Original Article

Randomized, single-blind, placebo-controlled trial on *Hominis placenta* extract pharmacopuncture for hot flashes in peri- and post-menopausal women ☆

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ARTICLE INFO

ABSTRACT

Background: *Hominis placenta* pharmacopuncture is widely used for climacteric symptoms. This study examined the efficacy and safety of pharmacopuncture with PLC (the extract of *Hominis placenta*) on hot flashes for perimenopausal and postmenopausal women.

Methods: This study was a randomized placebo-controlled single-blind trial, which recruited 128 perimenopausal and postmenopausal women, randomly assigned to receive pharmacopuncture with PLC or normal saline (NS) for eight weeks. The primary outcome was the mean changes in the hot flash score (HFS) and the secondary outcomes were the mean changes in the Menopause Rating Scale (MRS), follicle-stimulating hormone (FSH) levels, and estradiol (E_2) levels from baseline to eight weeks. Missing values were imputed using the last-observation-carried-forward method.

Results: After treatment (week 9), the HFS decreased significantly in both groups ($p = 0.000$). The residual HFS was $47.09 \pm 41.39\%$ and 56.45 ± 44.92 % in the PLC and control groups, respectively ($p = 0.262$). One month after the treatment (week 13), the score of the PLC group was reduced, but the score increased in the control group ($p = 0.077$). There were no statistically significant differences in the mean changes in MRS, FSH, and E_2 between the two groups. No serious adverse events related to this trial were noted.

Conclusion: In this study, *Hominis placenta* extract pharmacopuncture did not differ significantly from NS in reducing the hot flash score. While this therapy appears safe, the potential for long-term effect of PLC extract needs to be examined in a large randomized controlled trial with appropriate controls.

1. Introduction

As life expectancy increases, women live more than one third of their lives in the perimenopausal or postmenopausal period, and considerable attention has been given to menopause-related symptoms. The most common menopause-related symptom is hot flashes, which occurs in up to 80% of women.[1] Hot flashes commonly last from six months to 5 years[2] and often affect the quality of life. [3]

Acupuncture, one of the non-hormonal therapies, has been reported to reduce the symptoms of hot flashes in many clinical studies.[4,5] Pharmacopuncture combines acupuncture and medication and is a new therapy used widely in China and Korea.[6] The treatment involves the injection of a purified herbal medicine at the acupoints [7]. However, there is insufficient evidence for the use of pharmacopuncture to treat gynecological disorders, such as postmenopausal syndrome, and only a few studies have been conducted.[8,9]

Pharmacopuncture, especially with *Hominis placenta,* is a widely used therapy for gynecological disorders.[10] *Hominis placenta* is an extract of the human placenta comprising various cell pro-

liferators, blood clotting factors, and hormones and their precursors.[11] It has recognized efficacy in anti-oxidation, wound recovery, anti-inflammation, and in increasing the metabolism of tissue cells.[12–15] Indeed, *Hominis placenta* has been used widely for a long time in the clinical practice of Korean gynecology as an oral medication or pharmacopuncture.[16] Although *Hominis placenta* pharmacopuncture is used most commonly for perimenopausal or postmenopausal symptoms, it has been the subject of very little clinical research.[10,17] Therefore, this study examined the efficacy and safety of *Hominis placenta* pharmacopuncture used to treat hot flashes and other symptoms of perimenopausal and postmenopausal women.

2. Methods

This study was reviewed and approved by the Korean Ministry of Food and Drug Safety (approval 31743) and Institutional Review Board of Dongguk University Ilsan Korean Medicine Hospital (DUIOH 2018–07–003-002), Dunsan Korean Medicine Hospital of Daejeon University (DJDSKH-18-DR-15), and Gwangju Korean Medicine Hospital of Wonkwang University (2018/14). The protocol was registered at the Clinical Research Information Service, Republic of Korea (ID: KCT0003533). Informed consent was obtained from all participants. All procedures in the study were under the declaration of Helsinki. The protocol was developed in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) and Consolidated Standards of Reporting Trials (CONSORT) guidelines and checklists.

2.1. Study design

This study was a randomized, placebo-controlled, single-blind, multi-center, parallel-design clinical trial. The voluntary participants were recruited from three centers: Ilsan Korean Medicine Hospital of Dongguk University, Dunsan Korean Medicine Hospital of Daejeon University, and Gwangju Korean Medicine Hospital of Wonkwang University. In accordance with the protocol,[18] all participants who completed the screening period and met the selection criteria were assigned in a 2:1 ratio to two groups for eight weeks: the treatment group ($N = 85$) and the control group ($N = 43$).

