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**Urtica dioica in Comparison with Placebo and Acupuncture: A New Possibility for
Menopausal Hot Flashes: A Randomized Clinical Trial**

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Highlights files:

- In this 12 week, randomized clinical trial, that used herbal placebo and sham acupuncture, and included 72 postmenopausal women, we found that *Urtica dioica* can decrease menopausal hot flashes and increase the quality of life of postmenopausal women better than placebo-sham control but same as acupuncture.
- The combination of *Urtica dioica* and acupuncture did not add to the effects of those therapies.

Abstract:

Objectives: The purpose of this research was to investigate the effect of *Urtica dioica* in comparison with placebo, acupuncture and combined therapy on hot flashes and quality of life in postmenopausal women.

Methods: In a double-blinded randomized controlled trial, patients were treated for 7 weeks then followed up 4 weeks. Seventy-two postmenopausal women who reported at least 20 hot flashes attacks per week were randomly allocated into one of the 4 groups of *Urtica dioica* 450 mg/day and acupuncture 11 sessions (A), acupuncture and placebo (B), sham acupuncture and *Urtica dioica* (C), and sham acupuncture and placebo (D). The primary outcomes were the change in hot flashes score from baseline to the end of treatment and follow up; and the change in the quality of life (MENQOL) from baseline to the end of treatment. Secondary outcomes included changes in FSH, LH, and ESTRADIOL levels from baseline to the end of treatment. The trial was conducted from October 2017 to July 2018 in Acupuncture clinic of a teaching hospital in Iran.

Results: A total of 72 women 45 to 60 years old were enrolled, and 68 were included in the analyses. The median (IQR) hot flashes score decreased in the A group by 20.2 (31.7) and 21.1 (25.1), B group by 19 (18) and 17.3 (27), C group by 14.6 (25.4) and 20.8 (13), and D group by 1.6 (11.6) and 1 (13.3) at the end of treatment and follow up ($P<0.0001$, $P<0.0001$); no significant difference between A, B and C groups. The mean (SD) of MENQOL score decreased in the A group by 42.6 (21.1), B group by 40.7 (29.8), C group by 37.8 (26.8) and D group by 9.8 (14.3) at the end of treatment ($P=0.001$); no significant difference between A, B and C groups.

Conclusions: *Urtica dioica* can decrease menopausal hot flashes and increase the quality of life of postmenopausal women better than placebo-sham control but same as acupuncture. The combination of *Urtica dioica* and acupuncture did not add to the effects of those therapies.

Keywords: Menopause; Hot flashes; *Urtica dioica*; Acupuncture; Phytoestrogens

Clinical Trial Registration: ClinicalTrials.com, www.irct.ir, IRCT201707017265N10

1. Introduction

Normal menopause is described as 12 months of amenorrhea without bleeding episodes which is not associated with surgery, radiotherapy, and chemotherapy.¹

Menopause may be associated with complications such as vasomotor symptoms (flushing, night sweats), sleep disorders, vulvovaginal atrophy (dryness, itching, burning), sexual dysfunctions, mood disorders (depression, anxiety, irritability), and physical symptoms (back pain, fatigue, stiffness, and arthralgia).² The most important symptom is hot flashes which is the main and most prevalent symptom of postmenopausal and menopausal period. Hot flashes is described as the sudden onset of reddening of the scalp, neck and chest, which continues with an increase in heart rate and a feeling of severe warmth in the body, and sometimes ends with severe sweating and shivering. The duration of hot flashes varies from a few seconds to a few minutes and rarely lasts up to 1 hour. The frequency varies, and the severity increases overnight, during stress, and in warm environments.³ About 40% of women experience hot flashes at the beginning, and 60 to 80% at the end of the postmenopausal period, with about 20% requiring medical intervention.⁴ Hot flashes persist for 4-5 years in 50% of women; in about 25% it remains for more than 5 years, and in 10% it remains for up to 10 years.⁵ Smoking, lack of physical activity, socioeconomic factors such as low education and economic status, serum FSH concentration, climate, and genetic factors are related to the incidence and severity of hot flashes.⁶⁻⁸

