Utilization study of stems and leaves of tienchi ginseng
Evaluation of anti-hypertensive effects, toxicity, and safety of stems and leaves of tienchi ginseng

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Abstract

Tienchi ginseng tea (TGT) was prepared from the above-ground organs, stems and leaves, of Tienchi ginseng, which is a special product from China. Tienchi ginseng contains saponins as the main effective ingredient. In male stroke-prone spontaneously hypertensive rats, increases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly inhibited by the consumption of 4% TGT solution in drinking water for a prehypertensive age (6 weeks of age). In contrast, intake of TGT had no effect on SBP or DBP in normotensive Wistar Kyoto rats. These results were evaluated using a telemetric system. Saponins were divided into two groups; the 20(s)-protopanaxadiol (PPD) group, including ginsenoside Rb1, Re, Rb2, Rb3, and Rd that show hypotensive effects, and the 20(s)-protopanaxatriol (PPT) group, including ginsenoside Rg1 and Re that show the opposite effect to the PPD group. Furthermore, only PPD saponins were included in TGT, whereas both PPD and PPT saponins were present in the rhizome of Tienchi ginseng. Furthermore, an evaluation of safety using the Ames test and toxic potency using an acute toxicity test with the TGT extract revealed no gene mutagenicity, and the fatal dose was more than 2000 mg/kg. This study demonstrated that TGT has anti-hypertensive effects and provides better safety compared with the rhizome of Tienchi ginseng.

Keywords: tienchi ginseng, saponin, blood pressure, stroke-prone spontaneously hypertensive rat, telemetric system

I Introduction

Tienchi ginseng (Panax notoginseng (Burk.) F. H. Chen) is a special product from the province of Yunnan in China, and Tienchi ginseng cultivated in Bunzan prefecture has especially high quality. The rhizome of Tienchi ginseng is the main part used for herbal medicine, including for the treatment of tachycardia, cardiac angina, apoplexy, and atherosclerosis in clinical settings. The above-ground organs, stems and leaves, are mostly discarded. This study examined the possibility of using only the stems and leaves of Tienchi ginseng as Tienchi ginseng tea (TGT). Saponins, the main effective ingredients of Tienchi ginseng, are divided into two groups; the 20(s)-protopanaxadiol (PPD) group, including ginsenoside Rb1 ((3β,12β)-20-[(6-O-D-glucopyranosyl-D-glucopyranosyl)oxy]-12-hydroxydammar-3-yl-β-D-glucopyranosyl-β-D-glucopyranoside) and ginsenoside Rd ((3β,12β)-20-[(D-glucopyranosyloxy)-12-hydroxydammar-24-en-3-yl 2-O-β-D-glucopyranosyl-β-D-glucopyranoside)] and ginsenoside Rg1 ((3β,6α,12β)-3,12-dihydroxydammar-24-ene-6,20-diyl bis-β-D-glucopyranoside) and ginsenoside Re ((3β,6α,12β)-20-[(D-glucopyranosylxy)-3,12-dihydroxydammar-24-en-6-yl...
2-0-(6-deoxy-L-mannopyranosyl)-β-D-glucopyranoside)\(^2, 3)\) that show hypertensive potency. In our previous study\(^4\), the anti-hypertensive effect of TGT in male stroke-prone spontaneously hypertensive (SHRSP) rats was investigated and a significant decrease in systolic blood pressure (SBP) was found. Furthermore, only PPD-group saponins with ginsenoside Rb and Rb were included in TGT, whereas both PPD- and PPT-group saponins were included in rhizomes. Recently, new findings on ginsenoside have come to light; therefore, TGT was suggested to be a useful drink for the treatment of hypertension. In this study, the PPD group of ginsenoside R, Rb, and Rb was elucidated for inclusion in TGT. The anti-hypertensive effects of TGT were evaluated using a telemetric system, because there are no stress effects during the measurements compared with the tail cuff method, which also requires more rats. Therefore, a telemetric system was thought to be more objective and dependable compared with the tail cuff method.