2.2. Study population

This study included perimenopausal and postmenopausal women experiencing hot flashes, between 45 and 60 years of age, and having an average daily hot flash score (HFS) of 10 or higher for one week before the first visit.[19] The average HFS was obtained by multiplying the daily average hot flash intensity score (normal: 0 points, mild: 1 point, moderate: 2 points, severe: 3 points, very severe: 4 points) and the daily frequency of occurrence for each intensity.[20] The minimum HFS could be zero, and there is no maximum score limit. It was evaluated retrospectively at Visit 1. The following women were excluded: those diagnosed with psychogenic menopausal symptoms; suspected of having an organic disease; severe complications of the heart, liver, kidney, or other organs; a history of malignant tumors; thyroid disease or abnormal thyroid function, liver or kidney dysfunction; difficulties in participating in this trial due to diseases; a history of hypersensitivity to *Hominis placenta* or other drugs and food that may affect perimenopausal and postmenopausal symptoms; been administered hormones or hormone analogs or previous medicine within the washout period, within the past one month; having participated in another clinical trial within the past three months; not completing the required washout period for previous medication that may affect perimenopausal and postmenopausal symptoms; and those that were unsuitable for this trial.

2.3. Sample size calculation

For some clinical trials, especially when the control group is a placebo group, it is ethically desirable to have more participants in the treatment group compared to the placebo group. In prospective studies, there can be difficulty in recruiting sufficient patients to take part within a given time span. There are instances where randomly distributing the patients to the two treatment groups in the ratio 2:1 may be more time-efficient and cost effective than the usual ratio of 1:1.[21] Considering these ethical and recruitment issues, this study assigned all the participants in a 2:1 ratio to the treatment group and control group.

Because there was no previous study using *Hominis placenta* pharmacopuncture to treat hot flashes, studies that had used acupuncture alone [19] or oral medication of *Hominis placenta* extract [22,23] were used as references. The pharmacopuncture effect size was calculated by combining the estimates from the study of acupuncture with the weighted average from the studies of medication. A minimum of 68 women in the treatment group and 34 women in the control group were needed to identify a 20% difference with a power of 80% and $\alpha = 0.05$. With the calculation of 20% probable sample loss, the appropriate sample size was 128 subjects.

2.4. Study medication and intervention

The drug for this clinical trial, PLC, was an extract of *Hominis placenta* and was manufactured by the pharmaceutical company Unimed (Seoul, Republic of Korea) in accordance with Korean good manufacturing practice. PLC was manufactured from Drug Master File-registered raw materials (*Hominis placenta* extract) and was based on a Unicenta injection manufactured by the drug company Unimed. The Unicenta injection is an aqueous extract of *Hominis placenta* approved by the Korean Food and Drug Administration for sale and use in improving menopausal symptoms. *Hominis placenta* was isolated from healthy pregnant women after delivery and subjected to sterilization and hydrolysis extraction procedures. The normal saline (NS) for the control group consisted of sodium chloride 180 mg/20 ml (0.9%) and was procured from Daihan Pharm Co. (Seoul, Republic of Korea). A research pharmacist labeled the two test materials as PLC and NS.

For all subjects in the PLC group, 0.5 cc of PLC was injected twice weekly for 8 weeks at four acupoints (CV4, CV6, and bilateral Ex-BB1) using a 1-cc 30-gauge disposable syringe to a depth of up to 8 mm. The control group was injected with NS at the same points using the same technique. NS was injected as a control intervention because the acupuncture sensation after injection is similar to that of the *Hominis placenta* medication.[24] After the procedure, participants received infrared irradiation at a distance of 30 to 60 cm for 20 min to feel warmth in their lower abdomen. No other treatment was allowed during the test period. The acupoints were selected based on clinical experience, data from previous researches, and from the information provided in the standard text book.[25–27] The pharmacopuncture treatment was administered by an experienced Korean medicine doctor licensed by the Korean Ministry of Health and Welfare.

2.5. Randomization and blinding

Eligible participants were randomized into one of the two groups via stratified block randomization. The stratification factor was the hospital. An independent statistician generated a separate randomization sequence, and the randomization codes were sealed in opaque envelopes.

