การทดสอบความปลอดภัยของสารสกัดเถาวัลย์เปรียงในอาสาสมัครสุขภาพดี

Safety of *Derris scandens* Hydroalcoholic Extract in Healthy Volunteers

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Abstract

A phase 1 trial was performed in 12 healthy volunteers to primarily investigate safety of *Derris scandens* as well as to preliminary assess its effects on immune system. The volunteers received 400 mg/day of *D. scandens* hydroalcoholic extract (200 mg b.i.d.) for 2 months. No major side effects were reported from any of the subjects throughout the study. It was found that any significant changes in hematological and biochemical parameters were within normal limits. A significant rise in the frequencies of subjects with increasing amounts of IL-2, IL-4 and IL-6 after administration was shown. Our results suggested that the hydroalcoholic extract of *D. scandens* at the dose of 400 mg/day given to normal volunteers for 2 months was safe and could induce the secretion of cytokines that might help in modulating immune responses.

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บทคัดย่อ

ได้ทำการศึกษาเพื่อทดสอบความปลอดภัยและประสิทธิผลเบื้องต้นต่อระบบภูมิคุ้มกันของเถาวัลย์เปรียงในอาสาสมัครจำนวน 12 ราย โดยให้อาสาสมัครรับประทานแคปซูลเถาวัลย์เปรียงที่สกัดจากผงเถาวัลย์เปรียงด้วยร้อยละ 50 ของเอธานอล ครั้งละ 1 แคปซูล (200 มก./แคปซูล) วันละ 2 ครั้งเช้า-เย็น เป็นเวลา 2 เดือน พบว่าอาสาสมัครทั้ง 12 รายไม่มีอาการข้างเคียงใดๆ ระหว่างรับประทานสารสกัด ค่าทางโลหิตวิทยาและค่าทางชีวเคมีบางค่าที่เปลี่ยนแปลงจากก่อนได้รับสารสกัดนั้นพบว่าอยู่ในช่วงของค่าปกติ นอกจากนี้ยังพบความแตกต่างอย่างมีนัยสำคัญในจำนวนของอาสาสมัครที่มีปริมาณของ IL-2, IL-4 และ IL-6 ในซีรั่มเพิ่มขึ้น จากการศึกษานี้แสดงว่าสารสกัดเถาวัลย์เปรียงที่ขนาด 400 มก. ต่อวัน มีความปลอดภัยเมื่อรับประทานติดต่อกันนาน 2 เดือน และสามารถเหนี่ยวนำให้มีการหลั่งของ IL-2, IL-4 และ IL-6 ที่อาจมีส่วนช่วยควบคุมการทำงานของระบบภูมิคุ้มกันของร่างกาย

Introduction

Herbal preparations/extracts are commonly used in Thailand by various communities to alleviating health problems and strengthening health. Little has been studied on toxic effects during taking each preparation/extract, including the preparation/extract from *Derris scandens* Benth.

D. scandens is a woody vine growing throughout Southeast Asia (1,2). In Thai traditional medicine, the stem of *D. scandens* has been widely used as expectorant, antitussive, diuretic, antidysentery, and for health promotion (2).

It has been reported that hydroalcoholic extract of *D. scandens* stems showed immunostimulating activities on mouse and human immune cells (3,4). Lymphocyte proliferative response and IL-2 secretion were increased after stimulation of mouse splenic lymphocytes with the hydroalcoholic extract. Additionally, the extract was shown to stimulate lymphocyte proliferation and induced IL-2 secretion from normal peripheral blood mononuclear cells (PBMC). Furthemore, the *D. scandens* hydroalcoholic extract increased natural killer (NK) cell activities of normal individuals and HIV-1 infected patients *in vitro*.

Chronic toxicity was performed in rats by orally given the extract at various concentrations. The 6-months toxicity study indicated that the hydroalcoholic extract was safe even at the dose of 600 mg/kg/day that was equivalent to 75-100 times over the dose of humans (5).

Owing to the pharmacological and toxicological profiles of the *D. scandens* extract,

a phase I trial was conducted at the clinic of the Department of Medical Sciences using healthy volunterrs to substantiate its safety in humans as well as its effects on immunological parameters.

