ORIGINAL ARTICLE

A phase I trial of *Gynostemma pentaphyllum* Makino in healthy volunteers

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Abstract

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Songklanakarin J. Sci. Technol., March 2007, 29(Suppl. 1) : 83-93

We conducted a phase I trial of *Gynostemma pentaphyllum*, grown in northern Thailand, to evaluate its safety in three groups of healthy volunteers. Fourteen, fifteen and fourteen volunteers respectively received the water extract of *G. pentaphyllum* in capsules at the doses of 50, 200 and 400 mg twice daily for two months. There were no major adverse events reported from any of the three groups throughout the study. Significant changes in hematological parameters, natural killer cell activities and the numbers of $CD3^+$, $CD4^+$ and $CD8^+$ cells were not seen during taking the extract. Some biochemical parameters were significantly different from baseline data. Those values were, however, within normal limits and did not result in clinically significant conditions. Our results suggested that the water extract of *G. pentaphyllum* at the doses of 50, 200 or 400 mg twice daily given to healthy volunteers for two months was safe.

Key words : *Gynostemma pentaphyllum*, Jiaogulan, clinical trials, safety

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การศึกษาทางคลินิกระยะที่ 1 เพื่อประเมินความปลอดภัยของปัญจขันธ์ในอาสาสมัครสุขภาพดี

ว. สงขลานครินทร์ วทท. มีนาคม 2550 29(ฉบับพิเศษ 1) : 83-93

ได้ทำการศึกษาเพื่อทดสอบความปลอดภัยของปัญจขันธ์จากแหล่งปลูกทางภาคเหนือของไทยในอาสาสมัคร ซึ่งแบ่งออกเป็น 3 กลุ่ม โดยให้อาสาสมัครจำนวน 14, 15 และ 14 ราย รับประทานแคปซูลสารสกัดปัญจขันธ์ที่สกัดด้วยน้ำที่ขนาด 50, 200 และ 400 มก./ครั้ง วันละ 2 ครั้งเช้า-เย็น เป็นเวลา 2 เดือน ตามลำดับ พบว่าอาสาสมัครทั้ง 3 กลุ่มไม่มีอาการข้างเคียงใด ๆ ระหว่างรับประทานสารสกัด ไม่พบความเปลี่ยนแปลงของค่าทางโลหิตวิทยา การทำงานของเซลล์ NK และจำนวนของเซลล์ CD3, CD4 และ CD8 ขณะได้รับสารสกัด ค่าทางชีวเคมีบางค่าเปลี่ยนแปลงจากก่อนได้รับสารสกัดแต่อยู่ในช่วงของค่าปกติ และไม่ทำให้เกิดอาการที่แสดงถึงความผิดปกติอันเนื่องมาจากสารสกัดปัญจขันธ์ จากการศึกษานี้แสดงว่าสารสกัดปัญจขันธ์ที่ขนาด 50, 200 และ 400 มก./ครั้ง/วัน มีความปลอดภัยเมื่อรับประทานติดต่อกันนาน 2 เดือน

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Gynostemma pentaphyllum Makino, commonly known as Jiaogulan, is a perennial climber, widely grown in China, Japan, Korea and Southeast Asia. It is used for treatment of inflammation, cough, hyperviscosity of sputums and chronic bronchitis (Jiang-Xu, 1979; Lin *et al*., 1993). Gypenosides, dammarane-type saponins (Piacente *et al*., 1995; Hu *et al*., 1996; Liu *et al*., 2005) isolated from *G. pentaphyllum*, are major bioactive principles which have been reported to have various *in vitro* activities such as reducing cholesterol (Kimura *et al*., 1983; Huang *et al*., 2005), anti-tumor (Chen *et al*., 1999; Zhou *et al*., 2000; Wang *et al*., 2002; Chiu *et al*., 2003; Chen *et al*., 2004) anti-mutagenicity (Kulwat *et al*., 2005), anti-gastric ulcer (Rujjanawate *et al*., 2004), anti-thrombotic (Li and Jin, 1989; Tan *et al*., 1993), immunopotentiating (Zhang *et al*., 1990; Li and Xing, 1992; Sriwanthana *et al*., 2005) and anti-inflammatory (Lin *et al*., 1993) activities. Several lines of evidence demonstrated its efficacy in experimental animals and patients such as reducing levels of serum triglyceride and cholesterol in rats and quails (la Cour *et al*., 1995), hepato-protection in rats (Lin *et al*., 1993; Lin *et al*., 2000), cardiovascular protection in anesthesized guinea pigs (Circosta *et al*., 2005), recovering leukocyte counts and lymphocyte proliferation in cancer patients with radiotherapy or chemotherapy and in irradiated mice (Hou *et al*., 1991; Chen *et al*., 1996) and increasing immune responses to ovalbumin in mice (Sun and Zheng, 2005). *G. pentaphyllum* has, therefore, become popular as remedies for the above-mentioned conditions in many parts of the world including Thailand.

