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RESEARCH ARTICLE

Effects of Distilled *Cervi Pantotrichum Cornu* and *Rehmannia glutinosa* Pharmacopuncture at GB21 (*Jianjing*) on Heart Rate Variability: A Randomized and Double-blind Clinical Trial

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Rehmannia glutinosa

Abstract

Background/Purpose: The purpose of this study was to use heart rate variability (HRV) to investigate the effects of distilled Cervi Pantotrichum Cornu pharmacopuncture and *Rehmannia glutinosa* pharmacopuncture on the autonomic nervous system.

Materials and methods: Forty healthy male participants were divided into two groups: the participants of the C-group received distilled Cervi Pantotrichum Cornu pharmacopuncture and those of the R-group received *Rehmannia glutinosa* pharmacopuncture. The study design was a randomized, double-blind clinical trial. Each participant received one of the two solutions injected at GB21 *(Jianjing)*. The changes in HRV were measured seven times using the QECG-3: LXC3203 system (LAXTHA Inc. Korea). Time-dependent changes in HRV for each group were analyzed using the paired *t* test (significance level: $p < 0.05$), and the difference in the HRV fluctuations between the two experimental groups was evaluated using the independent sample test (significance level: $p < 0.05$).

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Results and conclusion: The results showed that Cervi Pantotrichum Cornu pharmacopuncture and *Rehmannia glutinosa* pharmacopuncture tended to activate the autonomic nervous system within the normal range. Cervi Pantotrichum Cornu pharmacopuncture tended to activate the sympathetic nervous system, whereas *Rehmannia glutinosa* pharmacopuncture tended to activate both the sympathetic and parasympathetic nervous systems.

1. Introduction

Pharmacopuncture is a therapeutic method to cure diseases using a syringe to inject a pharmacopuncture solution at acupoints or pain points on the basis of the theory of Korean medicine [1,2]. As the use of pharmacopuncture has recently increased, various studies are ongoing, especially studies regarding the autonomic nervous system (ANS). Several studies [3—7] have shown thatpharmacopuncture can have a significant influence on the heart rate variability (HRV). However, we were unable to find a study analyzing the effects of pharmacopuncture for herbal medicines of cold and warm natures. Among the herbal medicines that have the effect of moistening, Cervi Pantotrichum Cornu is a representative herbal medicine of a warm nature and *Rehmannia Glutinosa* is a representative herbal medicine of a cold nature[2].This study aimed to compare the different influences of these two pharmacopuncture medicines on the ANS according to the cold and warm characteristics of the two herbal medicines.

Cervi Pantotrichum Cornu is the dried unossified juvenile antlers of Sika deer (*Cervus nippon*) or red deer (*Cervus elaphus*), animals belonging to the Cervidae family, or of closely related animals belonging to the same genus. It tastes sweet and salty, and it is warm and nonpoisonous in nature. *Rehmannia glutinosa* is a perennial herbaceous plant belonging to the Scrophulariaceae family; fresh rhizoma, rootlets, and leaves are removed from the plant and subsequently washed to remove the soil. The taste of *Rehmannia glutinosa* is sweet and bitter, and it is cold and nonpoisonous in nature.

Accordingly, to compare the effects of each pharmacopuncture on the ANS, the author clinically observed the human body by comparing HRV before and after treatment. To this end, Cervi Pantotrichum Cornu pharmacopuncture or *Rehmannia glutinosa* pharmacopuncture was injected into GB21 (Jianjing, 肩井). Here, we report and compare the experimental results for two groups: participants injected with Cervi Pantotrichum Cornu pharmacopuncture and participants injected with *Rehmannia glutinosa* pharmacopuncture.

2. Materials and methods

The participants for this study, which was designed as a randomized and double-blind clinical trial, were male students enrolled at Woosuk University, Jeonju, Korea between April 2009 and June 2009. They were given a detailed explanation of the experiment; they participated voluntarily and gave a written consent; all showed normal sinus rhythm [8,9] on electrocardiography. Those who had central nerve injuries, who had a medical history of cardiovascular disease, endocrine disorder, or ANS disorder, who had taken, within 72 hours of the test, drugs or foods that could affect the ANS, or who were judged by the practitioners as being inappropriate for the test, were excluded. Finally, a total of 40 participants were included. They were randomly divided into two groups using a computer random numbers table: 20 participants in the Cervi Pantotrichum Cornu pharmacopuncture group (C-group) and 20 participants in the *Rehmannia glutinosa* pharmacopuncture group (R-group).The practitioners did not know the pharmacopuncture that the participants were using, and during the study, the participants did not know the group they were assigned.