With the economic growth of Japan, the dietary habits of Japanese people have tended towards higher lipid and protein levels. In addition, lifestyle-related diseases have recently become a serious problem. There are about 7 million Japanese people with hypertension according to the report by the Ministry of Health, Labor and Welfare of Japan in 2002. Above all, hypertension leads to fatal diseases such as cerebral hemorrhage, stroke, angina, myocardial infarction, and kidney failure. Recently, the market for health foods in Japan has expanded to over 1 trillion yen\(^5\), and many kinds of functional foods are sold. The previously discarded above-ground organs of Tienchi ginseng could be valuable as an inexpensive source of functional food. The purpose of the present study was to develop TGT as a health food and demonstrate the availability of its components for human consumption. The present study investigated the anti-hypertensive effect of TGT in SHRSP and Wistar Kyoto (WKY) rats and evaluated its safety using the Ames test and toxicity using an acute toxicity test with rats.

II Materials and Methods

1. Chemicals

Standard ginsenosides R, Rb, and Rb were purchased from WAKO Pure Chemical Industries (Osaka, Japan). The apparatus and methods to measure these ginsenosides were as described previously\(^6\).

2. Processing of TGT

The preparation methods of TGT and 4% TGT extract for animal experiments were as described previously\(^7\). The dose of 4% TGT is the maximum concentration that can be prepared efficiently and in large quantities in our laboratory. The saponin content of 4% TGT was measured by high-performance liquid chromatography (HPLC) and 5.39 mg ginsenoside Rb/100 ml and 14.71 mg ginsenoside R/100 ml were detected, but ginsenoside Rg and Re were not found. For Ames tests, freeze-dried TGT extract powder (Rb, 575.3 mg/100 g; R, 176.4 mg/100 g) was prepared in our laboratory. For the acute toxicity test, an extract of lyophilized TGT (Rb, 595.3 mg/100 g; R, 319.7 mg/100 g) was processed by Japan Jiffy Food Co., Ltd (Osaka, Japan).

3. Animals and treatment

Animals were handled in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka. Male SHRSP and WKY rats were originally provided by K. Okamoto (Professor Emeritus of Kyoto University, Kyoto, Japan), and maintained in specific pathogen-free conditions by brother-sister breeding in our Experimental Animal Laboratory. Animals had free access to laboratory chow (CE-2, CLEA Japan, Inc., Tokyo, Japan) and were kept in controlled conditions at 24 ± 1°C, 45 ± 5% relative humidity, and a 12-h lighting cycle (08:00-20:00).

4. Effect of TGT on the development of hypertension

Male SHRSP and WKY rats at 5 weeks of age were housed in individual cages and given tap water or 4% TGT freely during the experiment. Body weight and water intake were measured. SBP was determined by the tail pulse pick-up method\(^8\) at 6–11 weeks of age. Radiotelemetric measurements of SBP, diastolic blood pressure (DBP), heart rate (HR), and locomotive activity (LAT) were carried out. Continuous 24-h ambulatory SBP, DBP, HR, and LAT were monitored in free-moving conditions using a Dataquest IV system (Data Science International, St. Paul, MN, USA)\(^9\). Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Dainippon Pharmaceuticals, Osaka, Japan) and newly calibrated implants were placed surgically into the descending aorta of each rat at 12–14 weeks of age. Insertion of the catheter and hemostasis was usually achieved in seconds. Animals were then transported to a desiccated blood pressure measuring room (entry was strictly controlled) and housed individually in regular cages equipped with a telemetric receiver through a consolidation matrix to a personal computer. After the operations, rats were allowed to acclimatize for 2 weeks, and then the data on SBP, DBP, and HR were sampled for 10 sec every 5 min throughout the course of the study, and hourly averages were calculated\(^7\).