NS was administered identically to the PLC to maintain the blinding. The same amount of PLC or NS was injected by the physi-
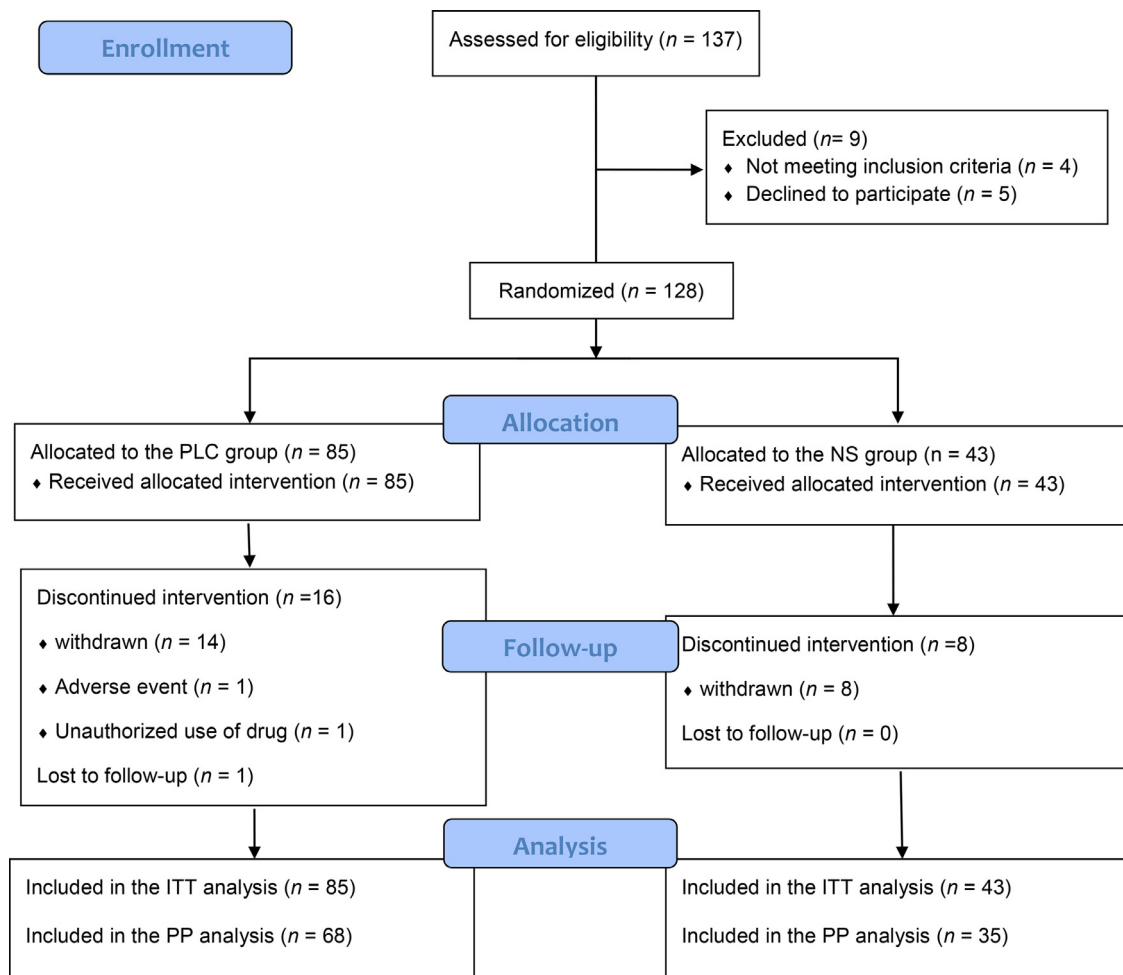
Fig. 1. Flow chart of the study participants, PLC: Placenta Extract, NS: Normal Saline, ITT: Intention-to-treat, PP: Per-protocol.

cian at the same acupoints using the same syringe gauge, so that participants could not feel any difference. Owing to differences in the color of the PLC and the saline solution, the syringes were covered with translucent tape to eliminate any bias by the participants. Therefore, in this trial, only participants were blinded to the treatment allocation.

2.6. Outcome measures

The primary outcome was the mean change in the HFS from baseline to eight weeks. The HFS was calculated from the hot flash diary.[28] The HFS for each day was calculated as the number of hot flashes multiplied by the average severity of the hot flashes on that day. The residual HFS (a percentage of the baseline score) was used for statistical analyses because the HFS does not have a highest value. The secondary outcomes were the mean changes in the Menopause Rating Scale (MRS), follicle-stimulating hormone (FSH) levels, and estradiol (E_2) levels, from baseline to eight weeks. The treatment effects on the patient's overall menopausal symptoms were assessed by administering the MRS questionnaire at baseline and at weeks 9 and 13 to both groups. The MRS is a health-related quality of life scale developed to measure the severity of menopause-related complaints in the early 1990s and has since been validated.[29] We used the international version of the MRS, which was translated into Korean.[30–32] The FSH and E_2 levels were evaluated by blood tests on screening and at week 9.