For the Cervi Pantotrichum Cornu pharmacopuncture used for this study, the top and upper sections of *Cervus elaphus sibiricus* were purchased. In the laboratory of the Korean Pharmacopuncture Institute (Gangseo-gu, Seoul 157-200, Korea), the Cervi Pantotrichum Cornu was dried and ground into a hyperfine powder using the extrusion process. The powder, 100 g, was put into the lower part of a reactor with 1.5L of water. The materials were macerated and subsequently decocted. The decocted solution was extracted at 107°C. From this solution, the supernatant, excluding minerals, was separated. It was adjusted to a salinity of 0.98 and a pH of 7.25—7.35 and was then subjected to tertiary filtration to obtain 1,000 mL of the Cervi Pantotrichum Cornu pharmacopuncture solution. Next, the filtered solution was dispensed into sterilized 20-mL vials using an auto dispenser and was sterilized at high pressure. *Rehmannia glutinosa* used in the test was cultivated in Andong and was selected by the pharmacy of Woosuk University's Oriental Hospital. The manufacturing

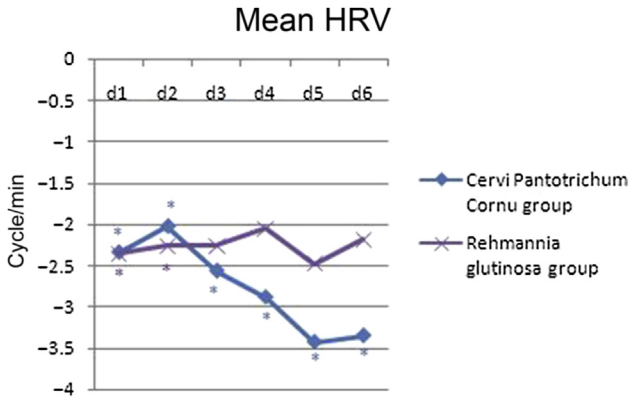

Figure 1 Comparison of mean HRV between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. * $p < 0.05$ (by paired t test). d1—d6 =differences between the values before and after six injections 5 minutes apart.

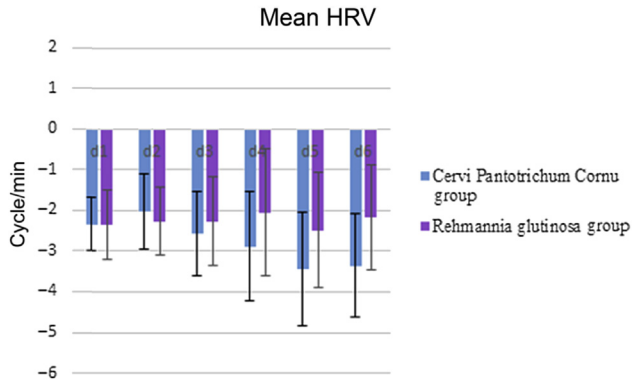

Figure 2 Comparison of mean HRV between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. Values are presented as mean ± standard error of the mean. d1−d6 = differences between the values before and after six injections 5 minutes apart.

process for the *Rehmannia glutinosa* pharmacopuncture was the same as that used for the Cervi Pantotrichum Cornu pharmacopuncture.

GB21 (Jianjing, 肩井) was selected as the acupoint into which the pharmacopuncture would be injected because of its function of relieving fire energy. However, it could raise fire energy depending on the characteristics of the injected solutions [10].GB21 is an important meeting point of the foot and the hand lesser yang meridians (Gallbladder and Triple energizer meridians), the foot yang brightness meridian (Stomach meridian), and the yang linking vessel [11]. Each solution, a total of 0.2 mL (0.1 mL to each side), was administered once to each group by inserting a sterile hypodermic syringe (DM Medicrat 1.0 mL, 26 gauge, Sin Dong Bang Medical Co., Korea) to a depth of 1.0 cm, and the associated pain was < 2 on the visual analog scale.