It has been known that phytochemical compositions and pharmacological activities of each medicinal plant of the same species may vary owing to the geographic location, the time of plant harvesting, the part used of the plant and the method used to extract the plant materials (Bauer, 2000). Hence, *G. pentaphyllum*, grown in various regions in Thailand, is needed for *in vitro* and *in vivo* studies before claiming its activities.

Studies of *G. pentaphyllum*, grown in Chiang Mai and Chiang Rai provinces of Thailand, demonstrated *in vitro* effects as immunomodulating (Sriwanthana *et al*., 2005), anti-tumor, anti-inflammatory, antioxidant activities as well as *in vivo* effect on reducing blood sugar levels in rats (Kalaya Anulukanapakorn; personnel communication). Chronic toxicity study of its water extract was performed in both male and female rats by orally giving the extract at the doses of 6, 30, 150

and 750 mg/kg/day. The results indicated that the water extract was safe even at the dose of 750 mg/kg/day (Attawish *et al*., 2004).

In the present study, we conducted the phase I trial to assess the safety of the *G. pentaphyllum* extract at three different doses in healthy volunteers as well as its effects on immunological parameters.

Materials and methods

Selection of Subjects:

Healthy volunteers attending the clinic of the Department of Medical Sciences were recruited for this study and were informed that the *G. pentaphyllum* extract was an herbal product. Summary of all laboratory results was explained to them in simple non-technical language. The volunteers were encouraged to ask questions if they needed further clarification. They were informed that they could withdraw at anytime during the trial while the clinical investigators could advise any volunteers to withdraw from the trial if he or she developed adverse events to the *G. pentaphyllum* extract.

Eligible subjects were male or female individuals with ages ranging from 20 to 45 years, were negative to HBsAg, HCV and HIV-1/2, not taking medications affecting their immune systems and without history of diabetes, cancer, allergy, heart, lung and hematological disorders. Subjects who had liver or renal abnormalities as detected by medical history, physical examination or blood chemistry were not included in the study. Female subjects who were pregnant or in lactation periods were excluded. No dietary supplements were allowed during the study. Written informed consent, approved by the Ethical Review Committee of the Thai Ministry of Public Health, was obtained from all volunteers prior to their enrollment and subjects were compensated for participating in the trial.

A study protocol was approved by the Ethical Review Committee of the Thai Ministry of Public Health on August 27, 2002.

Preparation of the extract

The extract was prepared as previously described (Sriwanthana *et al*., 2005). Briefly, aerial parts of *G. pentaphyllum* were washed thoroughly, cut into segments of appropriate size, dried in the oven at 40^{o}C and ground. Dried and ground aerial parts were extracted with distilled water using a reflux method for 2 h. Filtrate was collected and residues were further extracted with distilled water for 2 h. The filtrates collected from both extractions were pooled and dried under vacuum in a rotary evaporator. Quality of the extract was controlled by *in vitro* determining immunostimulating activities. The extract was tested for microbial, heavy metals and pesticides contaminations before formulating into capsules of standardized extract. One capsule contained either 50 or 200 mg of the dried extract.