2.7. Statistical methods

An independent statistician performed all statistical analyses using SAS (version 9.1.3; SAS Institute Inc. Cary, NC, USA). Missing values were imputed using the last-observation-carried-forward method. A student's t-test or Wilcoxon's rank sum test was conducted to assess the difference in the residual HFS and MRS changes between the PLC and control group. The FSH and E_2 levels were analyzed by dividing each group into the pre- and post-menopausal groups. The number of changes in the E_2 and FSH levels for each group before and after treatment was evaluated using a paired t-test or Wilcoxon signed-rank test. The difference in the change between the PLC group and control groups was analyzed using a Student's t-test or Wilcoxon's rank sum test. The analysis of covariance (ANCOVA) was used to control the baseline variables that can affect the primary outcome. The bias was reduced by correcting the difference in the base values using other variables as covariates. For safety evaluation, the proportion of patients with adverse events in each group was calculated and compared using a Chi-square test or Fisher's Exact test. In all tests, a significance level of 0.05 and a Confidence Interval of 95% were considered.

3. Results

3.1. Patients' flow and baseline characteristics

This study was conducted from October 2018 to January 2021. Fig. 1 presents a flow diagram of the overall study. One hundred

Table 1
Baseline characteristic of the participants.

	PLC group (N = 85)		Control group (N = 43)		p-value
	Mean ± SD (range)		Mean ± SD (range)		
Age (years)	53.31 ± 3.96 (45-60)		53.86 ± 4.41 (45-60)		0.473*
Height (cm)	156.56 ± 5.01 (147-168)		157.4 ± 5.09 (147-168)		0.380*
Weight (kg)	57.71 ± 6.53 (44-76)		58.93 ± 7.29 (44-74)		0.337*
BMI (kg/m2)	23.55 ± 2.51 (17.19-29.96)		23.83 ± 3.06 (15.97-29.05)		0.573*
Age of onset of hot flashes (years)	N = 84 49.44 ± 3.98 (30-57)		N = 42 49.31 ± 4.03 (40-59)		0.863*
Period of previous HRT (months)	N = 13 13.92 ± 19.35 (1-60)		N = 6 24.00 ± 27.88 (1-77)		0.371*
Exercise time (hours/week)	N = 40 3.90 ± 2.31 (1-10)		N = 21 3.29 ± 1.62 (1-7)		0.282*
	YES (n, %)	NO (n, %)	YES (n, %)	NO (n, %)	
Menopause	49 (57.65)	36 (42.35)	32 (74.42)	11 (25.58)	0.063#
Previous HRT	13 (15.29)	72 (84.71)	6 (13.95)	37 (86.05)	0.840#
Maternal history of hot flashes	17 (20.00)	68 (80.00)	13 (30.23)	30 (69.77)	0.849#
Smoking	-	85 (100.00)	-	43 (100.00)	-

HRT: Hormone replacement therapy ; BMI: body mass index; SD: standard deviation.
  * : p-value obtained from independent two-sample t-test.
  # : p-value obtained from a Chi-square test.

Table 2
The Residual Hot flash score at Baseline, Week 9 and 13.

ITT population	HFS (Mean ± SD)/residual HFS (%)		p-value
	PLC group (N = 85)	Control group (N = 43)	
Baseline (V2)	20.09 ± 12.51	27.23 ± 23.61	0.210*
Week 9 (V18)	8.16 ± 7.14/ 47.09 ± 41.39	16.00 ± 20.67/ 56.45 ± 44.92	0.134*/0.262*
Week 13 (V19)	7.51 ± 6.92/ 43.97 ± 40.40	17.30 ± 25.24/ 58.86 ± 48.88	0.285*/0.804*

ITT: Intention to Treat; V: Visit; HFS: Hot flash score; SD: standard deviation.
  * : p-value obtained from Wilcoxon's rank sum test.

and twenty-eight patients were enrolled in the study. There were 25 dropouts, leaving 103 patients who completed the study. The reasons for the dropouts are presented in Fig. 1. Table 1 lists the baseline characteristics of the 128 patients. There were no significant differences in the baseline characteristics between the PLC and control group.