The measurements were conducted using the QECG-3: LXC3203 system (LAXTHA Inc., Korea). After 20 minutes of rest, electrodes were attached to the participants. The measuring electrodes were attached to the right arm, right wrist, and left ankle, and the earth electrode was attached to the right ankle by utilizing the standard limblead method. The participants had another rest for 10 minutes, after which the first measurement (P0) was conducted 5 minutes prior to the injection. The two pharmacopunctures were injected into the participants of the respective groups, and measurements (P1−P6) were conducted every 5 minutes for a total of 30 minutes. Next, the differences (Δ1−Δ6) between the value measured before (P0) and the values measured after the (P1−P6) injections were obtained.

The results were analyzed using SPSSversion 15.0 for windows (SPSS Inc., Chicago, IL, USA). The details of measurements are as follows: two different categories were used for the HRV analysis. One was a time-domain analysis including the mean HRV, the standard deviation of all normal RR intervals (SDNN), and the quantified variable of geometrical characteristic of probability distribution plot of RR variability (HRV index). The second was a frequency-domain analysis including the natural logarithm (Ln) of the total power (TP), of the low-frequency oscillation power (LF), and of the high-frequency oscillation power (HF). They suggest the activity of each and total ANS. The differences between the measurements before and after each injection (Δ1−Δ6) were analyzed using the paired *t* test (significance level: $p < 0.05$).Furthermore, for the comparison of the differences between the two experimental groups (Δ1−Δ6) at each point of time (P1−P6), an independent sample test was performed (significance level: $p < 0.05$).

Table 1 Comparison of mean heart rate variability (HRV) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups.

Group	P0	Δ1	Δ2	Δ3	Δ4	Δ5	Δ6
Cervi Pantotrichum Cornu	74.483	−2.336 ± 0.650*	−2.021 ± 0.911*	−2.561 ± 1.035*	−2.881 ± 1.330*	−3.429 ± 1.390*	−3.352 ± 1.263*
Rehmannia glutinosa	73.507	−2.356 ± 0.840*	−2.263 ± 0.847*	−2.262 ± 1.082	−2.053 ± 1.554	−2.478 ± 1.419	−2.185 ± 1.288

Values are presented as mean ± standard error of the mean.
* $p < 0.05$ (by paired *t* test).
P0 = mean HRV before injection; Δ1−Δ6 = differences between the values before and after six injections 5 minutes apart.

Table 2 Comparison of standard deviation of all normal RR intervals (SDNN) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups.

Group	P0	Δ1	Δ2	Δ3	Δ4	Δ5	Δ6
Cervi Pantotrichum Cornu	46.781	3.346 ± 1.853	2.439 ± 2.539	7.609 ± 2.977*	8.676 ± 2.440*	11.899 ± 3.156*	9.654 ± 3.106*
Rehmannia glutinosa	46.497	4.236 ± 1.729*	5.004 ± 1.681*	10.572 ± 2.404*	9.633 ± 2.836*	12.367 ± 2.320*	14.200 ± 2.692*

Values are presented as mean ± standard error of the mean.
* $p < 0.05$ (by paired *t* test).
P0 = mean HRV before injection; Δ1−Δ6 = differences between the values before and after six injections 5 minutes apart.

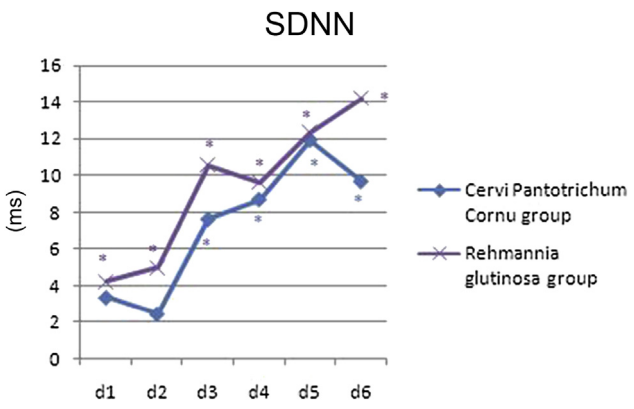

Figure 3 Comparison of the standard deviation of all normal RR intervals (SDNN) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. * $p < 0.05$ (by paired t test). d1—d6 = differences between the values before and after six injections 5 minutes apart.

