Safety and Toxicological Evaluation of VENETRON®

—A Botanical Health Product—

■

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ABSTRACT

VENETRON® is a commercial botanical product made from the leaves of *Apocynum venetum* L. It contains flavonoids, with the main functional components being hyperoside and isoquercitrin. The safety of VENETRON® were evaluated in animals and human clinical trials. Briefly, the 50% lethal dose of VENETRON® was determined to be greater than 2000 mg/kg body weight in an acute oral toxicity study of mice. An 8-week subchronic toxicity study in rats revealed the no-observed-adverse-effect-level for VENETRON® to be at least 250 mg/kg body weight/day. Drug interaction testing indicated that VENETRON® did not influence cytochrome P450 3A or P-glycoprotein in rats. Furthermore, 30 healthy volunteers in Japan participated in a safety study, ingesting 50 mg/day for weeks 1-8, followed by 150 mg/day for weeks 9-12. No adverse effects resulted from taking VENETRON®, and biochemical parameters remained within the reference ranges. Also through a placebo-controlled study with 17 healthy volunteers in Japan, ingesting 50 mg/day for 8 days, showed no adverse effects from taking VENETRON®. In addition, an 8-week study of 50 mg VENETRON®/day was also conducted in 39 individuals in Canada, the UK, and the USA. The adverse events and blood pressure records indicated no difference between the VENETRON® and placebo groups. These results demonstrate the safety of VENETRON® and provide overall support for the potential of VENETRON® in various health-related applications.
（**Jpn Pharmacol Ther 2018；46：127-35**）

KEY WORDS　*Apocynum venetum*, Flavonoids, Functional foods, Safety, VENETRON®

INTRODUCTION

Apocynum venetum L.（Apocynaceae）is a wild shrub distributed in north-western China. The leaves of *A. venetum* have been used to make tea in China since ancient times and also as a traditional herbal medicine for the treatment of hypertension and neurasthenia.[1)] In Japan, an aqueous extract of roasted *A. venetum* leaves is called to be "YANG LONG Level Care", and permitted as a FOSHU（Food for Special Health Uses）for hypertension in 2007. The mechanism underlying the antihypertensive effect of an *A. venetum* leaves

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Table 1　Body weights of mice treated orally with 2000 mg VENETRON®/kg body weight（VENETRON®）or purified water（Control）for 14 days

	Body weight（g）						
Male	Day 0	Day 7	Day 14	Female	Day 0	Day 7	Day 14
Control（n＝10）	27.8±0.6	32.3±1.5	36.6±1.3	Control（n＝10）	24.8±1.0	27.0±1.0	30.5±2.2
VENETRON®（n＝10）	27.8±0.6	32.8±1.3	37.4±1.5	VENETRON®（n＝10）	24.5±1.1	26.6±1.8	29.8±1.5

Data represent the mean ± the standard deviation

extract was studied recently.[2] The major active constituents of *A. venetum* leaves are flavonoids, which can scavenge free radicals and exert anti-inflammatory effects,[3,4] sedative effects,[5] and protective effects against human cell apoptosis.[6]

Flavonoids are well-known polyphenolic compounds, and mainly the phenolic groups in their structures are considered important for antioxidant activity,[7] as well as for other biological effects such as anti-cancer, inhibition of reactive oxygen species and LDL lipid peroxidation.[8–10] Flavonoids are abundant in fruits and vegetables, especially in the leaves of green tea and peels of citrus. Fruits and vegetables containing polyphenols are consumed popularly worldwide because of their health benefits.

VENETRON® is a commercial botanical product made from the leaves of *A. venetum* L. It mainly contains polyphenols such as catechin, epicatechin, gallocatechin, and flavonoids such as hyperoside, isoquercitrin, kaempferol, and quercetin.[11] Hyperoside and isoquercitrin, the major flavonoids in VENETRON®, are reported to exhibit antidepressant activity.[12,13] VENETRON® is used as a supplement, a pharmaceutical agent, and ingredient in various food products because of its positive effects on stress reduction and improvement of sleep quality supported by human clinical trials.[14–16]

The safety of herbal medicine and supplement needs to be carefully evaluated because of the wide usage of these botanical products. Many herbal medicine have been reviewed about the safety such as *Aloe vera*, ginseng, kava, and cat's claw and mentioned the latent problems of safety.[17,18] Therefore, the objective of this manuscript is to evaluate the safety of VENETRON®, including its acute and subchronic toxicities. We also review the safety of this product in clinical trials, as well as other toxicity considerations, including herb-drug interactions and cardiac glycoside analysis.