The pathophysiology of hot flashes is still unclear; however, studies indicate that there is a disorder of temperature regulation at the hypothalamic level due to the withdrawal of estrogen.⁹ The estrogen sensitive hypothalamic nerve network, which produces cyspeptin, Neurokinin B, and Dynorphin (KNDy neurons), adjacent to the thermoregulatory center, regulates the pulsatile secretion of GnRH and LH.¹⁰ The temperature adjustment range is more narrow in women with

hot flashes, which can cause compensatory responses such as shivering and hot flashes.^{11, 12} There are some medicines to reduce and treat hot flashes including hormone therapy¹³. Estrogen is effective in treating hot flashes, sleep disorders, mood changes, vaginal dryness, and improving quality of life in various forms.¹⁴ Estrogen therapy is effective in reducing the risk of colorectal cancers, decreasing osteoporosis, and reducing morbidity and mortality of coronary heart disease.¹⁵ However, it increases the risk of ovarian and endometrial cancers, coronary diseases, and stroke and thromboembolic diseases.¹⁶ Eighty percent of hot flashes cases respond to hormone therapy, but due to the complications and sometimes contraindications, a low percentage of women with hot flashes use this treatment. Therefore, the decision for hormone therapy to reduce the symptoms of menopause depends on the patient. For those with a history of skin cancer, cardiovascular diseases, thromboembolic or cerebrovascular diseases, alternative treatments such as antidepressants like paroxetine, citalopram, gabapentin, and clonidine are recommended,¹⁷ which are less desired due to complications, low to moderate effects in the treatment of hot flashes, and high costs.¹⁸ A large proportion of women with hot flashes seek complementary and alternative therapies such as medicinal herbs and acupuncture.^{19, 20}

Medicinal herbs with phytoestrogenic effects have been studied for the treatment of hot flashes. These medicinal herbs include sage, licorice, anise, red clover, soybean, etc which are phytoestrogens.²¹ *Urtica dioica*, known as Nettle, is a herbaceous perennial flowering plant in the family Urticaceae which is found worldwide. It seems to have phytoestrogenic components.²² Nettle contains many micronutrients and active ingredients such as phenols, vitamins (A, B2, B5), and minerals (calcium, potassium, magnesium, iron).²³ *Urtica dioica* contains phytoestrogen components, which are structurally or functionally similar to estrogen hormone and its active metabolite, and have phytoestrogens with anti-anxiety and calcium intake properties.²⁴

Phytoestrogens are also preventative for osteoporosis as well as breast and endometrial cancers.²⁵ We compared the effects of *urtica dioica* on menopausal hot flashes severity and frequency with placebo in a previous pilot study. The results suggested that *urtica dioica* might be effective in decreasing hot flashes severity and frequency.²⁶ There is no other human study regarding its therapeutic effect on menopausal hot flashes.

Acupuncture is another popular treatment for controlling hot flashes. Modern research suggests that acupuncture might affect the temperature regulation center of the hypothalamus by regulating the activity of serotonin and β -endorphins.^{27,28} Compared to sham acupuncture or control, therapeutic responses to acupuncture seem very different in different trials.^{29,30} Therefore, there is uncertainty towards the effects of acupuncture in menopausal hot flashes and other symptoms which requires further studies for clarification.

In Traditional Chinese Medicine, it is believed that herbal therapy cures the root of disease, while acupuncture cures symptoms. Therefore, it recommends a combination of acupuncture and herbal therapy.³¹

This study aimed to investigate the effect of *Urtica dioica* in comparison with placebo, acupuncture and combined therapy on hot flashes and quality of life in postmenopausal women.

2. Methods

2.1 Study design and participation

This study is a randomized controlled clinical trial on postmenopausal women conducted from October 2017 to July 2018 in Acupuncture Clinic of Imam Reza Hospital, Mashhad, Iran. The Ethics Committee of Mashhad University of Medical Sciences approved this research project (IR.MUMS.REC.1396.144). The study was also registered in the Iranian Registry of Clinical Trials (IRCT201707017265N10).