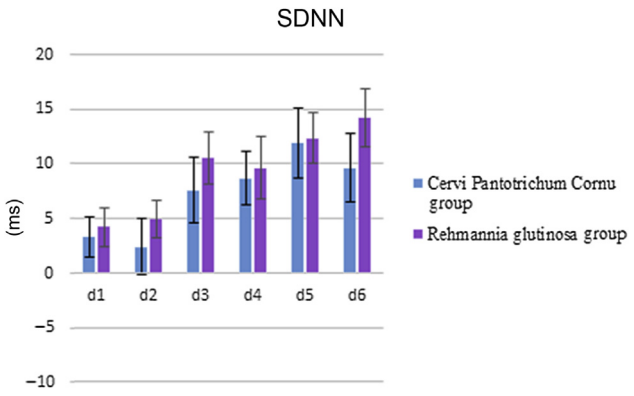

Figure 4 Comparison of the standard deviation of all normal RR intervals (SDNN) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. Values are presented as mean ± standard error of the mean. d1—d6 = differences between the values before and after six injections 5 minutes apart.

3. Results

The mean age of the participants was 22.75 ± 3.48 years, that of the C-group was 23.05 ± 4.19 years, and that of the R-group was 22.45 ± 2.67 years. The minimum age was 20 years, and the maximum age was 38 years.

The participants in the C-group showed significant decreases, compared with P0 (measurement before injection), in the mean HRV for all six measurements. By contrast, the R-group showed significant decreases, compared with P0, in the mean HRV only at the first and second measurements. However, the differences between the groups over time did not reach statistical significance (Table 1 and Figs. 1 and 2). In addition, the C-group showed significant increases, compared with P0, in SDNN at the third, fourth, fifth, and sixth measurements, whereas the R-group showed significant increases in SDNN at all six measurements. However, the differences between the two groups over time again did not reach statistical significance (Table 2 and Figs. 3 and 4). Furthermore, the C-group showed significant increases, compared with P0, in the HRV index at all six measurements, whereas the R-group showed significant increases only at the third, fifth, and sixth measurements. Here again, the differences between the two groups over time did not reach statistical significance (Table 3 and Figs. 5 and 6).

The C-group showed significant increases, compared with P0, in Ln(TP) at the first, third, fourth, fifth, and sixth measurements, whereas the R-group showed significant increases in Ln(TP) at all six measurements. A significant difference between the two groups was seen only at the second measurement(Table 4 and Figs. 7 and 8).In addition, the C-group showed significant increases, compared with P0, in Ln(LF) at the fourth, fifth, and sixth measurements as did the R-group. However, in this case, no significant differences between the groups over time were observed (Table 5 and Figs. 9 and 10). Furthermore, the C-group showed no significant changes in Ln(HF) between before and after injection, whereas the R-group showed significant increases in Ln(HF) at the third, fifth, and sixth measurements. The differences in Ln(HF) between the two groups at the second, third, fourth, and fifth measurements did achieve statistical significance (Table 6 and Figs. 11 and 12).

4. Discussion

The results of this experiment showed that the mean HRV was significantly decreased for 30 minutes after injection in the C-group, which means the heart rate can continuously and significantly decrease during a period of 30 minutes following the injection of Cervi Pantotrichum Cornu pharmacopuncture. By contrast, the mean HRV for the R-group significantly decreased only for 10 minutes following

Table 3 Comparison of heart rate variability index between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups.