5. Safety evaluation of TGT by Ames test

To examine the genotoxicity of TGT, a reverse mutation examination using Ames test was carried out in accordance...
with the Guideline for Genetic Toxicity for Medicines (No. 1604, issued on 1 November 1999) and based on the GLP for Good Laboratory Practice for MHLW in Japan (No. 21, issued on 26 March 1997). *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, to test histidine demand characteristics, were kindly provided by Prof. Bruce Ames (University of California, Berkeley, USA). *Escherichia coli* strain WA2uvrA, to test demand tryptophan characteristics, was obtained from the National Institute of Health and Science (Yoga, Tokyo, Japan) and is also commonly used in Ames test. To examine the influence of the addition of a drug-metabolizing enzyme from the liver, the rat liver microsome S9 fraction obtained from Sprague-Dawley rats, purchased from Kikkoman (Noda, Chiba, Japan), was used. The Ames test using the above five strains was performed as described previously. One hundred microliters of sample or positive control were added to a sterilized tube. Five hundred microliters of S9 mix or phosphate buffer (pH 7.4) and suspensions of each bacterial strain were added to the tube and mixed. The mixture was incubated at 37°C for 20 min. Two milliliters of soft agar was added to the tube and mixed. The mixture was put into a Petri dish and kept at 37°C for 48 h. The number of revertant colonies was counted, and the number of colonies presented is the average of two plates at each dosage. However, statistical analysis was not carried out. Here, gene mutagenicity was judged as positive when the number of revertant colonies increased more than two-fold compared with the negative control, and the dose dependence of the sample was identified. Positive controls for the bacteria and additional dosage of TGT are shown in Table 1. A dose-finding study of TGT, 8.19, 20.5, 51.2, 128, 320, 800, 2000, and 5000 μg/plate was carried out before the Ames test was performed. This experiment was performed at the Biosafety Research Center, Food, Drugs, and Pesticides.

6. Safety evaluation of TGT by acute toxicity test

This experiment was carried out in accordance with the Guideline for Testing of Chemicals 420 (17 December 2001) and based on the GLP for OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17, Issued in January 1988). Female CD rats (SD; SPF, Charles River Laboratories Japan Inc., Yokohama, Japan) at 7 weeks of age (n=5) were used. The lyophilized TGT extract was dissolved in distilled water and administered orally once at a rate of 2000 mg/kg body weight after fasting for 16 h, which was the maximum limit described in the Guideline. The rats were then observed once at 30 min, hourly up to 6 hrs after administration, and daily up to 14 days. Body weight was measured just before administration and at day 7 and day 14. The body surface, organs and tissues in the abdominal cavity, thoracic cavity, pelvic cavity, and braincase were observed after sacrifice by blood letting under anesthesia. Statistical analysis was not carried out. This experiment was also performed at the Biosafety Research Center, Food, Drugs and Pesticides.

7. Statistical analysis

All data are expressed as mean ± standard error (SE). Data for body weight, water intake, and SBP measured by the tail pulse pick up method were analyzed by Student's t-test. For the analysis of SBP, DBP, HR, and LAT measured by the telemetric system, Graph Pad Prism 4 software (Graph Pad Software Inc., California, USA) was used for 2-way ANOVA. Differences with a probability value <0.05 were considered to be significant.

III Results and Discussion

1. Effect of TGT on the development of hypertension

Changes in body weight and water intake between 6 and 17 weeks of age are shown in Fig. 1A and 1B, respectively. There were no significant differences between the control and

![Graph 1A](image)

![Graph 1B](image)

Fig. 1. Changes in the body weight and water intake of stroke-prone spontaneously hypertensive rats after administration of 4% Tienchi ginseng tea (TGT) extract