Advertisement for the trial was placed by posters in university-affiliated clinics and health centers, as well as social media. Participants were selected using convenience sampling method from women referring to the gynecology and acupuncture clinic of Imam Reza Hospital and were screened for eligibility criteria.

The study lasted for 12 weeks consisting of 7 weeks intervention and 4 weeks follow up. The assessment time points were as follows. During week 1, participants recorded the intensity and frequency of hot flashes. The intervention was given from the beginning of week 2 until the end of week 8, for a total of 7 weeks. At the end of treatment (week 9) and at the follow up visit (week 12), participants recorded their hot flashes severity and frequency. The MENQOL's questionnaire was completed at baseline, and at the end of treatment. The FSH, estradiol, and LH levels were measured before the intervention and at week 9 (end of intervention).

2.2 Inclusion criteria

Postmenopausal women (amenorrhea for 12 months or more)^{32, 33} who suffered from hot flashes included if they aged 45-60, experienced at least 20 hot flashes episodes per week, had Yin and Yang deficiency syndrome according to Chinese Medicine, and signed the informed consent for

participating in the study.^{33,34} There are 5 patterns for menopausal symptoms in Chinese Medicine. The most common one is Yin and Yang deficiency syndrome according to ACUFLASH study.³⁴ Participants with Yin and Yang deficiency syndrome experience hot flashes but cold hands and feet, night-sweating, frequent pale urination, flashes around the neck when talking, slightly agitated, chilliness, dry throat, dizziness, tinnitus, and backache symptoms.³⁴

2.3 Exclusion criteria

Oophorectomy, anti-cancer therapies due to malignancy, history of estrogen-related cancers in the past five years, a history of drug allergies to Urtica and metals, history of known mental disorder, hyperthyroidism and hypothyroidism, physical conditions and diseases which did not fit this study (thromboembolic disease, cardiac disease, hypertension, uncontrolled diabetes, etc.), vaginal bleeding with unknown cause over the past six months, taking any medication such as HT and SSRI for hot flashes during the past 8 weeks, history of acupuncture treatment, endometrial thickness over 10 mm in sonography.^{33, 34}

Ultrasonography was performed to determine the thickness of the endometrium and to evaluate the gynecologic disorders before intervention in each of the 4 groups. The participants recorded the number and severity of hot flashes based on VAS in a daily report form one week before start of the treatment. They were treated for 7 weeks and were followed up for 4 weeks.

2.4 Research tools

Data collection instruments included a daily report form for hot flashes frequency and severity, the Menopause-Specific Quality of Life Questionnaire (MENQOL), laboratory test, and a checklist for demographic data and study outcomes. The patient's daily report form is a reliable

and valid tool which has been used in various studies. The number of hot flashes attacks per day, and their severity based on Visual Analogue Scale (VAS) were recorded in the patient's daily report one week before the study, and at weeks 9 and 12. Based on the patient's daily report, the intensity of hot flashes attacks scored from 0 to 10 (0: very mild and 10: very severe and life disruptive). The MENQOL's questionnaire has 29 questions in three parts: somatic, psychological, and urogenital. The reliability of the Persian version of the MENQOL's questionnaire has been determined and confirmed according to the Cronbach's alpha of 0.9. Cronbach's alpha has been determined for each item of the questionnaire, and the validity of the Persian version has also been determined and confirmed.³⁵

2.5 Random allocation

Allocation of individuals to intervention groups was done via random allocation method using closed envelopes. Numbered envelopes were provided to the participants, where a completely random number was selected by choosing an envelope by the participants. Then, a third person, other than the intervener and the questioner, who set up the list of treatment type in terms of numbers, referred to the relevant list to identify the patient's group.

2.6 Blinding

In this study, participants, gynecologist, questioner, and statistician were blind regarding the study groups, but it was not possible to blind the acupuncturist.

In order to enhance the blindness of participants, both placebo tablets and sham acupuncture were used. Participants received *Urtica dioica* tablets or placebo tablets, plus true or sham acupuncture. The study groups were separated and participants were prevented from interaction.