Treatment of the subjects

A total of 43 subjects were enrolled in this study. They were randomly divided into 3 groups. Group 1, consisting of 14 subjects, was given the *G. pentaphyllum* extract in capsule at the dose of 50 mg twice daily (b.i.d.) for 2 months. Group 2, consisting of 15 subjects, was given the *G. pentaphyllum* extract in capsule at the dose of 200 mg b.i.d. for 2 months. Group 3, consisting of 14 volunteers, was given the extract in capsule at the dose of 400 mg b.i.d. for 2 months.

Assessment of compliance

A known number of capsules were provided to each subject at every visit. Compliance was assessed by counting the capsules returned at each follow-up.

Clinical assessment

At baseline and at biweekly visits, a physical examination was performed and a review of adverse events, concurrent medication and compliance was completed. Adverse events were all disorders of well being, 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 and 8 of the trial for complete blood count (CBC), red blood cell (RBC) and platelet counts, for biochemical and immunological assessment.

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 and uric acid.

Immunological assessment was performed by enumeration of $CD3^+$, $CD4^+$ and $CD8^+$ cells using a flow cytometer (EPICS-XL, Becton Dickinson, USA). Natural killer (NK) cell activities were determined by a chromium release assay as described in Sriwanthana and Chavalittumrong, 2001. The levels of serum IL-2, IL-4 and IFN-γ were examined using Human IL-2 ELISA, Human IL-4 ELISA and Human ELISA IFN-γ kits (R&D Systems, USA), respectively. Minimum detectable levels of serum IL-2, IL-4 and IFN-γ were less than 7, 10 and 8 pg/ml, respectively. In this study, individuals with cytokines levels greater than minimum detectable or their baseline levels were counted as numbers of subjects with increasing levels of either IL-2, IL-4 or IFN-γ.

Statistical analysis

Data were analyzed by an 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 Levene test. Bonferroni test was used for analysis of means when the variance was homogeneous, whereas Tamhane test was used for comparisons of the means when there was no homogeneity of the variance. The numbers of subjects with increasing levels of each cytokine were analyzed by Fischer's Exact test. The significant level was set at $P<0.05$.

Results

Study subjects

The phase I trial was undertaken at the clinic of the Department of Medical Sciences. Healthy volunteers who satisfied our inclusion criteria and who agreed to participate in the study by the written informed consent were recruited. Group 1 consisted of 3 males and 11 females with ages ranging from 20 to 45 years. Group 2 consisted of 6 males and 9 females with ages ranging from 20 to 44 years. Group 3 consisted of 4 males and 10 females with ages ranging from 20 to 45 years. Laboratory analyses were performed for the purpose of assessing the health status as compared with baseline data. Compliance during the study revealed that more than 90 % of the subjects in Group 1 took 100 percentage of the capsules while all of the subjects in Groups 2 and 3 complied with the study for the whole 2 months.

Adverse events

There was no anaphylactic reaction reported from any volunteers. No signs of severe adverse events were reported from subjects treated with the extract at the doses of 50 and 200 mg b.i.d. during the treatment period. For those who received the extract at the dose of 400 mg b.i.d., one subject described feeling nausea at week 4, one volunteer had acute tonsillitis at week 8 and two volunteers had a common cold at week 8.

Laboratory results

Effect on hematological parameters

Treatment with the *G. pentaphyllum* extract did not demonstrate significant changes in the number of white blood cell, % neutrophil, % lymphocyte, % monocyte, % eosinophil, the number of red blood cell, hemoglobin, hematocrit and platelet in all 3 groups of our study as compared with the baseline data (Tables 1-3).

Table 1. Hematological results of volunteers orally given *G. pentaphyllum* extract (50 mg b.i.d.) for 8 weeks (n = 14)