SHRS rats were given TGT to drink freely from 6 weeks of age. Each point and vertical bar indicates the mean ±SE for 6 rats in the control and 7 rats in the TGT group. A, body weight; B, water intake.
4% TGT groups for any of the categories measured, which is important in order to consider TGT as a food. A significant decrease in SBP was observed after 9 weeks of age; SBP decreased by 27 mmHg compared with the control at 11 weeks of age (Fig. 2). These results suggested that TGT had an anti-hypertensive effect. The telemetric measurement system was used to evaluate changes in blood pressure more objectively. This system can measure the blood pressure of rats without stress caused by restriction or heating compared with measurement by the tail pulse pick up method. In addition, the telemetric system is able to measure blood pressure continuously and directly by insertion of a catheter into the aorta, and data from 15 rats can be obtained simultaneously. Therefore, the influences of circadian changes in the rats can be monitored. Fig. 3 shows the typical changes in SBP, DBP, HR, and LAT monitored using the telemetric system. Cyclical changes were observed in all of the items measured. Obvious cyclical changes were shown in HR and LAT. Therefore, these values were expressed as an average of the daytime (08:00–20:00) and nighttime (20:00–08:00) values. HR and LAT at night tended to be higher than in the daytime, which was thought to be because rats are active at night and sleep in the daytime. There was no significant difference between the 4% TGT and control groups. Thus, TGT did not affect HR or LAT (Fig. 4). On the other hand, the values of SBP and DBP were expressed as daily averages, because they showed only slight cyclical changes. The values of 13.6–22.5 mmHg for SBP and 11.0–20.4 mmHg for DBP were significantly decreased in the TGT group compared with the control group. Therefore, TGT was evaluated objectively to have hypotensive potency (Fig. 5). An anti-hypertensive effect after the administration

![Fig. 2](image-url)

**Fig. 2.** Effect of Tienchi ginseng tea (TGT) on systolic blood pressure (SBP) in stroke-prone spontaneously hypertensive (SHRSP) rats

SHRSP rats were given 4% TGT extract to drink freely from 6 weeks of age. SBP was measured by the tail pulse pick-up method. Each point and vertical bar indicates the mean ±SE for 6 rats in the control and 7 rats in the TGT group.

![Fig. 3](image-url)

**Fig. 3.** Typical changes in the heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and locomotive activity (LAT) in stroke-prone spontaneously hypertensive (SHRSP) rats

HR, SBP, DBP, and LAT were monitored using a telemetric system. SHRSP rats were given 4% TGT extract to drink freely from 6 weeks of age. The data in the figure show hourly mean values.

![Fig. 4](image-url)

**Fig. 4.** Changes in heart rate (HR) and locomotive activity (LAT) in stroke-prone spontaneously hypertensive (SHRSP) rats

HR and LAT were monitored using a telemetric system. Each point and vertical bar indicates the mean ±SE of 6 rats in the control and 7 rats in the TGT group. bpm = beats per minute.

of a powder or extract of the rhizome of Tienchi ginseng has been reported previously\(^{11, 12}\). Ogawa et al. reported that SBP decreased significantly by 12 mmHg after the administration of rhizome powder at about 500 mg/day for 5 weeks, and the saponin intake was estimated as 82.9 mg Rgl, 12.4 mg Re, 53.9 mg Rb, and 20.7 mg Rd/kg body weight. Minami et al. reported that SBP decreased significantly by about 15 mmHg after the administration of 500 mg/kg/day of the same powder for 1 week from 19 weeks of age, and the saponin intake was 16.6 mg Rgl, 2.5 mg Re, 10.8 mg Rb, and 4.1 mg Rd/kg body weight. However, the effect was lower than that of TGT. In
the present study, the intake of 4% TGT was 30 ml/day, which corresponded to 0.65 mg Rb and 1.90 mg Rd/kg body weight/day. In this experiment, the anti-hypertensive effect of TGT could be evaluated more objectively using the telemetric system. In addition, TGT was thought to be a useful for assessing anti-hypertensive effects, because only inexpensive PPD was used in the production of TGT compared with more expensive rhizome extracts. There have been few studies on the bioactivity of the stem and leaf of Tienchi ginseng and especially in studies on anti-hypertensive effects. In addition, it has been demonstrated that the anti-hypertensive effect of PPD was induced by a Ca⁺ ion channel blockage activity\(^{13}\), and the vasodilating activity and hypotensive effect of Tienchi ginseng have already been reported\(^{14,15}\).