2.7 Treatment

After obtaining written informed consent, the participants were enrolled in the study and were randomly allocated to one of the following four groups: A. *Urtica dioica* tablets 450 mg (3 tablets)/day + Acupuncture 11 sessions; B. Placebo tablets + Acupuncture 11 sessions; C. *Urtica dioica* tablets 450 mg (3 tablets)/day + Sham Acupuncture 11 sessions; D. Placebo tablets + Sham Acupuncture 11 sessions. The treatment interventions lasted 7 weeks.

Participants in groups A and C received *Urtica dioica* in the form of Urtidine tablets (Barij Essence Co., Tehran, Iran) containing 150 mg of dried extract of standard *Urtica dioica* based on 22 mg of alanine amino acid. Participants in groups B and D received 3 placebo tablets per day, with the same shape, color, and weights as Urtidine tablets, supplied by Mashhad Faculty of Pharmacy.

According to LD50 (median lethal dose) of 1.721 g / kg to 1.929 g / kg of dried extract of *Urtica* as an intravenous injection in various studies³⁶ and the recommended dose of *Urtica* root in studies, we chose the minimum possible dose of 450 mg per day.^{23,37}

All participants received either acupuncture or sham acupuncture for 7 weeks; 2 sessions/week during the first 4 weeks of intervention, and one session/week during the remaining 3 weeks for 30 minutes in each session. Acupuncture needles were selected for treatment groups with the following specifications:

Groups A and B: disposable needles of 0.25 x 40mm (Suzhou, China)

Groups C and D: disposable needles of 0.25 x 25 mm needles (Suzhou, China)

Thirteen acupoints were selected based on Chinese Medicine resources, related articles, and the experiments of the faculty member acupuncturist who was skilled and experienced in the treatment of gynecological diseases (Table 1).^{29, 34} In each of the four groups, sterile gas was first applied on the acupoints. In the two groups receiving true acupuncture, the needle was passed through sterilized gas after reaching the skin surface and penetrated to a depth of 0.2-10 mm. Then, in order to reach the de qi sense, they were stimulated by hand. The de qi sense indicates the onset of the neural action potential in the nerves of the area and is felt which is expressed as a feeling of heaviness, numbness, stretchiness, fullness, and unpleasant pain like muscle fatigue. In the two groups receiving sham acupuncture, the needle was passed through the sterile gas and contacted with the skin in the same acupuncture points but returned to the top. It was then placed in the gas without touching the skin surface and without any manual stimulation to reach the de qi sensation. This sham design was adopted from a study by Liu et al.³⁸

The steps of the clinical trials were designed according to the Consolidated Standards of Reporting Trials (CONSORT) and statement of Revised Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA).

2.8 Outcomes

Primary outcomes included scores of hot flashes and MENQOL. The number and severity of hot flashes attacks were recorded on daily reports at weeks 1, 9, and 12.

The score of hot flashes was calculated by the number and severity of hot flashes based on the daily report form and VAS. Daily report along with hot flashes score is a valid and reliable method for evaluating hot flashes in studies.³⁹

To determine the MENQOL's scores, this questionnaire was completed by a questioner who was blind to the study groups before treatment and at the end of the treatment by asking from participants.

Secondary outcomes included FSH, LH, and Estradiol levels, which were measured at baseline and after the treatment (week 9) in order to determine hormonal changes. A blood sample of 4 ml was taken from all participants. The serum FSH, LH, and Estradiol was measured using Electrochemiluminescence (ECL), Cobas e 411, Roche, Germany. All participants were referred to a single laboratory, and a single device was used for hormonal assessment for all participants before and after the intervention.

2.9 Sample size calculation

Sample size was determined according to a study in 2011²⁹ and the formula of the two-sample t-test regarding a quantitative trait in two independent populations with an alpha equal to 0.05 and beta equal to 0.2, at least 15 individuals were selected per group. Considering the 10% drop rate, this number was increased to 17 people per each group.

2.10 Statistical method

SPSS version 16 was used for all statistical analyses. Normality of variables was investigated using one sample k-s test. Variables with normal distribution (MNEQOL score, ESTRADIOL levels) were introduced with mean and standard deviation. For examining the differences between the groups, ANOVA and Post hoc tests, and for before and after comparisons in each group, the paired t-test were used.