	Time after administration (weeks)					
	0 week (n=14)	**2 week (n=14)**	**4 week (n=14)**	**6 week (n=14)**	**8 week (n=14)**	**12 week (n=14)**
WBC (K/μL)	6.19±1.79	5.76±1.46	5.68±1.41	5.78±1.88	5.89±1.59	6.51±1.62
Neutrophil (%)	55.8±7.2	57.5±8.8	55.2±5.8	56.7±6.5	56.3±5.7	56.3±5.5
Lymphocyte (%)	32.0±6.8	31.80±8.47	33.7±6.0	31.9±6.7	32.3±6.2	32.4±3.8
Monocyte (%)	7.48±2.17	6.75±1.67	7.02±1.48	7.65±1.89	7.74±2.12	7.22±1.53
Eosinophil (%)	3.69±3.70	3.05±2.81	3.17±2.86	2.81±2.17	2.69±1.81	3.04±2.73
RBC ($x10^6$/μL)	4.76±0.55	4.70±0.53	4.92±0.57	4.62±0.49	4.80±0.57	4.83±0.53
Hemoglobin (g/dL)	13.75±1.14	13.52±1.12	14.01±1.39	13.25±1.23	13.65±1.34	13.77±1.24
Hematocrit (%)	40.7±3.5	40.2±3.6	41.9±3.8	39.3±3.6	40.9±4.2	41.2±3.8
Platelet (K/μL)	268±50	281±60	291±54	293±59	274±54	273±56

Each value represents mean ± SD

Table 2. Hematological results of volunteers orally given *G. pentaphyllum* extract (200 mg b.i.d.) for 8 weeks (n = 15)

	Time after administration (weeks)					
	0 week (n=14)	**2 week (n=14)**	**4 week (n=14)**	**6 week (n=14)**	**8 week (n=14)**	**12 week (n=14)**
WBC (K/μL)	7.30±2.17	6.31±1.32	6.14±1.52	6.48±1.39	7.46±2.65	7.02±1.80
Neutrophil (%)	55.8±11.2	52.9±7.4	50.4±8.5	55.3±8.8	56.8±8.8	54.3±9.8
Lymphocyte (%)	31.4±7.9	34.3±6.0	35.6±7.3	32.3±6.8	30.7±6.5	33.0±9.6
Monocyte (%)	7.83±2.49	7.81±2.0	8.65±2.67	7.66±1.05	7.76±1.91	7.85±1.37
Eosinophil (%)	4.28±6.17	4.32±5.28	4.03±5.44	3.68±4.81	3.75±4.49	3.71±4.41
RBC ($x10^6$/μL)	4.74±0.51	4.68±0.51	4.65±0.52	4.75±0.53	4.95±0.53	4.82±0.50
Hemoglobin (g/dL)	13.52±1.25	13.26±1.54	13.22±1.52	13.38±1.34	14.15±1.63	13.68±1.38
Hematocrit (%)	40.1±3.8	40.7±4.6	39.3±4.5	40.2±4.4	41.7±4.9	40.3±4.1
Platelet (K/μL)	320±61	303±61	311±64	323±75	339±74	352±65

Each value represents mean ± SD

Effect on blood chemistry

To assess the effects of the extract on blood chemistry, liver and renal functions, the levels of the biochemical profiles at biweekly visits were compared with those at baseline. There was no significant alteration in the levels of liver enzymes (AST, ALT and ALP), cholesterol, triglyceride, uric acid and BUN in any of the groups receiving the extract for 8 weeks as shown in Tables 4-6. It was found that the levels of total bilirubin (weeks 2 and 8), glucose (week 6), total protein (week 8), albumin (weeks 6 and 8), and creatinine (week 8) of Group 1 were significantly lower than those of the baseline results (Table 4). Serum creatinine levels of Groups 2 and 3 were significantly increased at week 8 (Tables 5 and 6).

Effect on immunological parameters

The numbers of $CD3^+$, $CD4^+$ and $CD8^+$ cells were not significantly increased from baseline in any of the extract-treated groups (Tables 7-9). Significant change in the number of $CD4^+$ cells

Table 3. Hematological results of volunteers orally given *G. pentaphyllum* extract (400 mg b.i.d.) for 8 weeks (n = 14)

