73±0.37	7.87±0.20	7.82±0.20	7.84±0.24	7.77±0.44	7.92±0.31*
Albumin (g/dL)	4.58±0.35	4.67±0.27	4.58±0.28	4.65±0.29	4.63±0.38	4.65±0.31
BUN (mg/dL)	10.19±1.97	11.08±2.40	10.44±3.15	10.64±2.31	10.11±2.29	10.76±2.92
Creatinine (mg/dL)	0.872±0.146	0.832±0.137*	0.846±0.126	0.821±0.131*	0.854±0.169	0.808±0.141*
Uric acid (mg/dL)	4.96±1.54	5.01±1.24	4.74±1.29	4.64±1.32*	5.08±1.54	5.04±1.49

Each value represents mean ± SD
*** significantly different from initial ($p<0.05$) by repeated measured ANOVA**

Table 3. Immunological results of normal volunteers orally given *P. grandiflora* extract for 8 weeks (n = 16)

	Time after *P. grandiflora* administration (weeks)					
	0 week	**2 week**	**4 week**	**6 week**	**8 week**	**12 week**
$CD3^+$ cells (cells/μl)	1469±378	1485±257	1522±402	1474±308	1405±315	1511±356
$CD4^+$ cells (cells/μl)	731±169	736±92	769±186	736±163	704±165	780±186
$CD8^+$ cells (cells/μl)	606±216	601±184	602±217	600±180	572±174	593±197

Each value represents mean ± SD

was about 10 µg/ml. The dose administered in this study was then calculated based on an average of blood volume, approximate percentages on absorption (50%) and excretion (50%), the chronic toxicity data in rats and an approval from the Ethics Committee for Research in Human Subjects in the Fields of Thai Traditional and Alternative Medicine, Ministry of Public Health. No major side effects or clinically significant symptoms were reported from any of the volunteers. The extract at the dose given was, therefore, found to be well tolerated.

Laboratory investigations on hematological parameters and biochemical profiles showed significant differences in the serum levels of AST, ALT, ALP, glucose, total protein, creatinine and uric acid. Increase in those values was not greater than twice the upper normal levels. Additionally, there was no observation of hepatotoxicity or renal toxicity, suggesting that the aqueous extract of *P. grandiflora* did not lead to clinically significant condition during the trial.

The effect on immunological parameters was assessed and a slight increase in the numbers of $CD3^+$ and $CD4^+$ cells demonstrated at week 4 and 12. IL-2, IL-4 and IFN-γ are cytokines known to play important roles in immune responses (Balkwill & Burke, 1989). Measured levels of serum IL-2, IL-4 and IFN-γ, on the other hand, were not increased during taking the extract. Our study suggested that the extract may not obviously enhance immune functions. It may be possible that the dose given to the volunteers was not able to induce significant effects of those parameters.

Both the clinical and laboratory results demonstrated that the *P. grandiflora* extract did not induce any significant changes in any of the volunteers receiving the extract at the dose of 500 mg/day for 2 months, suggesting that the extract is safe for healthy humans. To determine whether the extract possesses its capability to stimulate immune functions, a larger trial using different doses of the extract should be performed in normal volunteers. In addition, better understanding on the *P. grandiflora* effects on immunity should be further sought in immunocompromised persons such as HIV-infected individuals.

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