Safety Study of Apocynum venetum L. Extract in Healthy Adults

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The ethanol extract of *Apocynum venetum* L. leaves (VENETRON®, AVEX) is proved to have anti-depressant and anti-anxiety activities and used as an anti-stress healthy food. In order to evaluate the safety of A VEX, we conducted a safety study on 30 healthy male volunteers for consecutive 12 weeks intake. The following test items, height, body weight, blood pressure, pulse , bloodbiochemical test, hematology, urinalysis, self-rating depression scale (SDS) and state-trait anxiety inventory (STAI) were examined. The conscious and objective events were investigated according to the testee's diary. No harmful conscious and objective symptoms related to AVEX were observed. AVEX also did not cause any virtual changes in the blood and urine samples. Both SDS and STAI tests did not show any variation in these healthy volunteers. These results strongly indicate that AVEX does not show stimulant or sedative activities, and safe in healthy human.

Introduction

Apocynum venetum L., also called rafuma, is an upright shrub belonging to the family Apocynaceae that grows naturally in the temperate zone areas of Europe, China, and other Asian regions. In northwestern China, people have had the custom of drinking tea made from rafuma leaves since ancient times, and these leaves have been described as having hypotensive effects as well as beneficial effects on heart failure, diuresis and neurasthenia in the "Chinese Pharmacopoeia 2005" [1) 2)].

A forced swimming test in rats, a common model for evaluating anti-depressant effects, showed that rafuma leaf extract derived by ethanol extraction administered at 30-125 mg/kg for 2 weeks exerted anti-depressive effects, with significant reductions in the immobility time compared to controls [3)]. Moreover, examination of changes in neurotransmitter levels in the brain after 8 weeks of *A. venetum* ethanol extract (AVEX) administration at 15 mg/kg and 250 mg/kg showed reduced norepinephrine and dopamine concentrations in the hypothalamus, striate body and hippocampus [4)].

In forced swimming tests, the anti-depressant action of AVEX has been shown to be stronger than that of St. John's wort extract, which is known to possess anti-depressant properties [3) 5)]. AVEX has also been reported to show anxiolytic effects in an elevated plus maze test by Butterweck et al [6)].

Moreover, drug interactions have been reported with St. John's wort [7) 8)], whereas AVEX has been confirmed to have no effect on cytochrome P450 (CYP3A) or P-glycoprotein in rats[9)]. These results show that AVEX as an herb for mental care may be safer than St. John's wort.

The present study evaluated the effects of AVEX administered for 3 consecutive months on various clinical laboratory parameters and mental state, with the objective of investigating safety in humans.

2. Study methods

1) Subjects

Thirty-two male employees of Tokiwa Phytochemical Co., Ltd., (age range, 22-62 years) were enrolled in this study. The study was conducted under the approval of the ethics committee at Tokiwa Phytochemical. Subjects were recruited as volunteers from within the company, ensuring participation based on free will. The person in charge of the study provided individuals willing to participate with sufficient information on the objectives of the study, study design and privacy matters. Those individuals who provided written consent were then regarded as subjects. Before the study began, the subjects filled out a medical interview sheet for a survey of past medical history, current illnesses, current medications, etc., and were then judged as eligible or ineligible to participate in the study.

2) Study design

The study was conducted as an open-label study.

3) Investigational product

AVEX (VENETRON®; Tokiwa Phytochemical Co., Ltd.) is manufactured by extracting rafuma leaves from Shanxi Province in China using 60% ethanol and then purifying and drying the extract, and is characterized by the fact that hyperoside and isoquercitrin, flavonol glycosides, comprise ≥4% of the total content, as measured by high-performance liquid chromatography [3)]. Tablets containing 25 mg of AVEX each (Sankyo product; brand name, Venetron 25) were used for the study (Table 1).

4) Period and method of administration of the investigational product

The "Chinese Pharmacopoeia 2005" [2)] sets the dose for crude rafuma leaves at 6-12 g/day, equivalent to 480-960 mg of AVEX by yield conversion. An animal study showed anti-depressive effects with consecutive administration at 30-125 mg/kg/day [3)]. Moreover, a clinical study showed improvements in premenstrual syndrome at a dose of 50 mg/day in humans. The daily dose for the AVEX investigational product was thus set at 50 mg.

In the first 8 weeks, subjects received 2 tablets/day (1 tablet each in the morning and evening) so that the daily dose amounts to 50 mg as AVEX. In the remaining 4 weeks, the dose was tripled and

subjects received 6 tablets a day (3 tablets each in the morning and evening). The study was conducted over a period of 12 weeks between February 24 and May 19, 2005.