3.2. Outcome measures

3.2.1. Primary outcomes
(1) Residual Hot Flash Score

When the treatment was complete (Week 9), the residual HFS was 47.09 ± 41.39% in the PLC group and 56.45 ± 44.92 % in the control group ($p = 0.262$). In week 13, one month after the end of treatment, the residual HFS was 43.97 ± 40.40 % in the PLC group and 58.86 ± 48.88 % in the control group ($p = 0.804$). In week 13, the score of the PLC group was reduced by 3.12 ± 20.87, but the score was increased by 2.41 ± 21.60 in the control group ($p = 0.077$) (Table 2) (Fig. 2). The difference between weeks 9 and 13 was larger in Per Protocol (PP) group than in the Intention to Treat (ITT) analysis group. In the PP group, the score was reduced by 5.11 ± 20.75 in the PLC group, but increased by 2.96 ± 23.98 in the control group, indicating a significant difference between the two groups ($p = 0.044$).

(2) The mean change in Hot Flash Score

At baseline, there were no statistically significant differences in baseline HFS between PLC and control group. There were no statistically significant differences in the mean changes of HFS before and after treatment between PLC and control groups (Table 2, Supplementary Tables 1–2).
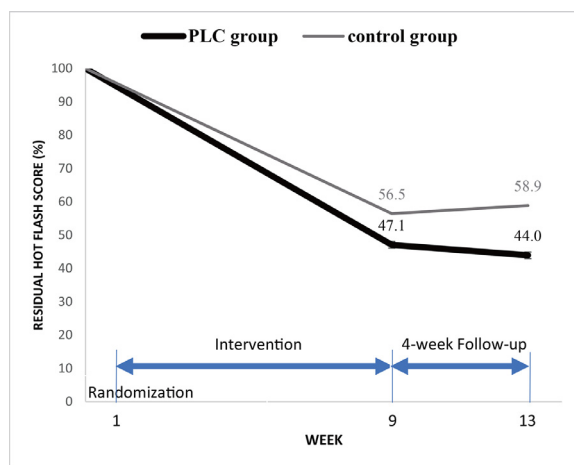
Fig. 2. The Residual Hot Flash Score at Week 9 and Week 13.

3.2.2. Secondary outcomes
(1) Menopause Rating Scale (MRS)

Changes in the MRS score of the subjects before and after treatment were examined. An analysis of the ITT group showed no significant difference between the two groups: 21.00 ± 8.52 for the PLC group and 23.70 ± 7.77 for the control group at baseline (Baseline) ($p = 0.084$). At the end of treatment (Week 9), the scores of the PLC and control group were 15.07 ± 8.70 and 17.91 ± 8.06, respectively, showing no significant difference ($p = 0.203$). The MRS scores of Weeks 9 and 13 were compared to determine if the treatment effect of each group had been maintained one month after treatment. One month after treatment (Week 13), the MRS scores of the PLC and control groups showed a slight decrease compared

Table 3
Adverse events.

	Diseases or Symptoms	Severity	PLC group (N = 85)		Control group (N = 43)		
			Patients N(%)	Events E	Patients N(%)	Events E	p-value
Patients	Acute gastroenteritis	moderate	1(1.18)	1	0(0.00)	0	
with	AST/ALT elevation	mild	2(2.35)	2	0(0.00)	0	
any	Back pain	mild	1(1.18)	1	0(0.00)	0	
AE	Bruise	mild	8(9.41)	8	2(4.65)	2	
	Cervical sprain	mild	1(1.18)	1	0(0.00)	0	
	Cold	mild	0(0.00)	0	1(2.33)	1	
	Cystitis	mild	0(0.00)	0	1(2.33)	1	
	Enterocolitis	mild	0(0.00)	0	1(2.33)	1	
	Itchy of pudenda	moderate	1(1.18)	1	0(0.00)	0	
	Lt. ankle ligament injury	moderate	1(1.18)	1	0(0.00)	0	
	Lt. flank urticaria	mild	1(1.18)	1	0(0.00)	0	
	Nausea	mild	1(1.18)	1	0(0.00)	0	
	Painful throat	mild	1(1.18)	1	0(0.00)	0	
	total		19(22.35)	19	5(11.63)	5	0.142*
Patients with any serious AE	Subarachnoid hemorrhage	severe	1(1.18)	1	0(0.00)	0	
	total		1(1.18)	0	0(0.00)	0	0.475*
Patient who died			0	0	0	0	-

E: number of events; AE: Adverse events; AST: ASpartate Transaminase; ALT: ALanine Transaminase; Lt.: Left * p-value obtained from a chi-squared test

to Week 9, indicating that the treatment effect had been well maintained (Supplementary Table 3).