PREPARATION OF VENETRON®

VENETRON® was prepared by Tokiwa Phytochemical Co. Ltd., Chiba, Japan.[14,15] Briefly, dried ground leaves of *A. venetum* were extracted with 60%（v/v）aqueous ethanol at 60℃ and filtered. The obtained solution was then further purified through synthetic adsorbent resin, and the eluent was concentrated to obtain a homogeneous dark brown powder, VENETRON®, which contained a minimum of 4% hyperoside and isoquercitrin by HPLC method.

IN VIVO SAFETY STUDIES IN ANIMALS

1　Acute single-dose toxicity

The occurrence for adverse effects following short-term exposure to VENETRON® was investigated by Japan Food Research Laboratories（Tokyo, Japan）using a single-dose oral toxicity study, conducted in accordance with the Organization for Economic Cooperation and Development（OECD）guidelines for the testing of chemical 401（1987）. VENETRON® was dissolved in purified water to make a 100 mg/mL test solution. Following a 4-h fast, male and female, four-week-old Institute of Cancer Research（ICR）mice weighing 24–27 g（10/sex/group）received a single gavage dose of the VENETRON® test solution, equivalent to 2000 mg/kg body weight（bw）. A control group received the same volume of purified water（0.6 mL）. The general behavior of the mice was observed continuously for 1 h after treatment and then intermittently for 4 h, followed by observations over a period of 24 h. The mice were further observed for up to 14 days after the treatment for any signs of adverse effects, including death. Body weight was measured on days 7 and 14 after the treatment with VENETRON®. Endpoint evaluations included clinical signs of toxicity, body weight, and mortality.

No mice died throughout the experimental period, and no remarkable changes were found in any organs

Table 2　Body and organ weights of rats treated orally with the indicated dose of VENETRON® for 8 weeks

	Body（g）	Body（g）	Liver（g）	Kidney（g）	Prostate（g）	Heart（g）	Spleen（g）
	0 days	After 8 weeks					
Control	140.5±2.3	400.0±5.5	15.85±0.57	2.85±0.03	0.548±0.036	1.26±0.03	0.799±0.030
15 mg/kg	142.5±3.0	402.1±5.5	16.09±0.46	2.89±0.11	0.561±0.019	1.26±0.04	0.802±0.039
30 mg/kg	135.9±1.9	401.6±8.0	15.83±0.40	2.81±0.09	0.597±0.022	1.28±0.02	0.867±0.034
60 mg/kg	144.3±2.2	396.2±8.0	15.20±0.37	2.87±0.07	0.592±0.021	1.21±0.02	0.826±0.021
125 mg/kg	137.9±3.6	395.7±13.7	15.96±0.62	2.68±0.07	0.575±0.036	1.26±0.03	0.744±0.035
250 mg/kg	137.5±2.7	372.9±10.1	15.70±0.48	2.68±0.07	0.596±0.029	1.22±0.03	0.811±0.030

Data represent the mean weight（g）± the standard error（n=8–10）

of mice; in details, at about 20 minutes after administration, depression due to spontaneous motor activity was observed in all experimental animals, but, after then, not only they recovered completely but also they did not show any abnormalities. Body weight changes in the VENETRON® group were approximately the same as those noted in the control group（**Table 1**）.

Based on these results, the 50% lethal dose（LD_{50}）of VENETRON® under the conditions employed in this study was greater than 2000 mg/kg bw for both male and female ICR mice; using a conservative 100-fold safety factor, this is equivalent to 20 mg/kg for humans（approximately 1200 mg VENETRON®/day for a 60-kg person）.