Materials and Methods

Selection of Subjects: Volunteers were informed that the *D. scandens* hydroalcoholic 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 which they need 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/she developed adverse reactions to the *D. scandens* extract.

Eligible subjects were male or female individuals with ages ranging from 20 to 45 years, were negative to HB_sAg, 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 detectable by 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 was obtained from each of twelve persons who met those criteria.

A study protocol was approved by the Ethical Review Committee of the Thai Ministry of Public Health on December 4, 2000.

Treatment of the subjects

All 12 subjects were given capsules of *D. scandens* (b.i.d.) for 2 months. The extract was prepared as previously described (4) and its quality was controlled by *in vitro* determining immunostimulating activities. The extract was formulated into standardized capsule dosage form. One capsule contained 200 mg of dry hydroalcoholic extract from *D. scandens* stems.

Clinical assessment

At baseline and at biweekly visits, a physical examination was performed and a review of adverse reactions, concurrent medication and compliance was completed. Ad-

verse reactions 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 aminotrasferase (ALT), alkaline phosphatase (ALP), bilirubin, creatinine, blood urea nitrogen (BUN), cholesterol, triglycerides, total protein, albumin, uric acid, glucose, sodium, potassium and chloride.

Immunological assessment was quantitated for $CD3^+$, $CD4^+$ and $CD8^+$ cells by a flow cytometer (EPICS-XL, Becton Dickinson, USA). The amounts of serum IL-2, IL-4 and IL-6 were examined using Human IL-2 ELISA, Human IL-4 ELISA and Human IL-6 ELISA kits, respectively (ENDOGEN, Inc., USA).

Statistical analysis

Data were analyzed by an SPSS program version 9.0. Statistical comparisons of means of laboratory measurements were performed using repeated measured ANOVA. The numbers of subjects with increasing amounts of each cytokine were analyzed by Fisher's Exact test.

Results

The study was designed to primarily determine the safety of the *D. scandens* hydroalcoholic extract in normal volunteers. Additionally, preliminary assessment of its efficacy on immunity was examined. Twelve volunteers, consisting of 6 males and 6 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 own control.

Adverse effects

There was no anaphylactic reaction reported from any volunteers. All of them were compliant with the study for the whole 2 months. At the 2nd week of the trial, one volunteer had a headache, one had lots of sweat and the other one had constipation. Three subjects reported sleepiness at week 2. Increasing in food consumption was observed in 3 persons (one was at week 2, another one was at week 4 and the other one was at week 8 of the study.) No other signs of side effects were noticed during the duration of the trial.

Laboratory results

Effect on hematological parameters

Significant changes in the number of white blood cell, neutrophil (%), lymphocyte (%), monocyte (%), eosinophil (%), the number of red blood cell and platelet were not observed during the trial. The level of hemoglobin and the percentage of hematocrit were significantly decreased at week 4 and 6 and at week 6 and 8, respectively, as compared with the baseline (week 0) measurements (Table 1). Significant increase in basophil (%) was found at week 2, 4, 6 and 8.

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 liver enzymes (AST, ALT and ALP), bilirubin, BUN, cholesterol, triglycerides, total protein, uric acid, glucose, and potassium (Table 2). The levels of creatinine were significantly increased at week 2 and 6. The levels of serum albumin at week 2, 4 and 6 were significantly decreased. Sodium levels at week 2 and 4 were significantly lower than the baseline level but those at week 6 and 8 were significantly elevated. Serum chloride levels were significantly decreased at week 2 and 4.

Effect on immunological parameters

There were no significant changes in the numbers and the percentages of $CD3^+$, $CD4^+$ and $CD8^+$ cells and the ratios of $CD4^+$ to $CD8^+$ cells during the trial (Table 3). No detectable levels of serum IL-2, IL-4 and IL-6 in all 12 volunteers were found at the onset of the study (Table 4). The levels of serum IL-2 were detected in 5, 4, and 4 subjects

above baseline at week 2, 4 and 6, respectively. The amount of serum IL-6 was detected in 7, 6, 2 and 10 volunteers at week 2, 4, 6 and 8 respectively. The levels of IL-4 were detectable in 1 and 2 volunteers at week 6 and 8, respectively. The frequency of the subjects with detectable IL-2 was significant in the 2^{nd}, 4^{th} and 6^{th} weeks of the study, while that of the subjects with detectable IL-6 was significant in the 2^{nd}, 4^{th} and 8^{th} weeks. There was no significant difference in the frequency of the volunteers with detectable IL-4.