(2) Follicle Stimulating Hormone (FSH)

The participants' FSH levels at the end of treatment (Week 9) were compared to the baseline levels. The PLC and control groups were again divided into pre- and post-menopausal groups and compared. In an analysis of the ITT group, a comparison of the groups showed no significant difference in all items (Supplementary Table 4).

(3) Estradiol (E_2)

The participants' E_2 values at the end of treatment (Week 9) were compared to the baseline status. As with FSH, the PLC and control groups were again divided into pre- and post-menopausal groups. In the analysis of the ITT group, the premenopausal women's E_2 level increased after treatment in the PLC group ($p = 0.456$). In the control group, however, E_2 level decreased after treatment ($p = 0.374$). The E_2 level after menopause was further divided and analyzed based on $E_2 < 5$ pg/ml of the basal state (V2). The E_2 level of postmenopausal women whose E_2 level was less than 5 pg/ml at baseline increased at Week 9 in both the PLC and control groups (each $p = 0.109$, $p = 0.068$). The E_2 level of postmenopausal women whose E_2 level at baseline was 5 pg/ml or higher increased slightly at Week 9 in the both groups ($p = 0.109$, $p = 0.906$, respectively). In all cases, there was no significant significance between the two groups (Supplementary Table 5).

3.3. Safety evaluation

Twenty-five cases of adverse events were reported, and there was one serious adverse event (SAE) (Table 3). One SAE, spontaneous subarachnoid hemorrhage, occurred in the test group. This SAE was due to a rupture of a middle cerebral aneurysm. This event was determined to be unrelated to PLC acupuncture treatment, and a previous study[30] reported that risk factors for cardiovascular disease did not change over 8 weeks of injecting human placenta extract for middle-aged women. The participant was withdrawn from the clinical study.

The types of adverse events reported are as follows (Table 3). Ten patients complained of bruising at the injection site. This adverse reaction could occur during the procedure and participants were notified about it in advance. For each participant who complained of urticaria and nausea, there was a possibility of a causal

relationship with the treatment, but the symptoms were mild and disappeared after follow-up. Other symptoms were judged to have low relevance to this study, and their degree was confirmed to be mild.

After analyzing the lab test results by dividing them into normal and abnormal based on the reference values for each testing institution, there was no statistically significant difference in any of the items before and after the clinical study. In the case of vital signs, the systolic blood pressure of the test and control group was reduced significantly after treatment ($p = 0.007$, $p = 0.003$, respectively), and the diastolic blood pressure of the test group was reduced significantly after treatment ($p = 0.008$). However, these were all changes within the normal range and were judged to have no clinical significance. In the other cases, there was no statistical significance in the changes before and after treatment. When comparing the two groups, the pulse rate after the end of the treatment (Week 9) was significantly higher in the test group than in the control group ($p = 0.027$), but this value was also within the normal range.

3.4. Additional analysis

For the exploratory analysis of the data, subgroup analysis was performed based on menopause status.

As a result, the amount of change in HFS before and after treatment between the test group and the control group was more pronounced in the pre-menopause group. When analyzing all participants, the difference between the PLC and control groups was less than one point in the HFS score change, but there was a difference of nearly three points in the pre-menopause group. However, this difference was not statistically significant ($p = 0.543$).

When pre-menopause participants were re-examined one month after the end of treatment (Week 13), HFS scores were further decreased in both the PLC and control groups compared to Week 9. Both groups maintained a significant decrease in scores compared to the baseline at Week 13 (PLC group $p = 0.003$, control group $p = 0.007$). On the contrary, in the post-menopausal participants, after the treatment (from week 9 to week 13), the HFS scores decreased significantly in the PLC group ($p = 0.035$), while the scores increased significantly in the control group ($p = 0.042$) (Table 4).

Table 4
The hot flash score difference between pre and post menopause.