2　Subchronic toxicity

The subchronic toxicity of orally administered VENETRON® was studied at the University of Münster, Germany, in 2001. Five doses（15, 30, 60, 125, and 250 mg/kg bw）of VENETRON® were administered as suspensions in purified water via gavage to male Sprague Dawley（200–220 g）rats（8–10/group）every day for 8 weeks. Purified water was administered to the control animals. Body weights were measured before and after the study. At the end of the study, the animals were euthanized and the liver, kidney, heart, spleen, and prostate were weighed. The statistical significance of any change in body or organ weight was assessed.

No statistically significant dose-related effects on body or organ weights were seen in any of the VENETRON® groups, as compared to the control group（**Table 2**）. The no-observed-adverse-effect-level（NOAEL）for VENETRON® under the conditions of this study was therefore at least 250 mg/kg bw/day for rats; using a conservative 100-fold safety factor, this is equivalent to 2.5 mg/kg for humans（approximately 150 mg VENETRON®/day for a 60-kg person）.

Table 3　Effect of a 2-week VENETRON® treatment on the pharmacokinetics of nifedipine

Treatment	C_{max}（ng/mL）	t_{max}（h）	AUC_{0-4h}（ng・h/mL）
Control	265.3±26.1	0.33±0.05	426.4±54.0
VENETRON®	216.0±31.9	0.70±0.36	425.5±93.3

Data represent the mean ± the standard error of the mean（n=3）
C_{max}=maximum plasma concentration of nifedipine
t_{max}=the time at C_{max}
AUC_{0-4h}=The area under the plasma concentration-time curve for 0–4 h
Data from reference 21

3　Drug interaction studies

The upsurge of herbal dietary supplements raise the concern of herb-drug interaction because of the high possibility of concomitant usage with conventional medication, and the researches of popular herb such as black cohosh, garlic, ginkgo, and ginseng have been mentioned to show herb-drug interaction according to the previous review.[19] Of different kinds of drug interaction index, the cytochrome P450（CYP）3A 4/5 is known to be important as it is involved in metabolizing about half of the conventional medications.[20] On the other hand, P-glycoprotein is also an important target in drug interaction study because of the protective roles such as limiting absorption of xenobiotics. It was known as the most studied ATP-binding cassette transporter due to the expression in a variety of tissues including small intestine, kidney, and liver.[20] Thus, drug interactions between VENETRON® and CYP3A or P-glycoprotein were studied using male Wistar rats（Hokudo, Sapporo, Japan）, which 3 to 5 rats were used in each experimental group, and were conducted in compliance with the Guidelines for the Care and Use of Laboratory Animals in the Health Sciences University of Hokkaido.[21] Nifedipine, a CYP3A sub-

strate, and methylprednisolone, a P-glycoprotein substrate, were commercially available.

1）Drug disposition of nifedipine

Six week-old rats were orally administered with VENETRON® suspended in distilled water at a dose of 3.3 mg/kg/day for 2 weeks. After VENETRON® administration, nifedipine（0.4 mg/kg）was administered. Then, the concentration of nifedipine in blood samples collected from the rats were analyzed by high-performance liquid chromatography（HPLC）with UV detection at 280 nm. Pharmacokinetic parameters, such as the maximum plasma concentration of nifedipine（C_{max}）and the time at C_{max}（t_{max}）, were obtained from the observed data. The area under the plasma concentration-time curve for 0-4 h（$AUC_{0-4\,h}$）was calculated. Based on these results, no significant differences in pharmacokinetic parameters were found between the VENETRON® and control groups（**Table 3**）. These results suggest that the administration of the recommended dose of VENETRON® does not affect CYP3A in rats.

2）Absorption of methylprednisolone

Six week-old rats were orally administered with VENETRON®（3.3 mg/kg/day）for 2 weeks. The jejunum was then excised, everted, and submerged in Tyrode's buffer containing methylprednisolone（100 μM）. The permeation of methylprednisolone across the intestine from the mucosal to the serosal surface was measured by sampling the serosal buffer at 15 and 30 min. The concentration of methylprednisolone was analyzed by HPLC with UV detection at 254 nm. No differences in methylprednisolone permeation were found between the control and VENETRON® groups at either 15 or 30 min, suggesting that the administration of VENETRON® does not affect P-glycoprotein in rats.