Discussion

We conducted the phase 1 trial to determine the safety of the hydroalcoholic extract of *D. scandens* given to 12 normal volunteers at the dose of 400 mg/day for 2 months. There were no major side effects reported from any of the volunteers. The study was, therefore, found to be well tolerated.

Laboratory investigations on hematological parameters and biochemical profiles showed significant differences in Hct (%), basophil (%), the levels of hemoglobin, creatinine, albumin, sodium and chloride. Increase or decrease in those values were within their normal ranges of did not result in a clinically significant condition during the trial.

Cytokines are integral components that play a central role in the regulation of cell differentiation, proliferation and intercellular communication network (6). They are required to mount and control immune responses. In this study, we measured the amount of serum IL-2, IL-4 and IL-6 to assess the effects of the *D. scandens* extract on functions of immune cells. IL-2 regulates proliferation and differentiation of lymphocytes (7). IL-4 promotes production of antibody (8,9,10). IL-6 is an inducer of plasma cell development and has been shown to influence IL-4 production (11,12,13). Our findings may suggest that the *D. scandens* extract could provide help in moderating secretion of Th1 and Th2 cytokines that are essential in activation and development of adaptive immune system.

Both the clinical and laboratory results demonstrated that the hydroalcoholic extract of *D. scandens* did not induce any significant changes in all volunteers receiving the extract at the dose of 400 mg/day (b.i.d.) for 2 months, suggesting that the extract is safe for normals. In addition, the extract showed its capability to induce cytokines secretion. A larger trial, however, should be performed to evaluate the effects of *D. scandens* on stimulating immunity in normal volunteers as well as in immunocompromised persons such as HIV-infected individuals.

Acknowledgements

We would like to acknowledge Ms. Supaporn Pittiporn of the Chaopraya Apaipubate Hospital, Prachinburi, for identification and providing *D. scandens* and Ms. Weena Treesangsri for assistance with laboratories. We thank Ms. Naiyana Wattanasri and her staff for their help on detection of HB_s Ag and anti-HCV antibody. We would also like to thank Ms. Sirirat Likanonsakul of the Bamrasnaradura Hospital for helping with flow cytometry.

Table 1 Hematological results of normal volunteers orally given *D. scandens* extract for 8 weeks

	Duration				
	week 0	week 2	week 4	week 6	week 8
WBC (K/μL)	7.00 ± 1.74	6.96 ± 1.54	7.06 ± 1.87	6.79 ± 1.60	6.51 ± 1.72
%Neutrophil	56.54 ± 7.69	57.14 ± 7.46	56.14 ± 10.41	55.18 ±7.70	57.51 ± 7.30
%Lymphocyte	33.92 ± 6.41	31.85 ± 6.11	31.91 ± 8.30	33.51 ± 6.05	31.32 ± 6.47
%Monocyte	6.37 ± 3.26	6.51 ± 1.58	6.72 ± 2.04	6.60 ± 1.66	6.70 ± 1.72
%Basophil	0.21 ± 0.30	0.92 ± 0.40*	0.92 ± 0.36*	0.84 ± 0.30*	0.99 ± 0.33*
%Eosinophil	2.96 ± 1.83	3.58 ± 1.89	4.32 ± 2.68	3.87 ± 2.16	3.47 ± 1.84
RBC($X10^6$/μL)	5.01 ± 0.56	4.95 ± 0.63	4.87 ± 0.67	4.78 ± 0.60	4.79 ± 0.65
Hemoglobin(g/dL)	13.69 ± 1.84	13.20 ± 1.94	12.87 ± 1.88*	12.98 ±1.86*	13.30 ± 1.91
%Hematocrit	42.58 ± 5.26	41.36 ± 5.92	40.55 ± 5.72	39.85 ± 5.46*	39.28 ±5.47*
Platelet(K/UμL)	232.09 ± 67.04	264.91 ± 41.85	247.91 ± 40.81	260.55 ± 37.72	255.82 ± 42.57