	Time after administration (weeks)					
	0 week (n=14)	**2 week (n=14)**	**4 week (n=14)**	**6 week (n=14)**	**8 week (n=14)**	**12 week (n=14)**
WBC (K/μL)	6.38±1.35	6.52±1.32	6.41±1.23	6.32±1.24	6.82±2.18	6.18±1.36
Neutrophil (%)	51.3±6.9	52.9±8.7	50.6±8.3	53.7±5.6	53±10.7	52.8±6.6
Lymphocyte (%)	36.9±6.3	35.5±8.2	37.3±6.3	34.3±5.6	35.1±7.6	35±3.8
Monocyte (%)	7.85±1.93	7.78±1.79	7.97±1.95	8.19±2.21	7.75±1.80	7.97±2.33
Eosinophil (%)	2.67±2.59	2.66±1.99	3.03±2.76	2.78±2.24	3.02±3.79	3.29±3.22
RBC ($x10^6$/μL)	4.71±0.48	4.68±0.41	4.69±0.48	4.67±0.37	4.63±0.43	4.74±0.46
Hemoglobin (g/dL)	13.54±1.74	13.69±1.61	13.06±1.78	13.74±1.45	12.85±1.55	13.64±1.57
Hematocrit (%)	40.4±5.1	40±4.3	40±4.7	40.1±4.1	39.1±4.1	40.6±4.4
Platelet (K/μL)	267±58	270±48	255±66	284±63	275±65	276±55

Each value represents mean ± SD

Table 4. Blood chemistry results of volunteers orally given *G. pentaphyllum* extract (50 mg b.i.d.) for 8 weeks (n = 14)

	Time after administration (weeks)					
	0 week (n=14)	**2 week (n=14)**	**4 week (n=14)**	**6 week (n=14)**	**8 week (n=14)**	**12 week (n=14)**
AST (U/l)	20.20±5.62	17.80±4.18	20.20±4.31	21.80±15.03	17.87±4.22	18.60±4.97
ALT (U/l)	16.47±10.87	15.07±7.58	14.47±8.19	15.13±8.12	15.93±10.15	15.67±7.26
ALP (U/l)	53.87±14.76	53.47±15.56	54.07±13.93	51.00±17.33	60.53±24.13	56.33±14.83
Total bilirubin (mg/dL)	0.831±0.289	0.533±0.133*	0.605±0.240	0.591±0.169	0.580±0.205*	0.663±0.249
Glucose (mg/dL)	89.44±6.48	86.23±7.65	85.72±8.30	85.55±6.26*	86.53±7.44	86.32±9.36
Cholesterol (mg/dL)	189±19	185±27	183±22	180±27	182±24	187±24
Triglyceride (mg/dL)	86.4±44.9	77.3±23.3	90.4±24.6	74.8±28.7	86.8±32.2	87.6±40.1
Total protein (g/dL)	8.45±0.67	8.23±0.56	8.17±0.58	8.03±0.56	8.03±0.62*	8.24±0.56
Albumin (g/dL)	4.58±0.29	4.49±0.21	4.44±0.18	4.31±0.19*	4.36±0.18*	4.51±0.25
BUN (mg/dL)	11.29±2.75	10.59±2.12	10.07±1.91	11.42±2.27	10.07±2.39	11.58±3.32
Creatinine (mg/dL)	0.965±0.142	0.932±0.126	0.953±0.149	0.931±0.128	0.885±0.138*	0.910±0.129

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

was demonstrated in the 50 mg b.i.d. group after stopping the administration (week 12) as shown in Table 7. The water extract of G. pentaphyllum had no significant effect on NK cell activities of all 3 groups. The effect of *G. pentaphyllum* on extract-induced cytokine secretion was assessed by measuring serum levels of IL-2, IL-4 and IFN-γ. The levels of serum IL-4 and IFN-γ were not higher than minimum detectable levels in all volunteers at the onset of the study and at the 2-month period of the study. At baseline, serum IL-2 level greater than its minimum detectable level was found in 1 (13.5 pg/ml) of 14 subjects in Group 1 but was not observed in the other groups. After receiving the extract, increasing in serum IL-2 was found in 2 (11.4 and 18.5 pg/ml), 1(10.4 pg/ml), 2 (10.4 and 18.5 pg/ml) and 3 (10.4, 10.4 and 10.4 pg/ml) volunteers of Group 1 at weeks 2, 4, 6, and 8,

Table 5. Blood chemistry results of volunteers orally given *G. pentaphyllum* extract (200 mg b.i.d.) for 8 weeks (n = 15)