3）Analysis of cardiac glycoside

Cardiac glycosides such as cymarin are found in plants from the *Apocynum* family, including *Apocynum cannabinum*, *Apocynum venetum* L. *var. basiculmon* Hara and *Apocynum lancifolium* according to the previous reports.[22-24] Although the toxicity of cardiac glycosides from plants is rare, it could be life-threatening poison with a consequent overdosing, which has been reported to inhibit sodium-potassium-adenosine triphosphatase and cause hyperkalemia.[22] Therefore, the content of cardiac glycosides as cymarin in VENETRON® was analyzed to evaluate its safety. Briefly, VENETRON® was dissolved in 50%（v/v）aqueous ethanol to make a 1 mg/mL test solution, which was filtered through a 0.45-μm nylon membrane and then analyzed by liquid chromatography-tandem mass spectrometry. The content of cymarin in VENETRON® was less than 5 ppm, consistent with the disappearance of VENETRON®-induced toxicity.[25]

HUMAN CLINICAL SAFETY STUDIES

1 Clinical study performed in Japan（Ⅰ）

A safety study of VENETRON® intake for 12 consecutive weeks was conducted in 30 healthy male volunteers in Japan, aged between 22 and 62 years old.[26] All volunteers received a full explanation of the procedures that were employed in the study, and the consent were obtained from all of the volunteers. The VENETRON® dose was 50 mg/day in weeks 1-8 and then 150 mg/day in weeks 9-12. Blood testing, urinalysis, body weight measurement, heart rate measurement, blood pressure measurement, and medical interviews were performed before the initial dose and subsequently at 4, 8, and 12 weeks later.

The examined parameters were weight, blood pressure, pulse rate, urinalysis（protein qualitative analysis, sugar, urobilinogen, bilirubin, specific gravity, pH, ketone body, and occult blood）, hematology（leukocyte, erythrocyte, and platelet counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration）, and blood biochemistry（total protein（TP）, albumin（ALB）, total bilirubin, aspartate aminotransferase（AST）, alanine aminotransferase（ALT）, alkaline phosphatase（ALP）, lactate dehydrogenase（LDH）, γ-glutamyl transpeptidase（γ-GTP）, blood sugar, total cholesterol, neutral fat, urea nitrogen, and creatine）. Subjective and objective events, as recorded in the participants' diaries, were also analyzed.

The blood biochemical data are presented in **Table 4**, and the urinalysis data are shown in **Table 5**. No harmful subjective or objective symptoms related to VENETRON® administration were observed in any of the participants throughout the trial period. Although significant reductions in total protein, and albumin levels were observed over the study period, these parameters remained within the relevant normal range（6.7-8.3 g/dL for total protein and 4.0-5.0 g/dL for total albumin）, based on the reference,[27] indicative of the product's safety. In addition, VENETRON® did not cause any virtual changes in the blood and urine samples during the test period. Therefore, these results strongly indicate that 50 mg/day and 150 mg/day VENETRON® is generally safe for healthy Asian

Table 4　Human blood biochemical data reported in healthy Japanese subjects

Test item	Day 0	Week 4	Week 8	Week 12
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
Neutral fat（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
TP（g/dL）	7.62±0.39	7.50±0.31	7.37±0.29*	7.37±0.33*
ALB（g/dL）	4.82±0.29	4.72±0.26	4.57±0.21*	4.67±0.23
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 sugar（mg/dL）	89.7±10.9	90.5±10.5	90.1±11.2	90.0±10.0

Data represent the mean±the standard deviation（n=30）

*P<0.05 compared with Day 0

Data from reference 26

Table 5　Human urinalysis data reported in healthy Japanese subjects

Test items	Day 0	Week 4	Week 8	Week 12
Protein	（−）28[a)] （±）3 （+）0	（−）28 （±）1 （+）2	（−）31 （±）0 （+）0	（−）29 （±）1 （+）0
Sugar	（−）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, sugar, bilirubin, ketone body, occult bleeding reaction:

Standard＝（−）Negative; Out of standard＝（±）False positive,（+）Slightly positive,（2+）Positive

Urobilinogen:

Standard＝（±）False positive,（+）Slightly positve; Out of standard＝（2+）Positive

[a)]Represent the number of subjects

Data from reference 26

Table 6　Frequencies of adverse events in Western subjects reported by themselves

	VENETRON® (n=20)	Placebo (n=19)
No. of adverse events	30	22
No. of patients reporting adverse events	9	9
Events rate as mild	28	20
Events rate as moderate	2	2
Events rate as severe	0	0
No. of adverse events explained as not related to study product	7	10
No. of adverse events rated to be 'possibly' related to study product	22	11
No. of adverse events rated to be 'probably' related to study product	1	1
No. of adverse events rated to be 'definitely' related to study product	0	0

people.