Group	P0	Δ1	Δ2	Δ3	Δ4	Δ5	Δ6
Cervi Pantotrichum Cornu	21.019	2.397 ± 0.912*	2.874 ± 1.320*	2.887 ± 1.017*	5.060 ± 1.506*	6.418 ± 1.670*	4.691 ± 1.076*
Rehmannia glutinosa	21.989	1.395 ± 1.027	1.738 ± 0.994	4.056 ± 1.309*	2.714 ± 1.763	3.559 ± 1.414*	4.327 ± 1.332*

Values are mean ± standard error of the mean.
* $p < 0.05$ (by paired t test).
P0 = mean HRV before injection; Δ1—Δ6 = differences between the values before and after six injections 5 minutes apart.

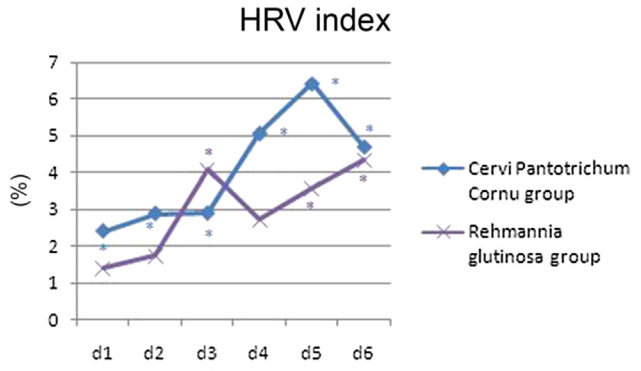

Figure 5 Comparison of heart rate variability index between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. * $p < 0.05$ (by paired t test). d1–d6 = differences between the values before and after six injections 5 minutes apart.

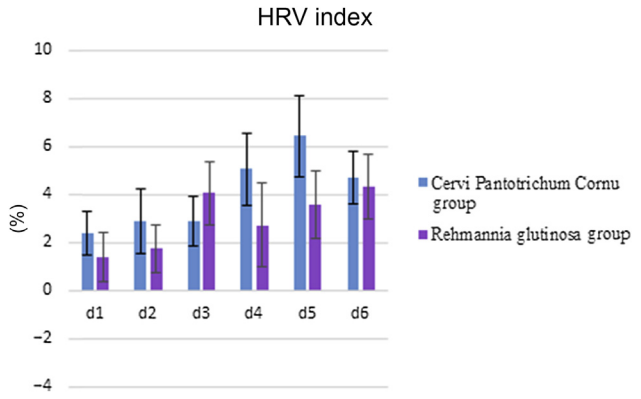

Figure 6 Comparison of the heart rate variability index between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. Values are presented as mean ± standard error of the mean. d1–d6 = differences between the values before and after six injections 5 minutes apart.

injection, which means that the heart rate can decrease significantly during a period of 10 minutes following the injection of *Rehmannia glutinosa* pharmacopuncture. In a comparison with existing studies regarding Cervi Pantotrichum Cornu pharmacopuncture, in this study, the mean HRV continuously decreased from the time of injection, whereas in the study by Kim et al [3], the mean HRV increased for the first 5 minutes after injection and then continuously decreased. However, it was not shown in this study, and finally the pattern of changes of the mean HRV showed decreasing in both studies, which means that the moistening nature of Cervi Pantotrichum Cornu pharmacopuncture was activated. The decreasing pattern within normal limits of the mean HRV in the C-group suggests that Cervi Pantotrichum Cornu pharmacopuncture can affect the ANS that results to be healthy condition.

For the participants of the R-group in this study, the mean HRV decreased significantly during a period of 10 minutes following injection. In prior studies, the mean HRV of the R-group showed significant decreases at the point of P1 only, and the authors suggest that it is the effect of needling stimulus [4,12]. In this study, the mean HRV decreased in the early stage of injection. Both results suggest similar effects of *Rehmannia glutinosa* pharmacopuncture in activating the ANS in the early stage. Further long-term studies with more participants are needed to distinguish the effects of the solution. As for the reduction in the heart rate, a significant difference was noted between the two pharmacopunctures, indicating that the two pharmacopunctures reduce the heart rate similarly. However, over time, Cervi Pantotrichum Cornu pharmacopuncture had a higher effect on the heart rate than did the *Rehmannia glutinosa* pharmacopuncture.