2. Effect of TGT on the blood pressure of WKY rats

There was no significant difference in blood pressure between the control and TGT groups measured by the tail pulse pick up method for rats of 5–11 weeks of age (Fig. 6). Using the telemetric system, HR at night (309–322 bpm) was about 16% higher than that in the daytime (263–281 bpm), and LAT in night (19–28 counts) was also about 3 times higher than that in the daytime (4–8 counts). In addition, for the same SHRSP rats, there was no significant difference between the two groups for HR and LAT. On the other hand, a significant difference was observed in DBP and SBP at 112–114 and 128 days. However, the difference was slightly less than 10 mmHg (Fig. 7). Therefore, TGT did not affect the blood pressure of normotensive rats.

3. Chemical components

HPLC chromatograms of TGT are shown in Fig. 8. Ginsenosides Rc, Rb, and Rb were found and the retention times of these were 31.6, 34.5, and 35.9 min, respectively, and the contents of ginsenosides Rb, Rc, Rb, Rb, and Rd were 349.6, 1719.6, 365.9, 1669.2, and 68.9 mg/100 g of TGT, respectively.

4. Safety evaluation of TGT by Ames test

In dose-setting experiments, a definite increase in the mutagenicity of all bacteria was not observed with or without S9 treatment in the TGT group. On the other hand, the
positive control gave rise to definite mutagenicity. Therefore, the maximum dose was set at 5000 μg/plate. In the reverse mutation test, a clear increase in mutagenicity was not observed in any bacteria compared with the negative control at any dosage with or without S9 treatment. In contrast, the positive control clearly showed mutagenicity in each bacterium (Table 1). Ginsenosides Rg3 and Rb1 have been reported to decrease the mutagenic activity of AF-2. From the above data, TGT extract was judged to have no mutagenic activity under the conditions in this study.

5. Safety evaluation of TGT by acute toxicity test

TGT was observed as a mortality-free substance during this experiment. The excretion of loose stool or loose stool with a blackish color was observed in 1 and 2 rats out of 5 rats, respectively. However, these were thought to be temporary changes because they were not observed after the next day. Furthermore, the blackish feces were considered to have been stained by the TGT extract. All rats showed normal body weight gain, and no damage was observed from visual observation of the organs and tissues during dissections at the end of the experiment. These findings indicated that the TGT extract has a very low acute toxicity and the fatal dose was thought to be over 2000 mg/kg in the present conditions. Takasugi et al. reported that when 12.5 mg/kg per rat of Tienchi ginseng rhizome extract was administered, blood pressure decreased temporarily, accompanied by a decrease in blood flow in the carotid artery and heart rate (7). Takase et al. carried out an acute toxicity test with Tienchi ginseng rhizome by forced single oral administration, and they concluded that toxicity was not observed after the administration of 80,000 mg/kg per rat. Imada et al. also reported that the extract of Tienchi ginseng rhizome administered at 97,000 mg/kg per rat showed no toxicity (9). Furthermore, Imada et al. performed an acute toxicity test with Tienchi ginseng extract by forced oral administration to rats for a month, and toxicity was observed at more than 6500 mg/kg per rat. From the above findings, the acute toxicity of TGT was

![High-performance liquid chromatography (HPLC) chromatograms of saponins in Tienchi ginseng tea (TGT)](image)

**Fig. 8.** High-performance liquid chromatography (HPLC) chromatograms of saponins in Tienchi ginseng tea (TGT)

Peak 1, ginsenoside Rb1; peak 2, ginsenoside Re; peak 3, ginsenoside Rb1; peak 4, ginsenoside Rb1; peak 5, ginsenoside Rd. The conditions for the measurement are described in the Materials and Methods.

### Table 1. Mutagenic activity of Tienchi ginseng tea extract toward Salmonella typhimurium and Escherichia coli strains