2　Clinical study performed in Japan（Ⅱ）

A randomized, double-blind, placebo-controlled, crossover study of 50 mg/day VENETRON® intake for 8-days was conducted in 17 Japanese adult volunteers.[16] All participants received a full explanation of the procedures that were employed in the study, and the resulting informed consent was documented and signed by all of the participants. The anthropometrical, blood biochemical, and urinalysis examinations were performed at each examination day before and after the ingestion period.

The following anthropometric parameters were recorded: body weight（kg）, body mass index: BMI, body fat percentage（%）, blood pressure（mmHg systolic/diastolic）, and pulse rate（beats per min）.

The following biochemical parameters were analyzed: AST, ALT, γ-GTP, ALP, LDH, leucine aminopeptidase, total bilirubin, direct bilirubin, indirect bilirubin, cholinesterase, zinc sulfate turbidity test, total protein, blood urea nitrogen, creatinine, uric acid, creatine kinase, sodium, potassium, chloride, calcium, inorganic phosphorus, iron, serum amylase, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, triglyceride level, blood glucose level, hemoglobin A1c, and glycoalbumin. In addition, the following hematological parameters were analyzed: leukocytes, erythrocytes, hemoglobin, hematocrit, platelet, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and differential leukocyte ratio.

The following urinalysis parameters were analyzed: protein qualitative analysis, glucose, urobilinogen, bilirubin, ketone body, pH, and occult blood.

Subjective and objective events, as recorded in the participants' diaries, were also analyzed.

No harmful subjective or objective symptoms related to VENETRON® administration were observed in any of the participants throughout the trial period. Although some significant changes in exanimated parameters were observed over the study period, these parameters remained within the medical normal range based on the reference.[27] Therefore, VENETRON® ingestion with a dose of 50 mg/day for 8 days, did not lead to abnormal findings in anthropometrical, blood biochemical and urinalysis examinations.

3　Clinical study performed in Canada, the UK, and the USA

A randomized 8-week study was conducted to determine the safety of oral VENETRON® administration to subjects in Western countries including Canada, the UK, and the USA. The determined endpoints were weekly adverse events and blood pressure. Thirty-nine subjects（13 male and 26 female）were randomized to two groups and blindly assigned to either the VENETRON®（n=20）or placebo（n=19）group. VENETRON® was administered at 50 mg/day. All subjects joined a meeting with the study physician and discussed the information of the study, and the consent were obtained from all of the subjects.

As shown in **Table 6**, the VENETRON® and placebo groups showed no difference in the number of adverse events that were rated as 'probably' or 'defi-

nitely' related to sample administration. Although the total number of 'possibly' related adverse events was higher in the VENETRON® group, the number of adverse events that were rated as moderate or severe was the same in the VENETRON® and placebo groups, indicating that there was no difference between the VENETRON® and placebo groups.

In addition, analysis of blood pressure at day 0, week 4, and week 8 showed no significant changes. No subjects dropped out of the study due to side effects. The results of this study suggest that the use of VENETRON® is generally safe for Western individuals.