Each value represents mean + SD

* significantly different from initial ($p < 0.05$)

Table 2 Blood chemistry reslts of volunteers orally given *D. scandens* for 8 weeks

	Duration				
	week 0	week 2	week 4	week 6	week 8
AST (U/L)	20.91 ± 3.81	22.45 ± 4.16	20.82 ± 4.77	24.09 ± 5.17	22.91 ± 7.16
ALT (U/L)	21.64 ± 6.93	23.91 ± 9.78	25.73 ± 11.31	23.55 ± 6.83	23.64 ± 7.37
ALP (U/L)	78.45 ± 21.16	75.91 ± 20.23	74.18 ± 19.81	77.73 ± 18.45	74.18 ± 17.95
Bilirubin (mg/dL)	0.54 ± 0.19	0.63 ± 0.32	0.61 ± 0.26	0.63 ± 0.27	0.64 ± 0.27
Creatinine (mg/dL)	1.01 ± 0.13	1.04 ± 0.14*	1.05 ± 0.15	1.08 ± 0.15*	1.05 ± 0.13
BUN (mg/dL)	11.39 ± 2.51	12.14 ± 2.79	11.45 ± 2.51	12.77 ± 1.92	12.14 ± 2.19
Cholesterol (mg/dL)	201.93 ± 24.47	211.54 ± 28.05	209.56 ± 20.98	209.36 ± 26.55	210.75 ± 33.92
Triglyceride (mg/dL)	94.75 ± 41.92	76.73 ± 20.91	86.50 ± 29.56	102.12 ± 30.49	124.41 ± 53.60
Total protein (g/dL)	8.24 ± 0.33	8.20 ± 0.33	7.97 ± 0.39	8.18 ± 0.28	8.33 ± 0.29
Albumin (g/dL)	4.59 ± 0.32	4.33 ± 0.30*	4.32 ± 0.31*	4.42 ± 0.26*	4.56 ± 0.30
Uric acid (mg/dL)	5.69 ± 1.65	5.70 ± 1.60	5.71 ± 1.76	6.09 ± 1.91	6.23 ± 1.96
Glucose (mg/dL)	92.83 ± 5.47	91.87 ± 5.34	91.59 ± 6.45	93.90 ± 4.83	94.44 ± 4.39
Sodium (mmol/L)	143.00 ± 1.61	140.36 ± 1.36*	138.64 ± 1.50*	145.82 ± 2.40*	147.82 ± 3.68*
Potassium (mmol/L)	4.21 ± 0.32	4.24 ± 0.33	4.15 ± 0.19	4.25 ± 0.31	4.59 ± 0.22
Chloride (mmol/L)	106.73 ± 1.62	103.18 ± 1.60*	102.91 ± 1.58*	105.91 ± 2.66	104.73 ± 4.08

Each value represents mean ± SD

* significantly different from initial ($p < 0.05$)

Table 3 Immunological results of volunteers orally given *D. scandens* for 8 weeks

	Duration				
	week 0	week 2	week 4	week 6	week 8
$CD3^+$ cells	1578 ± 314	1522 ± 380	1507 ± 410	1558 ± 349	1374 ± 327
$CD4^+$ cells	820 ± 184	789 ± 205	801 ± 240	822 ± 244	733 ± 214
$CD8^+$ cells	664 ± 167	633 ± 187	618 ± 173	646 ± 128	559 ± 134
CD4/CD8	1.30 ± 0.43	1.32 ± 0.44	1.35 ± 0.37	1.32 ± 0.46	1.35 ± 0.42

Each value represents mean ± SD

Table 4 Frequencies of volunteers with increasing cytokine levels after receiving *D. scandens*

	Duration				
	week 0	**week 2#**	**week 4**	**week 6**	**week 8**
IL-2	0/12	5/11*	4/12*	4/12*	0/12
IL-4	0/12	0/11	0/12	1/12	2/12
IL-6	0/12	7/11*	6/11*,@	2/12	10/12*

Each value represents numbers of volunteers with increasing amount of each cytokine as compared with the amount on week 0

only 11 sera were tested for IL-2, IL-4 and IL-6 levels

@ only 11 sera were tested for IL-6 levels

* significantly different from initial ($p < 0.05$)

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