The two groups showed increases in SDNN after injection. SDNN for the C-group significantly increased from 15 minutes after injection, whereas SDNN for the R-group significantly increased immediately after injection. Based on an existing study [13], an increase in SDNN means an increase in the activity of the ANS. Thus, both the pharmacopunctures stimulate the activity of the ANS so that the stress endurance improves; *Rehmannia glutinosa* pharmacopuncture activates the ANS more rapidly than Cervi Pantotrichum Cornu pharmacopuncture does. Furthermore, no significant differences between the two groups were observed over time; thus, both the pharmacopunctures activated the autonomic nerve to a similar extent.

The HRV index for the C-group increased significantly immediately after injection, whereas that for the R-group was observed to change from 15 minutes after injection. Thus, the two pharmacopunctures raised the activity of the vagus nerve in the heart and activate cardiac function. Because no significant differences in the HRV index between

Table 4 Comparison of the natural logarithm (Ln) of the total power (TP) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups.

Group	P0	Δ1	Δ2†	Δ3	Δ4	Δ5	Δ6
Cervi Pantotrichum Cornu	7.442	0.206 ± 0.093*	0.115 ± 0.134	0.316 ± 0.143*	0.380 ± 0.125*	0.517 ± 0.154*	0.447 ± 0.140*
Rehmannia glutinosa	7.487	0.198 ± 0.092*	0.215 ± 0.079*	0.378 ± 0.095*	0.321 ± 0.106*	0.491 ± 0.098*	0.542 ± 0.107*

Values are mean ± standard error of the mean.
* $p < 0.05$ (by paired t test).
† $p < 0.05$ (by independent sample test).
P0 = mean HRV before injection; Δ1–Δ6 = differences between the values before and after six injections 5 minutes apart.

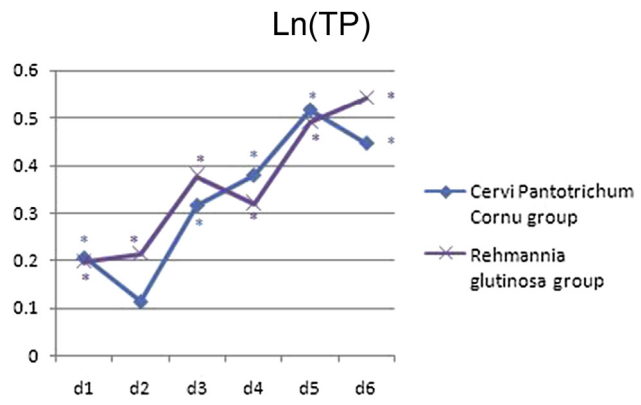

Figure 7 Comparison of the natural logarithm (Ln) of the total power (TP) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. * $p < 0.05$ (by paired t test).

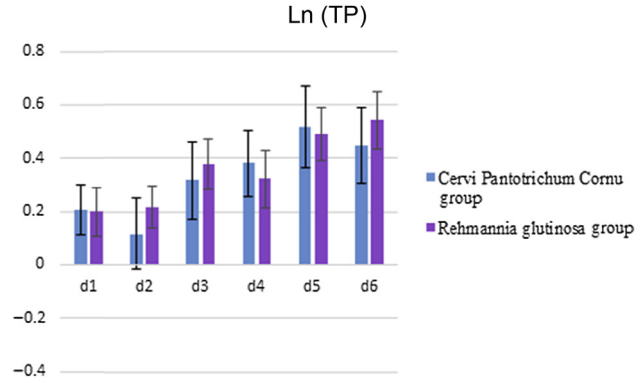

Figure 8 Comparison of the natural logarithm (Ln) of the total power (TP) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. Values are presented as mean ± standard error of the mean.

the participants in the two experimental groups were observed over time, one can conclude that the two pharmacopunctures activated cardiac function to similar extents.

The Ln(TP) for the C-group was significantly increased at all measurement times, except 10 minutes after injection, whereas that for the R-group was significantly increased at all measurement times after injection. Thus, both the pharmacopunctures activates the ANS. In this experiment, the Ln(TP) for the C-group showed a slightly lower, but still increased, value at 10 minutes after injection, which was a statistically significant difference between the two groups. Our results showed a slight difference from the results of another study, which reported that the Ln(TP) for Cervi Pantotrichum Cornu pharmacopuncture was significantly increased at all times after injection [3]. Nevertheless, overall, the result of this study that both the pharmacopunctures showed increased values of the Ln(TP) is similar to that of the other study [3].