DISCUSSION

Interest in the health benefits of botanical products has increased in recent years. Examples include green tea,[28–31] apple,[32] and grape seed products.[33] The activities of these products are likely attributable to their polyphenol or flavonoid components. Hence, the development of processed herbal supplement that contain higher levels of flavonoids is also increasing, along with the associated safety research.[34,35]

A. venetum leaves have been traditionally 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 patients with hypertension who took tea made from *A. venetum* leaves.[36] In addition, an *A. venetum* beverage product, YANG LONG Level Care, which contains hyperoside and isoquercitrin as the functional ingredients, is known to act as an antihypertensive in humans. A double-blind human clinical trial was performed with 91 subjects who ingested 500 mL/day of YANG LONG Level Care for 12 weeks; this contained 15 mg hyperoside and 15 mg isoquercitrin and all of the results supported the safety of this *A. venetum* beverage product.[37] In another double-blind human clinical trial, 45 subjects consumed three times this dose of YANG LONG Level Care. These results also confirmed the safety of YANG LONG Level Care.[38] These previous studies reported the safety of the *A. venetum* product, indicating the high safety of *A. venetum* in beverage application. As a different kind of herbal supplement of *A. venetum*, VENETRON® is also applied to promote health and showed unique biological activity on stress reduction and improvement of sleep quality. Therefore, the aim of the manuscript was to review the safety and toxicology of VENETRON®.

The LD_{50} of VENETRON® was estimated to be > 2000 mg/kg bw for ICR mice, based on an acute single-dose toxicity study; no abnormal changes were observed in any of these animals. This LD_{50} value was equivalent to about 1200 mg VENETRON®/day for a 60-kg person. A subchronic toxicity study in rats suggested that the NOAEL for VENETRON® was more than 250 mg/kg bw/day, a level equivalent to 150 mg VENETRON®/day for a 60-kg person. In addition, herb-drug interactions between VENETRON® and drugs were studied previously.[21] Nifedipine, a substrate of CYP3A, and methylprednisolone, a substrate of P-glycoprotein, were employed in the study and the results indicated that VENETRON® had no influence on hepatic CYP3A or P-glycoprotein in rats. The level of cymarin in VENETRON®, a kind of cardiac glycoside which shows cardiotonic effect, inducing a contractile response increase the heart rates, was reported to be less than 5 ppm in a previous report.[25] This result also indicates that VENETRON® is safe. In summary of *in vivo* studies of VENETRON®, the toxicity tests by acute and subchronic toxicity study showed the minimum safe dosage of VENETRON® was supposed to be 150 mg/day for 60-kg person, and no herb-drug interaction was observed in neither CYP3A nor P-glycoprotein between VENETRON®, which both the targets are important index of drug interaction study. Furthermore, the trace amount of cardiac glycoside in VENETRON® also indicated the non-toxicity of the herbal material.

In a human clinical trial in Japan, 50 mg/day VENETRON® was administered by healthy Japanese adults at 1–8th weeks and then 150 mg/day at 9–12th weeks.[26] The measured biochemical parameters indicated that there were no adverse effects of taking VENETRON® at these levels. Although statistically significant reductions in the pulse rate, total protein, and albumin levels were observed, the values remained within normal ranges during the test period. The overall blood and urinalysis data showed normal values and indicated a high level of safety during VENETRON® administration. Moreover, by a randomized, double-blind, placebo-controlled, crossover study of a dosage of 50 mg/day VENETRON® for 8-days, orally ingested by healthy Japanese subjects, the anthropometrical, blood biochemical, and urinalysis examinations were performed and the results indicated there were no adverse effects of taking VENETRON® at these levels.[16]

There was an issue about the variable drug or dietary supplement effects on people based on race or

sex.[39] Thus, a safety study of Western subjects was also performed using a dose of 50 mg/day for 8 weeks. Analysis of the reported weekly adverse events showed equal numbers of adverse events that were rated as moderate or severe in the VENETRON® and placebo groups, and no group differences in the numbers of adverse events rated as 'probably' or 'definitely' related to VENETRON® administration. In these human clinical trials demonstrated that 50 mg/day VENETRON® was safe in individuals from Asian and Western regions.

In the present review, the *in vivo* safety of the *A. venetum* product, VENETRON®, was investigated and other toxicity reports, including a drug interaction study and human clinical trials, were reviewed. These results demonstrated the high level of safety and non-toxicity of VENETRON®. Moreover, the source of this plant product, *A. venetum*, has been used since ancient times for its multiple health benefits. Thus, VENETRON® could be used safely as a food additive, dietary supplement, beverage product, and even as a pharmaceutical product. The confirmed safety of VENETRON® may help to promote research into other botanical products.

【Conflict of interest】 Tokiwa Phytochemical Co. Ltd., of which T. L., J. Y. and T. I. are employees. K. L., S. N. and V. B. declare that there are no conflicts of interest.