Non-normal variables (hot flases score, FSH, and LH levels) were introduced with median and interquartile range (IQR) indices. In order to examine the differences between groups, Kruskal-

Wallis, and Mann-Whitney test, and for pre-test and post-test comparisons in each group, Wilcoxon test were used.

The percentage of hot flashes score reductions in the 9th and 12th weeks compared to the baseline were calculated in the 4 groups. This method has previously been used in other studies.^{29, 40}

3. Results

Ninety participants were visited to check for inclusion and exclusion criteria. Eighteen participants did not meet the eligibility criteria. Seventy-two women were included and randomly allocated into four groups. The study flowchart is presented in Figure 1.

3.1 Participants' demographic information

The basic characteristics of participants are presented in Table 2. Study groups were similar in terms of basic characteristics.

3.2 Comparison of hot flashes scores among the studied groups

The median and IQR of hot flashes scores in the study time points and the differences are presented in Table 3.

The hot flashes score indicated a significant decline in the 9th week compared to the baseline in the A, B and C groups in the intergroup assessment, while the placebo group did not show a significant decrease. The change in hot flashes score in the 9th week from baseline was significant among study groups, which was due to the difference between groups A & D; B & D; and, C & D.

In the 12th week, hot flashes score in the A, B and C groups showed a significant decrease; while the D group did not show a significant change. There was a significant difference between the hot flashes score in the 12th week compared to the baseline among study groups which was due to the difference between A and D; B and D; and C and D groups.

Hot flashes score did not show any significant decline at the 12th week (follow-up visit) in comparison to 9th week (end of treatment) in the A, B and C groups; suggesting the stability of the treatment results for 4 weeks after treatment.

3.3 Comparison of MENQOL's score among the study groups

Mean scores of MENQOL were significantly reduced at week 9 from baseline in each of the four groups; the greatest decrease was observed in groups A, B and C, respectively (Table 3). The change in the mean MENQOL's score at week 9 from baseline was different among study groups ($P=0.001$). Post-hoc analysis suggested that this difference was significant in comparison between groups A and D; B and D; and C and D groups.

3.4 Comparison of the percentage of hot flashes score's reduction among the studied groups

The percentage of hot flashes score's reduction in the study groups is presented in Table 4.

The percentage of hot flashes score's reduction based on VAS at week 9 from 1 ($p = 0.001$), and at week 12 from 1 ($P<0.0001$) were significant. The Mann-Whitney analysis showed this difference was significant at week 9 and 12 compared to the baseline between A and D; B and D; and, C and D.

3.5 Comparison of sex hormones among the study groups

The mean FSH and LH did not change significantly at week 9 from baseline in any of the 4 groups (table 3). However, the difference in the mean of ESTRADIOL at week 9 from baseline was significant in groups B and C. The differences in the mean of FSH, LH and Estradiol were not significant at week 9 from baseline among study groups.

3.6 Blinding Test

At the end of intervention, all participants were asked whether they received real tablet or placebo; and true acupuncture or sham. Out of 16 people in group A, one individual reported that the drug and acupuncture were both placebo. From 16 people in group B, all reported that their medicine and acupuncture were real. Of 18 women in group C, all reported that their medicine and acupuncture were real. Of 18 women in group D, all said that their acupuncture was real and 2 people said that their tablets were placebo. Therefore, it seems that blinding was successful in this study.

3.7 Adverse events:

Adverse events and other feelings experienced by participants did not show any significant difference among study groups, except for increased energy and pain and ecchymosis (table 5).

4. Discussion

In this study, we investigated the effects of *Urtica dioica* and acupuncture separately and in combination based on hot flashes score and MENQOL's score compared to the control group receiving the placebo and sham acupuncture. At the end of the treatment, the hot flashes and MENQOL's scores were reduced by *Urtica dioica*, acupuncture, and combined therapy more than control compared with baseline; however, those treatments did not have any preference over each other. In the follow-up performed at week 12, the hot flashes score in the 3 treatment groups were better than placebo compared with baseline, however, there was no significant difference among interventions. Hormonal changes were not significantly different among the study groups.