5) Test methods

Blood test, urinalysis, body weight measurement, heart rate measurement, blood pressure measurement and medical interview were performed before the initial dose, and 4, 8 and 12 weeks after the initial dose. Tests were performed before noon on the days of testing. Subjects were instructed not to eat anything within 5 h before testing. Height was measured only before the initial dose. Items evaluated on blood testing and urinalysis were as follows: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma--glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), triglyceride, total cholesterol, total protein, albumin, total bilirubin, urea nitrogen, creatinine and glucose as biochemical examinations; white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count as hematological examinations; and urinary pH, specific gravity of urine, qualitative test for protein, qualitative test for glucose, urobilinogen, bilirubin, ketone bodies and qualitative test for occult blood as urinalyses. The Self-Rating Depression Scale (SDS) [10] and State-Trait Anxiety Inventory (STAI) [11] were also administered at the time of testing to study whether the investigational product exerts mental excitatory or sedative effects on healthy individuals. The SDS used a questionnaire comprising 20 questions, with the subject choosing an answer on a 4-point scale (1, “none or occasionally” to 4, “almost always”). Answers were tallied, with higher scores indicating a stronger state of depression. The STAI evaluated two types of anxiety: anxiety at the time of measurement (state anxiety); and anxiety as a personality trait (trait anxiety). Judgments were based on answers on a 4-point scale (1, “almost none” to 4, “frequent” for trait anxiety; and 1, “definitely no” to 4, “definitely yes” for state anxiety) for 20 questions. Answers were tallied for each set of questions, with higher total scores indicating greater anxiety. During the study period, the subject entered the following information in a diary: whether they took the investigational product; subjective illness during the study period; and other remarks.

6) Statistical analysis

Measured values for body mass index (BMI), blood pressure, heart rate, hematological examination, blood biochemical examination, urinalysis, SDS and STAI are each presented as mean ± standard deviation. For each of these items, the paired t-test was performed for differences between the mean before treatment initiation and that at each time point. Furthermore, the Kruskal-Wallis method was used for multiple-group comparisons, and the Tukey-Kramer method was used for multiple comparisons between multiple groups. In these tests, the level of significance was set at 5%

(two-sided test).

3. Results

Of the 32 subjects, 31 completed the 8-week study period from the beginning of treatment (the period for AVEX 50 mg/day) and 30 completed the 4-week study period from Week 9 (the period for AVEX 150 mg/day). The 2 subjects who dropped out requested discontinuation of the study themselves, and the study was not considered causally related to the discontinuation.

The status of administration of the investigational product in the 30 subjects who completed the study was as follows: 100% (280/280 tablets), 7 subjects; ≥90% (252/280 tablets), 18 subjects; ≥80% (224/280 tablets), 3 subjects; and ≥70% (196/280 tablets), 2 subjects. The rate of administration over the entire study was 94% (7882/8400 tablets).

1) Subjective adverse events

Subjective adverse events during the study period identified in the diaries of the 30 subjects who completed the study are shown in Table 2. Influenza, common cold and pollinosis occurred frequently along with symptoms associated with these conditions; namely, pyrexia, throat pain, headache and rhinitis. Besides these, diarrhea, constipation, asthma, eczema, stomatitis and sty developed during the study period. To treat these adverse events, the subjects consulted a physician or took a medicine (prescription or over-the-counter) at individual discretion. As a result, all symptoms proved temporary and resolved during the study period.

2) Evaluation of safety

(1) Body weight, blood pressure, heart rate

Effects on body mass index (BMI), blood pressure and heart rate are shown in Table 3. No significant changes were observed in BMI and blood pressure. However, blood pressure above the normal range (systolic blood pressure, ≥140 mmHg; diastolic blood pressure, ≥90 mmHg) showed decreasing tendencies 12 weeks after the start of treatment, as shown in Table 4 (decreases were observed in 7 of 7 subjects with systolic blood pressure ≥140 mmHg and in 4 of 6 subjects with diastolic blood pressure ≥90 mmHg). Regarding heart rate, significant decreases were observed 8 weeks after the start of treatment, but these changes were within the reference range.

(2) Hematological examination

There were no significant changes in white blood cell count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC or platelet count (Table 5).