The Ln(LF) for the participants in both the groups showed a tendency to significantly increase from 20 minutes after injection. In addition, the C-group tended to show a higher Ln(LF) than the R-group; however, the values for the two groups did not show significant differences over time, indicating that both the pharmacopunctures activated the sympathetic nervous system to similar extents. According to a previous study [4], although the result showed no significance, a similar pattern of Ln(LF) was found in this study. Moreover, a long-term effect (20—30 minutes after the injection) in this study suggests that the pattern of increasing Ln(LF) was caused by the effect of *Rehmannia glutinosa* pharmacopuncture. Further studies are needed to distinguish the exact value of the influence.

The Ln(HF) for the C-group showed no significant changes compared to the value before injection, repeatedly increasing and decreasing. However, the Ln(HF) for the R-group increased compared to the value before injection, and the differences between the two groups were significant from 20 minutes after injection. Thus, Cervi Pantotrichum Cornu pharmacopuncture did not affect the activity of the parasympathetic nervous system, whereas *Rehmannia glutinosa* pharmacopuncture activated it from 15 minutes after injection. As for the differences between the two groups over time, no significant differences were observed from 10 minutes after injection; thus, the influences of the two pharmacopunctures on the parasympathetic nervous system were clearly different.

In conclusion, we found that both the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* pharmacopunctures activated the ANS within the normal range. Both main herbs of the two pharmacopunctures have moistening effect in common so that the two pharmacopunctures are assumed to have a control effect in the condition of

Table 5 Comparison of the natural logarithm (Ln) of the low-frequency oscillation power (LF) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups.

Group	P0	Δ1	Δ2	Δ3	Δ4	Δ5	Δ6
Cervi Pantotrichum Cornu	6.207	0.056 ± 0.099	0.211 ± 0.118	0.283 ± 0.137	0.467 ± 0.159*	0.544 ± 0.157*	0.359 ± 0.151*
Rehmannia glutinosa	6.304	0.040 ± 0.129	0.168 ± 0.126	0.248 ± 0.137	0.302 ± 0.140*	0.504 ± 0.140*	0.440 ± 0.127*

Values are presented as mean ± standard error of the mean.
* $p < 0.05$ (by paired t test).
P0 = mean HRV before injection; Δ1—Δ6 = differences between the values before and after six injections 5 minutes apart.

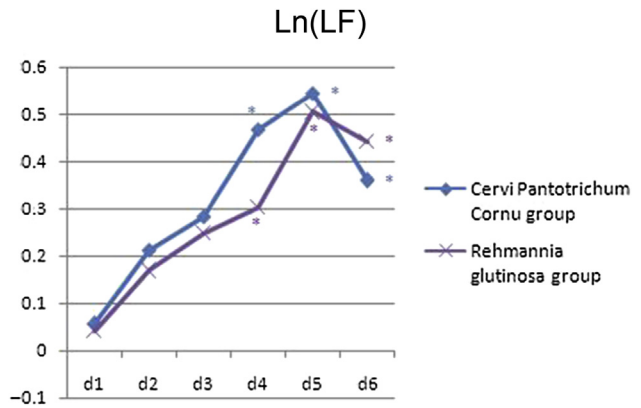

Figure 9 Comparison of the natural logarithm (Ln) of the low-frequency oscillation power (LF) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. * $p < 0.05$ (by paired *t* test). d1—d6 = differences between the values before and after six injections 5 minutes apart.