The results of our study are comparable to the results of Nedeljkovic et al. in 2013.⁴¹ In their study, postmenopausal participants were treated for 4 weeks in four groups of acupuncture, sham acupuncture, CHM, and CHM placebo for 12 weeks and were followed for 12 weeks. The difference in scores of MRS in the acupuncture group was statistically significant in comparison to the sham acupuncture group and the herbal treatment group, but the herbal therapy group did not significantly differ with the placebo. Indeed, concerning acupuncture's superiority to sham acupuncture on menopausal symptoms, our study confirmed the results of the Nedeljkovic's study. In our study and Nedkovich's, the effect of acupuncture on menopausal symptoms was better than sham acupuncture, while most previous studies indicated that acupuncture did not excel sham acupuncture.^{29,40,42-44} Comparing acupuncture with herbal therapy, our study showed different results from Nedeljkovic's. In the study of Nedeljkovic, the effect of acupuncture was better than the herbal therapy, while in our study, acupuncture and *Urtica dioica* had similar effects on menopausal symptoms. The differences in results may refer to the different designs of

these studies. The design of our study seems to be better for comparison among herbal medicine and acupuncture, since the herbal medicine group received sham acupuncture, and the acupuncture group also received the placebo for herbal medicine in our study. This design minimizes the effect of placebo. Perhaps the lack of receiving sham acupuncture in the herbal treatment group was the reason for the acupuncture's superiority over herbal medicine in the Nedeljkovic's study. In addition to a better design, the sample size of our study was also larger than the Nedeljkovic's, rendering our study more reliable. The Nedeljkovic's study included all Chinese Medicine patterns for menopause and presented a pattern-based heterogenic treatment in the acupuncture group, which makes it difficult to judge the results due to the small sample size. However, in our study, only post-menopausal participants with the "Yin and Yang Deficiency pattern" were included. Therefore, although we used the TCM acupuncture like Nedeljkovic et al., the acupuncture points were the same in all participants in our study. In addition, the herb was different in our studies. Nedeljkovic et al. tested a combination of 14 herbal medicines under the name of CHM, while we used a single herb called *Urtica dioica*, which was able to reduce the symptoms of hot flashes better than placebo and as much as acupuncture.

Another comparable study is the study by same author Azizi et al, (2011) who examined the effect of an 8-weeks treatment by a combined therapy of acupuncture and herbal therapy versus herbal therapy alone and HT in premenopausal and post-menopausal women.⁴⁵ They found that the reduction of Kupperman index score was same by the combined therapy and HT, but better than herbal medicine alone. Differently, in the present study, combination therapy presented same effects as herbal medicine. The design of the present study makes it more reliable for comparing herbal medicine and acupuncture because of use of both sham acupuncture and placebo. There was no sham acupuncture and placebo in Azizi study (2011).

The largest acupuncture trial on hot flashes was the ACUFLASH study in 2007 in which 12 weeks of acupuncture treatment were compared with daily care in 267 participants with a 12 months follow up.³⁴ We used the results of this study to select among different Chinese Medicine patterns and acupuncture points. Since the most common pattern in the ACUFLASH study was Yin and Yang deficiency, we selected this pattern as an inclusion criterion. The most commonly used points in the ACUFLASH study were PC6, HT6, LI4, LU7, REN4, KI3, KI6, KI7, LR3, SP6, and ST36, which were also used in our study⁴⁶. We added 2 acupoints to the frequent points of the ACUFLASH study, because we selected the Yin and Yang deficiency syndrome. In the study of ACUFLASH, the difference in the MRS score in the acupuncture group was statistically significant ($p < 0.05$). Our study confirmed this result. The strength of our study design compared to ACUFLASH is the presence of a sham acupuncture group, although the sample size of our study was less than that of ACUFLASH. The number of acupuncture sessions in our study was almost the same as in the ACUFLASH study.