(3) Blood biochemical examination

There were no significant changes in AST, ALT, γ-GTP, ALP, LDH, triglyceride, total cholesterol, total bilirubin, urea nitrogen, creatinine and glucose. Total protein showed significant decreases at 8

and 12 weeks after the start of treatment, but both changes were within the reference range (6.7-8.3 g/dL according to the Chiba Prefectural Manual for Unification of Laboratory Values). Albumin, as with total protein, also showed a significant decrease at 8 weeks after the start of treatment, but the change was within the reference range (4.0-5.0 g/dL according to the Chiba Prefectural Manual for Unification of Laboratory Values) (Table 6).

(4) Urinalysis

There were no significant changes in specific gravity of urine, urinary pH, qualitative test for protein, qualitative test for glucose, bilirubin and qualitative test for occult blood. Individually, worsening of test values to 2+ in urobilinogen and ketone bodies was observed in 1 subject each at 12 weeks after the start of treatment (Tables 7 and 8).

(5) SDS

According to the criteria for the clinical evaluation of SDS scores, scores <40 indicate absence of depressive state, 40-49 indicate mildly depressive state and ≥50 indicate depressive state requiring treatment. Mean SDS score before the start of treatment was 37.2, and the scores were within normal range in most subjects. No significant changes were observed during the study period (Table 9).

(6) STAI

According to the criteria for the clinical evaluation of state anxiety and trait anxiety scores, 42-50 points are considered “high” and ≥51 points are considered “very high” for state anxiety, and 45-54 points are considered “high” and ≥55 points are considered “very high” for trait anxiety. Mean scores before starting AVEX administration were 41.8 and 43.8 for state anxiety and trait anxiety, respectively. Both values were within the normal range. There were no significant changes in either state or trait anxiety during the study period (Table 9).

4. Discussion

1) Subjective adverse events

The high incidences of influenza, common cold and pollinosis along with the various symptoms associated with these conditions observed in the results of the present study were attributable to an overlap of influenza and pollinosis seasons with the study period (February-May 2005). The year 2005 was a year in which cedar pollen had a record-breaking overwhelming influence[12], and exposure to a massive amount of cedar pollen presumably induced pollinosis and common cold-like symptoms in the subjects. Headache, diarrhea, constipation, throat pain, asthma, eczema, stomatitis, sty and rhinitis developed as other symptoms of poor physical condition. However, all of these symptoms were transient and resolved during the study period, and were not consistent with the dose of the investigational product or time-dependent changes. No definitive judgment could be made because of the absence of a control group, but the investigational product did not seem to be causally related to these adverse events.

2) Evaluation of safety

Rafuma leaves have traditionally been used to lower blood pressure in the form of tea or hot water extract. According to the Chinese Material Medica Dictionary, improvements were reported in many patients with hypertension who received tea made using rafuma leaves (3.75-7.50 g/day) [1]. Moreover, hypotensive effects of the flavonoid component in rafuma extract and leaves were recently reported in rat models of spontaneous hypertension [13)-18)]. In our study, no statistically significant difference attributable to the investigational product was observed with regard to blood pressure. However, decreasing tendencies were observed at 12 weeks in those individuals who already had high blood pressure before taking the investigational product (maximum blood pressure, ≥140 mmHg; minimum blood pressure, ≥90 mmHg). No marked changes were observed in those individuals whose blood pressure was in the normal range from the beginning. However, these results were consistent with those of studies conducted by Kagawa et al. [15)] and Kwan et al. [16)] and suggestive of a possible beneficial effect on relatively high blood pressure even in humans. On the other hand, a significant decrease was observed in heart rate 8 weeks after administration, although the change was slight and within the normal range. According to Nishibe et al., intravenous injection of quercetin, an aglycone of flavonol glycoside contained in rafuma, to rats led to decreases in heart rate [17)]. The quercetin glycoside contained in AVEX may thus have had an effect on the heart rate.

In the blood biochemical examination, significant decreases were observed in total protein and albumin levels. However, both these changes were within reference ranges. Regarding total protein, the concentration of protein including albumin was measured, which means that changes in total protein levels reflect changes in albumin levels. In the above-mentioned results, therefore, decreases in albumin levels were considered to be directly reflected in the total protein levels. The decrease in albumin levels may be due to liver disorder, but no marked changes were observed in other hepatic function test values. AVEX was thus considered unlikely to have affected liver functions. AVEX was not considered as the cause of the decrease in total protein levels, as no dose-response relationship was identified from these test results, although no definitive judgment could be made given the absence of a control group.

There were no statistical significant differences in other biochemical, general, urinary and hematological test parameters. Worsening in levels of urobilinogen and ketone bodies was observed 12 weeks after the initial dose in some subjects, but a comprehensive judgment made in consideration of all test items showed that no marked abnormal changes in body functions occurred during the AVEX study period.