	ITT population		PLC group N = 36	Control group N = 11	Logistic regression (95% CI)	*p*-value
Premenopausal participants	Baseline (V2)	Mean ± SD	18.64 ± 8.36	20.09 ± 9.60	0.981 (0.908-1.059)	0.936*
	Week 9 (V18)	Mean ± SD	8.28 ± 6.25	12.64 ± 12.50	0.942 (0.869-1.021)	0.808*
	Baseline- Week 9	Mean ± SD	10.36 ± 10.67	7.45 ± 6.19	1.033 (0.958-1.113)	0.543*
	Week 13 (V19)	Mean ± SD	7.33 ± 5.96	12.18 ± 11.78	0.928 (0.852-1.012)	0.936*
	V2-V18-V19	*p*-value	0.000[†]	0.008[†]		
	V2-V18	*p*-value	0.002[††]	0.012[††]		
	V2-V19	*p*-value	0.003[††]	0.007[††]		
	V18-V19	*p*-value	0.336[††]	0.436[††]		
			N = 49	N = 32		
Postmenopausal participants	Baseline (V2)	Mean ± SD	21.16 ± 14.83	29.69 ± 26.48	0.978 (0.953-1.004)	0.440*
	Week 9 (V18)	Mean ± SD	8.08 ± 7.79	17.16 ± 22.86	0.957 (0.918-0.997)	0.159*
	Baseline- Week 9	Mean ± SD	13.08 ± 15.95	12.53 ± 23.00	1.002 (0.978-1.026)	0.996*
	Week 13 (V19)	Mean ± SD	7.63 ± 7.60	19.06 ± 28.38	0.952 (0.909-0.996)	0.341*
	V2-V18-V19	*p*-value	0.000[†]	0.000[†]		
	V2-V18	*p*-value	0.000[††]	0.000[††]		
	V2-V19	*p*-value	0.000[††]	0.000[††]		
	V18-V19	*p*-value	0.035[††]	0.042[††]		

ITT: Intention to Treat; V: Visit; CI: confidence interval; SD: standard deviation.
* : *p*-value obtained from Wilcoxon's rank sum test.
[†] : *p*-value obtained from Friedman's test by ranks.
[††] : *p*-value obtained from Wilcoxon signed (paired) rank test.

Since there was a difference between the two groups in the maintenance of HFS reduction after 1 month of intervention, we performed an exploratory additional analysis on the maintenance of the treatment effect after the intervention using covariate analysis according to the protocol. ANCOVA was performed to evaluate the effects of the intervention. The change values in HFS between Week 13 and Week 9 were entered as the dependent measure, group was entered as the fixed variable, and the previous HRT, menopause, and exercise were entered as covariates. The rationale for using these covariates was based on literature[33] identifying previous HRT, menopause, and exercise as influential factors for the hot flash. The result of ANCOVA when previous HRT history and exercise were included in the model as covariates was significant, indicating that the group variances were significantly different ($F = 4.449$, $p = 0.037$; $F = 3.965$, $p = 0.049$, respectively).

4. Discussion

4.1. Summary of the main results

This study examined the effectiveness and safety of PLC pharmacopuncture. After the pharmacopuncture treatment, the hot flash scores and residual hot flash scores of PLC and control groups were reduced significantly. The changes before and after treatment were larger in the PLC group than in the control group, but the differences between the two groups were not statistically significant. Moreover, the PLC treatment effect was maintained for one month after the end of treatment (Week 13). When comparing the residual hot flash score at the time of treatment (Week 9) and after one month (Week 13), the score increased again in the control group, but the score decreased in the PLC group. Therefore, the therapeutic effect of the PLC pharmacopuncture was maintained for one month after treatment. Hence, the long-term effects of PLC pharmacopuncture will need to be examined through additional research. In addition, safety evaluation revealed no major side effects.

4.2. Potential mechanism and implication for research

This study was a randomized controlled clinical study using *Hominis placenta* pharmacopuncture, which is used widely for hot flashes in menopausal disorders. This is meaningful as the first clinical study using pharmacopuncture conducted after receiving Investigational new drug (IND) approval from the Ministry of Food and Drug Safety of Republic of Korea. After the pharmacopuncture treatment, the hot flash scores and residual hot flash scores of the PLC and control groups were reduced significantly. The changes before and after treatment were larger in the PLC group than in the control group, but the differences between the two groups were not statistically significant. Moreover, the PLC treatment effect was maintained for one month after the end of treatment (Week 13). When comparing the residual HFS at the time of treatment (Week 9) and after one month (Week 13), the score increased again in the control group, but the score decreased in the PLC group. The difference between two groups were statistically significant in the PP analysis, but not in the ITT analysis. This finding is due to the difference in analysis methods and should be interpreted with caution. The ITT analysis includes patients who, in fact, did not receive the experimental treatment, and one would expect them to have attenuated values.[34] The missing data of noncompliers could result in a greater uncertainty of the trial conclusions. Some studies have demonstrated that the PP analysis tends to provide, on average, higher estimates of effect than the ITT analysis.[34–36] Therefore, the therapeutic effect of the PLC pharmacopuncture was maintained for one month after treatment, and this could be the effect of the PLC acupuncture solution. Hence, the long-term effects will need to be examined through additional research.