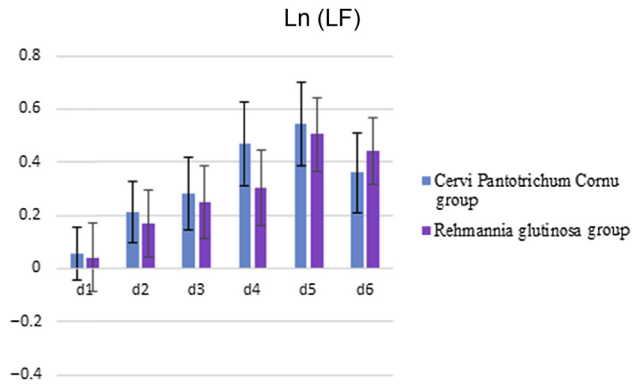

Figure 10 Comparison of the natural logarithm (Ln) of the low-frequency oscillation power (LF) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. Values are presented as mean ± standard error of the mean. d1—d6 = differences between the values before and after six injections 5 minutes apart.

hypofunction or disharmony of the ANS. However, they have different characteristics. Cervi Pantotrichum Cornu pharmacopuncture activates sympathetic nerve only, whereas *Rehmannia glutinosa* pharmacopuncture activates

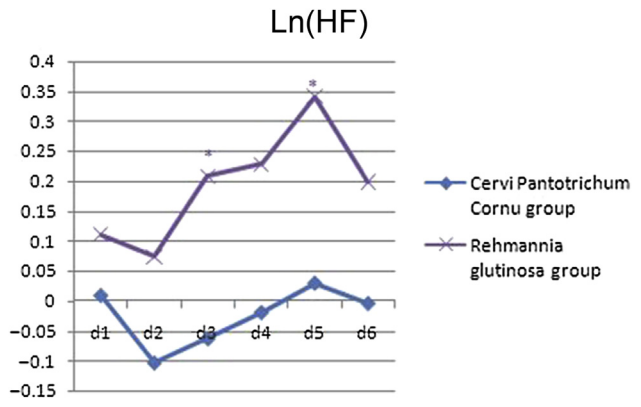

Figure 11 Comparison of the natural logarithm (Ln) of the high-frequency oscillation power (HF) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. * $p < 0.05$ (by paired *t* test). d1—d6 = differences between the values before and after six injections 5 minutes apart.

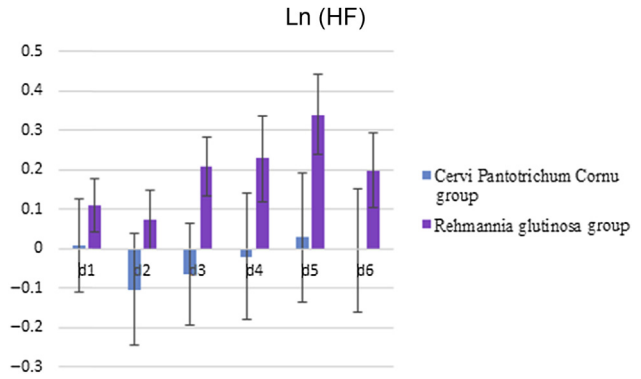

Figure 12 Comparison of the natural logarithm (Ln) of the high-frequency oscillation power (HF) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups. Values are presented as mean ± standard error of the mean. d1—d6 = differences between the values before and after six injections 5 minutes apart.

both sympathetic and parasympathetic nerve. We assumed that a different nature of herbal medicine had affected to the result; one is warm and the other is cold. The two pharmacopunctures with different natures need to be demonstrated in further studies.

Table 6 Comparison of the natural logarithm (Ln) of the high-frequency oscillation power (HF) between the Cervi Pantotrichum Cornu and *Rehmannia glutinosa* groups.

Group	P0	Δ1	Δ2†	Δ3†	Δ4†	Δ5†	Δ6†
Cervi Pantotrichum Cornu	5.666	0.008 ± 0.118	−0.103 ± 0.141	−0.063 ± 0.129	−0.019 ± 0.160	0.029 ± 0.163	−0.004 ± 0.155
Rehmannia glutinosa	5.587	0.110 ± 0.066	0.074 ± 0.074	0.207 ± 0.074*	0.228 ± 0.109	0.340 ± 0.101*	0.198 ± 0.095

Values are presented as mean ± standard error of the mean.
* $p < 0.05$ (by paired *t* test).
† $p < 0.05$ (by independent sample test).
P0 = mean HRV before injection; Δ1—Δ6 = differences between the values before and after six injections 5 minutes apart.

Disclosure statement

The authors declare that they have no conflicts of interest and no financial interests related to the material of this manuscript.

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