Moreover, the fact that there were no significant changes in SDS or STAI during the study period showed that AVEX had no excitation or sedative effects on healthy individuals.

These results from the present study suggest that AVEX is safe at ≤150 mg/day.

5. Conclusions

We conducted a study of AVEX administered orally over 8 weeks at 50 mg/day followed by 4 weeks at 150 mg/day in 30 healthy men. As a result, there were no abnormal findings suggestive of an effect of the investigational product in hematological, blood biochemical or urinary parameters, or in body weight, blood pressure or heart rate.

Table 1 Ingredients of the investigational product (per tablet)

Ingredient	Content (%)	Weight (mg)
AVEX	12.4	25
Glycyrrhiza extract	1.2	2.5
Corn starch	41.6	84
Lactose	11.2	22.7
Microcrystalline cellulose	29.5	59.5
Edible oil and fat	3.1	6.3
Shellac	0.9	1.72
Glycerin fatty acid ester	0.1	0.28
Total	100	202

Table 2 Number of episodes of subjective adverse events in the study period

Adverse events	Number of episodes
Common cold	13
Headache	9
Pollinosis	8
Influenza	4
Throat pain	4
Rhinitis	3
Pyrexia	2
Diarrhea	1
Chills	1
Asthma	1
Stomatitis	1
Sty	1
Constipation	1
Eczema	1
Cough	1

Table 3 Changes in BMI, blood pressure and heart rate over time

Item	Before start of administration	4 weeks after administration	8 weeks after administration	12 weeks after administration
BMI	24.9±2.9	24.7±2.8	24.6±2.8	24.7±2.9
Systolic blood pressure (mmHg)	125.9±16.0	127.3±14.4	125.8±12.8	126.5±9.6
Diastolic blood pressure (mmHg)	76.5±14.5	82.8±15.0	77.9±9.4	79.7±10.8
Heart rate (beats/min)	74.3±7.9	71.0±6.4	69.8±5.2*	70.3±5.2

n=30.
Mean±SD.
*: $P<0.05$.

Table 4 Individual blood pressure data (mmHg)

Subject no.	Before starting administration		4 weeks after administration		8 weeks after administration		12 weeks after administration	
	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP
1[b]	124	90	138	96	132	88	128	94
2	116	58	118	74	108	68	116	68
3	128	68	136	78	124	70	120	74
4	114	60	116	76	122	76	128	80
5[a]	142	88	138	90	134	86	126	80
6	118	72	132	80	116	72	126	70
7	138	70	110	70	122	80	124	84
8[ab]	162	120	166	124	152	94	148	108
9	128	88	122	80	128	80	120	76
10[a]	146	68	146	90	142	86	134	90
11	108	62	114	70	116	60	128	64
12	102	60	128	82	124	70	118	66
13	110	68	116	58	122	72	124	68
14	104	70	122	74	118	74	120	80
15[ab]	146	94	124	88	124	86	132	82
16	122	82	120	70	128	84	136	84
17	106	66	114	78	124	78	122	76
18	118	78	120	76	122	74	122	78
19	118	60	114	80	130	88	126	80
20[ab]	158	100	166	112	152	94	152	94
21	118	74	106	60	112	64	124	60
22	124	80	134	86	152	92	144	90
23	108	62	126	88	114	70	106	80
24[b]	134	92	146	106	142	78	134	84
25	118	88	132	88	118	78	122	80
26	116	60	112	62	100	62	128	64
27	126	80	130	84	116	70	128	80
28[ab]	148	92	132	112	138	92	128	96
29[a]	144	68	124	80	112	78	116	74
30	120	84	118	80	124	80	116	88

SBP: systolic blood pressure
DBP: diastolic blood pressure

[a] Individuals with SBP >140 before the start of administration
[b] Individuals with DBP >90 before the start of administration

Table 5 Changes in hematological test values over time

Item	Before starting administration	4 weeks after administration	8 weeks after administration	12 weeks after administration
White blood cell count (/μL)	6314±1421	6146±1633	6050±1286	5948±1640
Red blood cell count ($\times10^4$/μL)	502.7±36.0	503.9±29.8	497.2±29.6	496.2±38.7
Hemoglobin (g/dL)	15.77±0.92	15.83±0.91	15.52±0.78	15.52±0.96
Hematocrit (%)	47.01±2.64	47.11±2.25	46.11±1.95	46.00±2.76
MCV (fL)	93.74±5.59	93.68±5.42	92.90±5.14	92.97±5.62
MCH (pg)	31.45±1.60	31.48±2.06	31.27±1.81	31.37±1.97
MCHC (%)	33.56±0.70	33.61±0.98	33.65±0.64	33.74±0.77
Platelet count ($\times10^4$/μL)	25.48±5.54	24.94±5.46	24.98±4.97	25.33±5.38

n=30.
Mean±SD.