<table>
<thead>
<tr>
<th>Dose (μg / plate)</th>
<th>TA100</th>
<th>TA1535</th>
<th>WP2rapA</th>
<th>TA98</th>
<th>TA1537</th>
</tr>
</thead>
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<tr>
<td></td>
<td>-S9</td>
<td>+S9</td>
<td>-S9</td>
<td>+S9</td>
<td>-S9</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0</td>
<td>128</td>
<td>14</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>19.5</td>
<td></td>
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</tr>
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<td>39.1</td>
<td></td>
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<tr>
<td>2500.0</td>
<td></td>
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<tr>
<td>Positive control</td>
<td>AF-2&quot; 2-AA&quot; NaNO₃ 2-AA AF-2 2-AA AF-2 2-AA 9-AA 9-AA 2-AA</td>
<td></td>
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<tr>
<td>Dose (μg / plate)</td>
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<td>0.5</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean revertant (colonies / plate)</td>
<td>697 979 456 330 139 716 700 362 300 139</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamid; ** 2-Aminoanthracene; *** Sodium azide; **** 9-Aminoacridine hydrochloride

Table 1. Bacterial reverse assay with Tienchi ginseng tea extract

The Ames test using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 was used. One hundred microliters of sample or positive controls was added to a sterile tube. Five hundred microliters of S9 mix or phosphate buffer (pH 7.4) and cell suspensions of each bacteria strain were added to the tube and mixed. The mixture was incubated at 37°C for 20 min. Two milliliters of soft agar was added to the tube and mixed. The mixture was kept at 37°C for 48 h. The numbers of revertant colonies were counted, and are presented here as the average of two plates for each dosage.
considered to be extremely low. However, Liu et al. reported that Rb1 showed a teratogenic effect during the mouse organogenetic period. Therefore, Tienchi ginseng should be used with caution in pregnant women in the first trimester before more data in humans is available. Information about toxicological concerns with Tienchi ginseng was reported in the database of safety and effectiveness of health foods of the National Institute of Health and Nutrition. Pesticide residue monitoring inspections including 200 items for imported foods and the voluntary standard for heavy metals (arsenic [1.0 mg/kg], cadmium [0.1–0.4 mg/kg], lead [0.5–3.0 mg/kg], and total mercury [0.1 mg/kg]) in TGT were applied, but no toxic levels were recorded. In a preliminary experiment, 325 ml of 0.7% TGT extract was administrated per day for 1 month to humans (n=5) and there was no influence on liver function tests such as glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and γ-glutamyl transpeptidase (γ-GTP). Further experiments using TGT intake in drinking water for 90 days are underway in humans. In the present study, an anti-hypertensive effect of TGT was observed, and the safety of TGT was also demonstrated. Therefore, the consumption of TGT in drinking water may be useful as a health supplement, but further studies are also required to set the effective dose of TGT.

IV References


論文

田七人参の茎、葉の有効活用の研究
田七人参の茎と葉の降圧効果、毒性および安全性の評価
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キーワード：田七人参、サポニン、血圧、脑卒中発症高血圧自然発症ラット、テレメトリーシステム

概要

中国の特産である田七人参茶（TGT）はその地上部の茎と葉から作られた。田七には主要成分としてサポニンが含まれる。オスの腸発症高血圧自然発症ラット（SHRSP）において昇圧期（6週齢以降）に4% TGTを飲用させることで、収縮期血圧（SBP）および拡張期血圧（DBP）の上昇を有意に抑制した。一方、血圧が正常なWister Kyotoラット（WKY）にはSBPおよびDBPに影響は見られなかった。これらの結果を、テレメトリーシステムを用いて評価した。サポニンは2つのグループに分類される。一つは血圧を下げる作用を持つ20(S)-protopanaxadiol (PPD)群で、ジンセノサイドRb1、Rc、Rb2、Rb3およびRdを含む。もう一つは、血圧を上げる作用を有する20(S)-protopanaxatriol (PPT)群でジンセノサイドRg1、Reを含む。これらは相反する作用を示す。さらに、田七人参の根茎部にはPPD群およびPPT群のサポニンが両方とも含まれるのに対し、TGTにはPPD群のサポニンのみが含まれる。また、さらにTGTを用いた安全性評価をAmes試験で、また毒性試験を急性毒性試験で行った。その結果、変異原性は認められなかった。また半数致死量は体重1kgあたり2000mg以上であることが分かった。本研究により、田七人参の地上部には血圧降下作用が期待でき、かつ安価な健康食品として提供できる可能性を示唆するものである。