Further studies on the mechanism of treatment will also be needed. In premenopausal subjects, the E_2 level was increased in the PLC group at the end of treatment (Week 9) before menopause but decreased in the control group. Although the difference between the two groups was not statistically significant, PLC pharmacopuncture treatment appeared to be more effective in raising E_2 than saline acupoint infusion in premenopausal women. In addition, the improvement effect on FSH, which is another endocrine index related to menopausal symptoms, was not confirmed in both groups. Therefore, further research on the related mechanism will be needed.

4.3. Limitations

Only a few previous studies have reported the efficacy of *Hominis placenta* extract in reducing climacteric symptoms.[30,37] In the present study, we confirmed the improvement of menopausal

symptoms after PLC pharmacopuncture; however, compared with NS pharmacopuncture at same acupoints, there was no significant difference in efficacy. The difference in the extent of the HFS changes did not show statistical significance, and this could be due to the sample size determination. When calculating the sample size, only the acupuncture study was used to estimate the effect size of the control group, and the PLC group effect size was assumed by adding the weighted average value of oral administration studies of the *Hominis placenta* extract to the estimated amount of the acupuncture study because of the absence of previous studies.

The treatment effect of pharmacopuncture is expressed by the combined physical and pharmacological effects of the injected medicine and the acupuncture effect. Pharmacopuncture has both medical and acupuncture effects by simultaneously stimulating the meridians and acupuncture points both physically and chemically[7]. In addition, the stimulation intensity increases according to the amount of the acupuncture injection,[38] and there is a difference in the therapeutic effect.[39] Therefore, it is necessary to consider the physical acupoint stimulation effect of the pharmacopuncture extract. NS injection in the control group would have given the same physical acupoint stimulation effect in addition to the acupuncture effect.[40,41] Therefore, it is thought that the sample size calculation would not be appropriate to assess only the difference in chemical effect of pharmacopuncture extract. The placebo effect according to blinding and the very large standard deviations of outcomes should also be considered when interpreting the results. Therefore, follow-up studies that reflect the results of this study will be needed. An appropriate sample size calculation considering control settings will be needed. The outcome of this study can be used to calculate the sample size of similar studies in future. To confirm the effect of PLC pharmacopuncture treatment, and not just the effect of PLC extract, it would also be beneficial to set up another intervention as a control group under a clinically meaningful hypothesis. Considering that acupuncture is mainly used together with acupuncture in real world clinics, comparing pharmacopuncture vs. acupuncture treatment or adding pharmacopuncture to acupuncture treatment with single acupuncture treatment confirms the effectiveness of pharmacopuncture treatment and at the same time it will also be clinically meaningful. In addition, it will be necessary to evaluate the economic feasibility considering the efficacy and cost.

Conclusions

PLC and NS pharmacopuncture reduced the hot flash score significantly. This study showed that the *Hominis placenta* extract did not differ from NS in reducing the hot flash scores. Additional studies on the efficacy of PLC pharmacopuncture for hot flashes are needed.

Acknowledgments

CRediT authorship contribution statement

Su-Ji Choi: Writing – original draft, Writing – review & editing, Data curation, Investigation, Methodology. **Dong-Il Kim:** Supervision, Writing – review & editing, Investigation, Methodology, Conceptualization. **Sang Ho Yoon:** Investigation. **Chang-Min Choi:** Investigation. **Jeong-Eun Yoo:** Investigation, Writing – review & editing.

Conflict of interest

The authors declare that there are no conflicts of interest.

Funding

Ethical statement

This study was reviewed and approved by the Korean Ministry of Food and Drug Safety (approval 31743) and Institutional Review Board of Dongguk University Ilsan Korean Medicine Hospital (DUIOH 2018–07–003-002), Dunsan Korean Medicine Hospital of Daejeon University (DJDSKH-18-DR-15), and Gwangju Korean Medicine Hospital of Wonkwang University (2018/14). Informed consent was obtained from all participants.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.imr.2022.100891.

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