	Time after administration (weeks)					
	0 week (n=14)	**2 week (n=14)**	**4 week (n=14)**	**6 week (n=14)**	**8 week (n=14)**	**12 week (n=14)**
AST (U/l)	18.85±1.95	18.08±5.12	18.23±4.92	19.0±3.06	17.15±3.21	18.23±4.30
ALT (U/l)	14.54±4.45	15.46±7.63	15.62±10.33	13.92±4.97	12.54±5.36	15.62±7.87
ALP (U/l)	73.77±16.70	73.77±16.30	75.62±14.63	75.62±15.84	80.15±16.83	75.46±14.57
Total bilirubin (mg/dL)	0.486±0.167	0.598±0.197	0.489±0.194	0.665±0.333	0.508±0.203	0.512±0.235
Glucose (mg/dL)	79.69±9.14	81.87±6.88	81.98±6.67	78.38±4.72	78.86±5.46	80.70±5.74
Cholesterol (mg/dL)	189±22	176±27	169±17	181±18	178±24	179±22
Triglyceride (mg/dL)	63.6±24.9	62.8±23.8	55.3±20.0	55.1±16.9	56.7±19.6	67.0±28.3
Total protein (g/dL)	7.72±0.31	7.63±0.41	7.54±0.36	7.62±0.35	7.78±0.38	7.81±0.26
Albumin (g/dL)	4.43±0.14	4.42±0.20	4.37±0.17	4.38±0.19	4.50±0.17	4.31±0.13
BUN (mg/dL)	11.48±2.80	11.52±2.85	10.85±1.88	12.08±2.15	11.53±2.84	12.63±2.40
Creatinine (mg/dL)	0.940±0.110	0.976±0.135	0.936±0.151	0.973±0.133	0.979±0.130*	0.982±0.148
Uric acid (mg/dL)	5.12±1.00	5.16±0.76	5.06±0.99	5.03±1.02	5.01±0.92	5.21±1.02

Each value represents mean ± SD
*** significantly different from initial value (p<0.05)**

Table 6. Blood chemistry results of volunteers orally given *G. pentaphyllum* extract (400 mg b.i.d.) for 8 weeks (n = 14)

	Time after administration (weeks)					
	0 week (n=14)	**2 week (n=14)**	**4 week (n=14)**	**6 week (n=14)**	**8 week (n=14)**	**12 week (n=14)**
AST (U/l)	20.93±6.122	25.64±15.29	20.50±6.26	22.71±7.58	21.00±7.23	21.14±9.94
ALT (U/l)	23.29±15.92	28±28.95	21.86±14.31	21.79±16.59	20.79±14.08	23.79±23.84
ALP (U/l)	66.86±9.01	68±12.69	67.29±10.11	67.29±13.18	67.36±13.20	67.79±12.40
Total bilirubin (mg/dL)	0.609±0.246	0.614±0.167	0.635±0.226	0.636±0.229	0.665±0.228	0.644±0.211
Glucose (mg/dL)	86.32±8.31	82.97±5.54	82.90±9.98	86.22±7.39	83.82±7.05	85.68±7.21
Cholesterol (mg/dL)	192±31	189±22	186±27	193±27	192±27	193±26
Triglyceride (mg/dL)	65.13±16.49	71.67±22.86	67.67±20.09	68.88±27.96	67.23±23.42	69.96±22.55
Total protein (g/dL)	8.09±0.31	7.88±0.27	7.84±0.34	8.10±0.31	8.17±0.28	8.31±0.27
Albumin (g/dL)	4.50±0.25	4.45±0.22	4.41±0.26	4.45±0.20	4.41±0.22	4.60±0.14
BUN (mg/dL)	10.70±3.09	10.06±2.38	11.57±3.53	10.35±2.83	9.04±1.96	11.41±3.34
Creatinine (mg/dL)	0.919±0.133	0.892±0.124	0.954±0.143	0.916±0.113	0.924±0.133*	0.983±0.138
Uric acid (mg/dL)	4.66±0.83	4.48±0.89	4.85±0.93	4.66±0.85	4.50±0.96	4.5±1.03

Each value represents mean ± SD
*** significantly different from initial value (p<0.05)**

respectively (Table 7). The subjects with increased IL-2 levels during the treatment in Group 1 were not the one with detectable IL-2 level at the baseline visit. At week 8, increased levels of IL-2 were found in 2 (280 and 33 pg/ml) of 14 subjects of Group 3 (Table 9). The two subjects were not the ones with either acute tonsillitis or common cold.