REFERNCES

1）The Pharmacopoeia Committee of the Health Ministry of People's Republic of China, editor. Pharmacopeia of People's Republic of China. Part 1. Beijing: Chemical Technologic Publisher; 2005. p.147.
2）Lau Y, Ling W, Murugan D, Kwan C, Mustafa M. Endothelium-dependent relaxation effect of *Apocynum venetum* leaf extract via Src/PI3K/Akt signalling pathway. Nutrients 2015; 7: 5239-53.
3）Jin XN, Yan EZ, Wang HM, Sui HJ, Liu Z, Gao W, et al. Hyperoside exerts anti-inflammatory and anti-arthritic effects in LPS-stimulated human fibroblast-like synoviocytes *in vitro* and in mice with collagen-induced arthritis. Acta Pharmacol Sin 2016; 37: 674-86.
4）Ku SK, Zhou W, Lee W, Han MS, Na M, Bae JS. Anti-inflammatory effects of hyperoside in human endothelial cells and in mice. Inflammation 2015; 38: 784-99.
5）Xie W, Zhang X, Wang T, Hu J. Botany, traditional uses, phytochemistry and pharmacology of *Apocynum venetum* L.(Luobuma): a review. J Ethnopharmacol 2012; 141: 1-8.
6）Hao X, Kang Y, Li J, Li Q, Liu E, Liu X. Protective effects of hyperoside against H_2O_2-induced apoptosis in human umbilical vein endothelial cells. Mol Med Rep 2016; 14: 399-405.
7）Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: determination of radical Scavenging efficiencies. Methods Enzymol 1990; 186: 343-55.
8）Lotito SB, Frei B. Relevance of apple polyphenols as antioxidants in human plasma: contrasting *in vitro* and *in vivo* effects. Free Radic Biol Med 2004; 36: 201-11.
9）Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B, Bartsch H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. Eur J Cancer 2000; 36: 1235-47.
10）Serafini M, Laranjinha JA, Almeida LM, Maiani G. Inhibition of human LDL lipid peroxidation by phenol-rich beverages and their impact on plasma total antioxidant capacity in humans. J Nutr Biochem 2000; 11: 585-90.
11）Kamata K, Seo S, Nakajima J. Constituents from leaves of Apocynum venetum L. J Nat Med 2008; 62: 160-3.
12）Butterweck V, Jürgenliemk G, Nahrstedt A, Winterhoff H. Flavonoids from Hypericum perforatum show antidepressant activity in the forced swimming test. Planta Medica 2000; 66: 3-6.
13）Butterweck V, Nishibe S, Sasaki T, Uchida M. Antidepressant effects of *Apocyum venetum* leaves in a forced swimming test. Biol Pharm Bull 2001; 24: 848-51.
14）Yoto A, Ishihara S, Li-Yang J, Butterweck V, Yokogoshi H. The stress reducing effect of γ-aminobutyric acid and *Apocyum venetum* leaf extract on changes in concentration of salivary chromogranin A. J Physiol Anthropol 2009; 14: 55-9.
15）Yamatsu A, Yamashita Y, Maru I, Yang J, Tatsuzaki J, Kim M. The improvement of sleep by oral intake of GABA and *Apocyum venetum* leaf extract. J Nutr Sci Vitaminol 2015; 61: 182-7.
16）Nakata A, Yamashita S, Suzuki N, Liang T, Kuniyoshi T, Yang J, et al. Effect of an *Apocynum venetum* leaf extract (VENETRON®) on sleep quality and psychological stress improvement: a randomized, double-blind, placebo-controlled crossover study. Jpn Pharmacol Ther 2018; 46: 117-25.
17）Asif M. A brief study of toxic effects of some medicinal herbs on kidney. Adv Biomed Res 2012; 1: 44.
18）Dunnick JK, Nyska A. The toxicity and pathology of selected dietary herbal medicines. Toxicol Pathol 2013; 41: 374-86.
19）Gurley BJ. Pharmacokinetic herb-drug interactions (part 1): origins, mechanisms, and the impact of botanical dietary supplements. Planta Med 2012; 78: 1478-89.
20）Gurley BJ, Fifer EK, Gardner Z. Pharmacokinetic herb-drug interactions (part 2): drug interactions involving popular botanical dietary supplements and their clinical relevance. Planta Med 2012; 78: 1490-514.
21）Kobayashi M, Saitoh H, Seo S, Butterweck V, Nishibe S. Apocynum venetum extract does not induce CYP3A and P-glycoprotein in rats. Biol Pharm Bull 2004; 27: 1649-52.