Table 6 Changes in blood biochemical test values over time

Item	Before starting administration	4 weeks after administration	8 weeks after administration	12 weeks after administration
AST (IU/L)	25.7±9.6	25.7±8.7	22.4±5.6	23.9±8.9
ALT (IU/L)	33.8±25.7	32.2±21.4	27.0±15.4	31.2±23.7
γ-GTP (IU/L)	62.5±59.1	57.4±54.6	47.6±38.8	51.9±45.8
ALP (IU/L)	235.5±85.3	219.3±61.0	219.7±61.6	220.0±65.5
LDH (IU/L)	186.9±29.7	189.6±28.9	180.2±28.0	183.5±28.2
Triglyceride (mg/dL)	116.7±97.2	123.1±85.5	106.8±49.6	115.1±77.5
Total cholesterol (%)	194.0±38.9	195.0±39.6	189.0±36.2	191.3±37.0
Total protein (g/dL)	7.62±0.39	7.50±0.31	7.37±0.29*	7.37±0.33*
Albumin (g/dL)	4.82±0.29	4.72±0.26	4.57±0.21*	4.67±0.23
Total bilirubin (mg/dL)	0.72±0.23	0.72±0.24	0.69±0.26	0.75±0.25
Urea nitrogen (mg/dL)	14.1±3.1	14.2±3.1	14.5±3.0	14.4±2.6
Creatinine (mg/dL)	0.840±0.081	0.835±0.090	0.832±0.086	0.880±0.088
Blood glucose (mg/dL)	89.7±10.9	90.5±10.5	90.1±11.2	90.0±10.0

n=30.
Mean±SD.
*: $P<0.05$.

Table 7 Changes in general urine test values over time

Item	Before starting administration	4 weeks after administration	8 weeks after administration	12 weeks after administration
Protein	(-) 28 (±) 3 (+) 0	(-) 28 (±) 1 (+) 2	(-) 31 (±) 0 (+) 0	(-) 29 (±) 1 (+) 0
Glucose	(-) 29 (±) 1 (+) 1	(-) 31 (±) 0 (+) 0	(-) 31 (±) 0 (+) 0	(-) 30 (±) 0 (+) 0
Urobilinogen	(-) 0 (±) 31 (+) 0 (2+) 0	(-) 0 (±) 31 (+) 0 (2+) 0	(-) 0 (±) 31 (+) 0 (2+) 0	(-) 0 (±) 29 (+) 0 (2+) 1
Bilirubin	(-) 31 (±) 0	(-) 31 (±) 0	(-) 31 (±) 0	(-) 30 (±) 0
Ketone bodies	(-) 31 (±) 0 (+) 0 (2+) 0	(-) 31 (±) 0 (+) 0 (2+) 0	(-) 31 (±) 0 (+) 0 (2+) 0	(-) 29 (±) 0 (+) 0 (2+) 1
Occult blood	(-) 30 (±) 1	(-) 31 (±) 0	(-) 31 (±) 0	(-) 29 (±) 1

Protein, glucose, bilirubin, ketone bodies, occult blood:

Standard: negative (-)

Non-standard: false positive (±); slightly positive (+); positive (2+)

Urobilinogen

Standard: false positive (±); slightly positive (+)

Non-standard: positive (2+)

Table 8 Changes in urine biochemical test values over time

Item	Before starting administration	4 weeks after administration	8 weeks after administration	12 weeks after administration
Specific gravity	1.019±0.007	1.019±0.006	1.018±0.006	1.020±0.006
pH	5.95±0.60	6.08±0.71	6.16±0.86	6.03±0.96

n=30.

Mean±SD.

Table 9 SDS and STAI evaluations

Item	Before starting administration	4 weeks after administration	8 weeks after administration	12 weeks after administration
SDS	37.2±7.6	36.3±8.0	35.9±8.5	35.8±8.7
STAI: state anxiety	41.8±8.5	40.7±7.7	40.5±8.3	41.1±8.9
STAI: trait anxiety	43.8±10.0	42.4±10.9	42.0±10.5	42.1±10.7

n=30.

Mean±SD.