Table 7. Immunological results of volunteers orally given *G. pentaphyllum* extract (50 mg b.i.d.) for 8 weeks (n = 14)

	Time after administration (weeks)					
	0 week (n=14)	**2 week (n=14)**	**4 week (n=14)**	**6 week (n=14)**	**8 week (n=14)**	**12 week (n=14)**
$CD3^+$ cells (cells/µl) [a]	1245±303	1171±373	1229±364	1194±292	1215±320	1340±266
$CD4^+$ cells (cells/µl) [a]	652±136	629±177	655±165	658±164	645±159	723±156*
$CD8^+$ cells (cells/µl) [a]	488±188	448±191	472±193	449±152	468±171	510±134
NK activity (Lytic unit/10^7PBMC) [a]	11.87±7.54	18.51±14.80	5.00±0.00	11.32±14.82	10.60±6.41	57.16±62.25
IL-2 (pg/ml)	1/14 [b]	2/14 [b]	1/14 [b]	2/14 [b]	3/14 [b]	0/14 [b]
IL-4 (pg/ml)	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]
IFN-γ (pg/ml)	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]

[a] Each value represents mean ± SD
[b] Each value represents numbers of volunteers with cytokines levels greater than minimum detectable levels / total numbers of volunteers
*** significantly different from initial value ($p<0.05$)**

Table 8. Immunological results of volunteers orally given *G. pentaphyllum* extract (200 mg b.i.d.) for 8 weeks (n = 15)

	Time after administration (weeks)					
	0 week (n=14)	**2 week (n=14)**	**4 week (n=14)**	**6 week (n=14)**	**8 week (n=14)**	**12 week (n=14)**
$CD3^+$ cells (cells/µl) [a]	1435±322	1486±437	1503±422	1429±374	1501±421	1568±502
$CD4^+$ cells (cells/µl) [a]	693±174	731±249	742±248	717±226	754±258	800±297
$CD8^+$ cells (cells/µl) [a]	598±150	610±204	609±174	583±160	621±166	645±212
NK activity (Lytic unit/10^7PBMC) [a]	43.47±20.45	55.93±28.16	47.17±29.76	88.51±75.59	66.73±34.44	46.05±24.49
IL-2 (pg/ml)[b]	0/15	0/15	0/15	0/15	0/15	0/15
IL-4 (pg/ml)[b]	0/15	0/15	0/15	0/15	0/15	0/15
IFN-γ (pg/ml)[b]	3/15	0/15	0/15	0/15	0/15	0/15

[a] Each value represents mean±SD
[b] Each value represents numbers of volunteers with cytokines levels greater than minimum detectable levels / total numbers of volunteers

Discussion

Products of *G. pentaphyllum* distributed in Thailand and other countries are mostly made from aerial parts and formulated as capsules, tonic beverages or tea preparations. Variations in biological activities of each product may result from location and harvesting time of the plant, extraction procedures and manufacturing processes (Bauer, 2000). Less has been known for clinical effects and safety of different preparations of *G. pentaphyllum* products using materials grown in Thailand. Therefore, our phase I clinical study was conducted to determine the safety of the water extract of *G. pentaphyllum*, cultivated in Chiang Mai and Chiang Rai provinces, at 3 different doses which were 50, 200 and 400 mg b.i.d. and orally given to 14, 15 and 14 healthy subjects, respect-

Table 9. Immunological results of volunteers orally given *G. pentaphyllum* extract (400 mg b.i.d.) for 8 weeks (n = 14)