22）Oerther SE. Plant poisonings: common plants that contain cardiac glycosides. J Emerg Nurs 2011; 37: 102–3.
23）Imai K, Ikeda N. Chemical constituents of *Apocynum venetum* L. *var. basiculmon* Hara. Takamine Kenkyusho Nenpo 1957; 9: 31–4.
24）Xu C, Sun N. Studies of cardiac glycoside of *Apocynum lancifolium* Rus. Yaoxue Xuebao 1996; 13: 585–95.
25）Irie K, Sato T, Tanaka I, Nakajima J, Kawaguchi M, Himi T. Cardiotonic effect of Apocynum venetum L. extracts on isolated guinea pig atrium. J Nat Med 2009; 63: 111–6.
26）Li-Yang J, Tanaka I, Butterweck V, Seo S, Kimura N, Uchida T. Safety study of Apocynum venetum L. extract in health adults. Journal of Nutritional Food 2009; 12: 1–9.
27）Esumi M.(3)Recommended reference interval in the wing 2003. Iryo 2005; 59: 75–7.
28）Chow H, Cai Y, Hakim IA, Crowell JA, Shahi F, Brooks CA, et al. Pharmacokinetics and safety of green tea polyophenols after multiple-dose administration ofepigallocetechin gallate and polyphenon E in healthy individuals. Clin Cancer Res 2003; 9: 3312–9.
29）Sang S, Hou Z, Lambert JD, Yang CS. Redox properties of tea polyphenols and related biological activities. Antioxidant Redox Signal 2005; 7: 1704–14.
30）Sutherland BA, Rahman RMA, Appleton I. Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration. J Nutri Biochem 2006; 17: 291–306.
31）Zaveri NT. Green tea and its polyphenolic catechins: medicinal uses incancer and noncancer applications. Life Sci 2006; 78: 2073–80.
32）Shoji T, Akazome Y, Kanda T, Ikeda M. The toxicology and safety of apple polyphenol extract. Food Chem Toxicol 2003; 42: 959–67.
33）Bentivegna SS, Whitney KM. Subchronic 3-month oral toxicity study of grape seed and grape skin extracts. Food Chem Toxicol 2002; 40: 1731–43.
34）Fujii H, Sun B, Nishioka H, Hirose A, Aruoma O. Evaluation of the safety and toxicity of the oligomerized polyphenol Oligonol. Food Chem Toxicol 2007; 45: 378–87.
35）Saiyed Z, Sengupta K, Krishnaraju A, Trimurtulu G, Lau F, Lugo J. Safety and toxicological evaluation of Meratrim®: an herbal formulation for weight management. Food Chem Toxicol 2015; 78: 122–9.
36）Jiangsu New Medical College, editor. Chinese material medica dictionary. Shanghai Science and Technology Publishing House; 1978. p.1355–6.
37）Kajimoto O, Nakazawa Y, Onizuka S, Yakahashi Y, Kagawa T, Nishibe S, et al. Hypotensive effects of beverage containing flavonoids of YAN LONG Tea on high normal blood pressure and mild hypertensive subjects. Journal of Japan Society of Health Sciences 2005; 21: 115–28.
38）Kagawa T, Tagawa C, Kanazawa K, Nakazawa Y, Nishimura A, Kajimoto Y, et al. Influence of a beverage containing YANG LONG flavonoids on human blood pressure and evaluation of its safety when consumed in excess（1）: in case of excessive intake when the beverage is consumed three times per day. Journal of Japan Society of Health Sciences 2007; 23: 117–29.
39）Yasuhara H. 1. Overview of ICH E5. Jpn J Clin Pharmacol Ther 2001; 32: 143–4.

Received 10 November 2017; Accepted 25 December 2017

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