	Time after administration (weeks)					
	0 week (n=14)	**2 week (n=14)**	**4 week (n=14)**	**6 week (n=14)**	**8 week (n=14)**	**12 week (n=14)**
$CD3^+$ cells (cells/μl) [a]	1517±411	1492±470	1591±463	1420±331	1535±410	1478±409
$CD4^+$ cells (cells/μl) [a]	748±246	765±308	811±272	725±248	769±291	755±246
$CD8^+$ cells (cells/μl) [a]	620±241	598±250	627±240	555±173	597±192	568±226
NK activity (Lytic unit/10^7PBMC) [a]	26.06±15.68	25.95±13.49	39.19±23.37	33.45±19.43	49.91±55.43	34.67±21.78
IL-2 (pg/ml)	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]	2/14 [b]	
ND						
IL-4 (pg/ml)	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]
IFN-γ (pg/ml)	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]	0/14 [b]

[a] Each value represents mean ± SD
[b] Each value represents numbers of volunteers with cytokines levels greater than minimum detectable levels/ total numbers of volunteers
ND: not done

ively for 2 months. A preliminary assessment of its efficacy on immunity was additionally examined.

No major adverse events which were related to taking the *G. pentaphyllum* capsules were reported in any groups of the study, suggesting that the 2-month use of *G. pentaphyllum* was well tolerated.

The results of hematological parameters and the numbers of $CD3^+$, $CD4^+$ and $CD8^+$ cells did not demonstrate significant changes in any of the 3 groups during orally administration of the extract for 8 weeks, indicating that *G. pentaphyllum* had no effects on hematological system. Significant difference in the number of $CD4^+$ cells found at week 12 of Group 1 may not be relevant to the effect of the extract. It may possibly be due to other stimulating agents or body factors resulting in an elevation of $CD4^+$ cells because the duration of taking *G. pentaphyllum* capsules were 8 weeks.

G. pentaphyllum extract did not significantly alter serum levels of liver enzymes which were AST, ALT and ALP. AST and ALT are indicators of acute and toxic damage to liver cells, while ALP could be used for assessment of long-term or chronic toxic effect on the liver (Zimerman, 1984; Mayne, 1994). Serum levels of creatinine and BUN were used to assess renal function (Faulkner and King, 1996). Our findings observed significant changes in creatinine in the groups receiving the extract at the doses of 200 and 400 mg b.i.d. at week 8 but the changes were within normal ranges and did not differ in a clinically significant manner during the trial. In addition, differences in the levels of total protein, albumin, total bilirubin, glucose, cholesterol, triglyceride and uric acid remained within normal limits.Taken together, the blood chemistry results may suggest that the extract at the doses given does not produce any short-term or long-term toxicity on the liver, the kidney or other health status.

The activity of NK cells is known as one of the first lines of host defense mechanisms against a variety of infections. We measured the effect of *G. pentaphyllum* on NK cell activity in all volunteers. No changes in NK cell activity were found in any of the extract-treated groups. Our result suggested that the extract at the doses given to healthy subjects in this study may not be able to enhance NK cell activity.

Cytokines are known to play a role in controlling immune responses. In this study, we measured the levels of serum IL-2, IL-4 and IFN-γ

in order to assess the effects of the *G. pentaphyllum* extract on the functions of immune cells. IL-2 and IFN-γ regulates proliferation and differentiation of lymphocytes while IL-4 promotes production of antibody (Vilcek and Le, 1994). In our study, we found that the number of subjects with higher IL-2 levels in serum during the trial was small and was not a dose-related pattern. It may indicate that the water extract of *G. pentaphyllum* at the doses given in this study did not induce cytokine secretion in healthy volunteers. Further investigation may need to assess *in vivo* effective doses of the extract on NK cell activity as well as induction of cytokines secretion.

The results of the present phase I study of *G. pentaphyllum* grown in northern Thailand revealed no adverse events in any of the 3 groups. Laboratory parameters were within normal ranges or was borderline statistically significant different. Furthermore, the extract did not induce any clinically significant changes during the 2-month period. Our study suggested that the extract at the doses given is safe for normals. Whether the water extract of *G. pentaphyllum* grown in Thailand possesses a potential effect on the immune system as previously reported (Hou *et al.,* 1991) should be further investigated in immunocompromised hosts such as cancer patients with either radiotherapy or chemotherapy or people living with HIV/AIDS.

Acknowledgements

We would like to acknowledge Ms. Sirirat Likanonsakul of the Bamrasnaradura Hospital for helping with flow cytometry.

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