Changes in FSH and LH levels in the 9th week compared to baseline were not significantly different among groups. The difference of the estradiol level in the *Urtica dioica* and acupuncture groups was significant at the 9th week compared to baseline, although there was no significant difference among the 4 groups (Table 3). Reviewing previous studies suggested that a number of studies have indicated the suppressive effects of phytoestrogens on the hypothalamus axis,^{26,47,48} but some also express their ineffectiveness on the axis⁴⁹⁻⁵¹. Due to the lack of difference between the *Urtica dioica* group and placebo in reducing estradiol, our study confirms the mentioned second group of studies. Also, regarding the effect of acupuncture on the hypothalamus axis, some studies suggest suppression of the hypothalamus-hypophysis-ovarian axis,^{45,52,53} while

other studies indicate the lack of effect of acupuncture on the axis,^{27,54,55} which are in line with our study.

Considering our results suggesting the lack of effect of *Urtica dioica* and acupuncture on hypothalamic-hypophysis-ovarian axis hormones, other mechanisms seem to be involved to control hot flashes by these two interventions. Hot flashes are caused by dysfunction of the heat regulation at the hypothalamic level, which can develop with the estrogen's withdrawal.^{12,56} One of the major theories about hot flashes mechanism is that with reductions of estrogen concentrations in menopause, the concentration of endorphins in the hypothalamus decreases, leading to increased serotonin release and norepinephrine, followed by a drop in the set point of the hypothalamus's heat regulator, leading to heat dissipation or hot flashes.^{12,56} The withdrawal effect of estrogen on the hypothalamus is the narrowing of the temperature regulation center, where even a slight rise in temperature increases the activity of the norepinephrine of the brain and potentiates the sympathetic response of the body to generate heat.³⁰ Acupuncture probably affects and regulates the activity of serotonin and β -endorphins in the central nervous system on the heat regulation center of the hypothalamus.^{27,28} The *Urtica dioica* contains antioxidant components such as flavonoids and phytoestrogens such as beta-Sitosterol and lignins.²³ Various studies have shown the effectiveness of antioxidants and phytoestrogens in improving hot flashes.²¹

One of the strengths of our study was the creative design of four groups with sham and placebo content. All 4 groups referred to the clinic twice a week to receive the treatment, and all individuals contacted the intervener at the same frequency. Further, all four groups referred to the same intervener and were evaluated in terms of symptoms and possible complications. In our study, the effect of *Urtica dioica* was compared with the placebo, and the effect of acupuncture

was compared with sham acupuncture. The design also let it possible to compare acupuncture with the herb by maintaining the blinding of participants.

Individuals included in our study were all selected from one Chinese Medicine pattern for menopause. This design led to more similarity among study groups compared to studies in which several acupuncture protocols were used according to different traditional patterns. It also suggested the possibility of evaluating a traditional treatment within a modern methodology. We did not include individuals with a history of receiving acupuncture treatment. This contributed to the proper implementation of blinding in this study, which enhances the reliability of our results.

Limitations

One of the shortcomings of our study was the use subjective outcomes; the frequency and severity of hot flashes were determined by participants. Adding some objective outcomes may result in better evaluation and increased reliability of results in future studies. The failure to conduct pre/post urine analyses for concentrations of the herbal agent is also a limitation.

Furthermore, the duration of the follow-up was relatively short in our study; longer follow-ups are required to ensure the therapeutic outcomes. Another potential limitation for data interpretation would be the pulsatility effects of hormonal levels at pre- and post-menopausal period. The assessment of other estrogen components such as estriol and estrone would also be considered as a limitation.

Conclusions

In this study, *Urtica dioica* decreased menopausal hot flashes and increased the quality of life of postmenopausal women better than placebo-sham control but same as acupuncture. The combination of *Urtica dioica* and acupuncture did not add to the effects of those therapies.

Authors' contributions

Conception and design of the clinical trial: Hoda Azizi, Rahele Kargozar, Roshanak Salari

Analysis: Lida Jarahi

Quality assessment of the clinical trial: Hoda Azizi, Rahele Kargozar, Roshanak Salari, Seyedeh Azam Pourhosseini, Mahdi Yousefi, Monirsadat Sahebkar Khorasani

Writing of the paper: Rahele Kargozar, Hoda Azizi, All authors read and approved the final version of the manuscript.

Conflicts of interest: none.

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Legends

Figure 1: CONSORT flow chart of the study

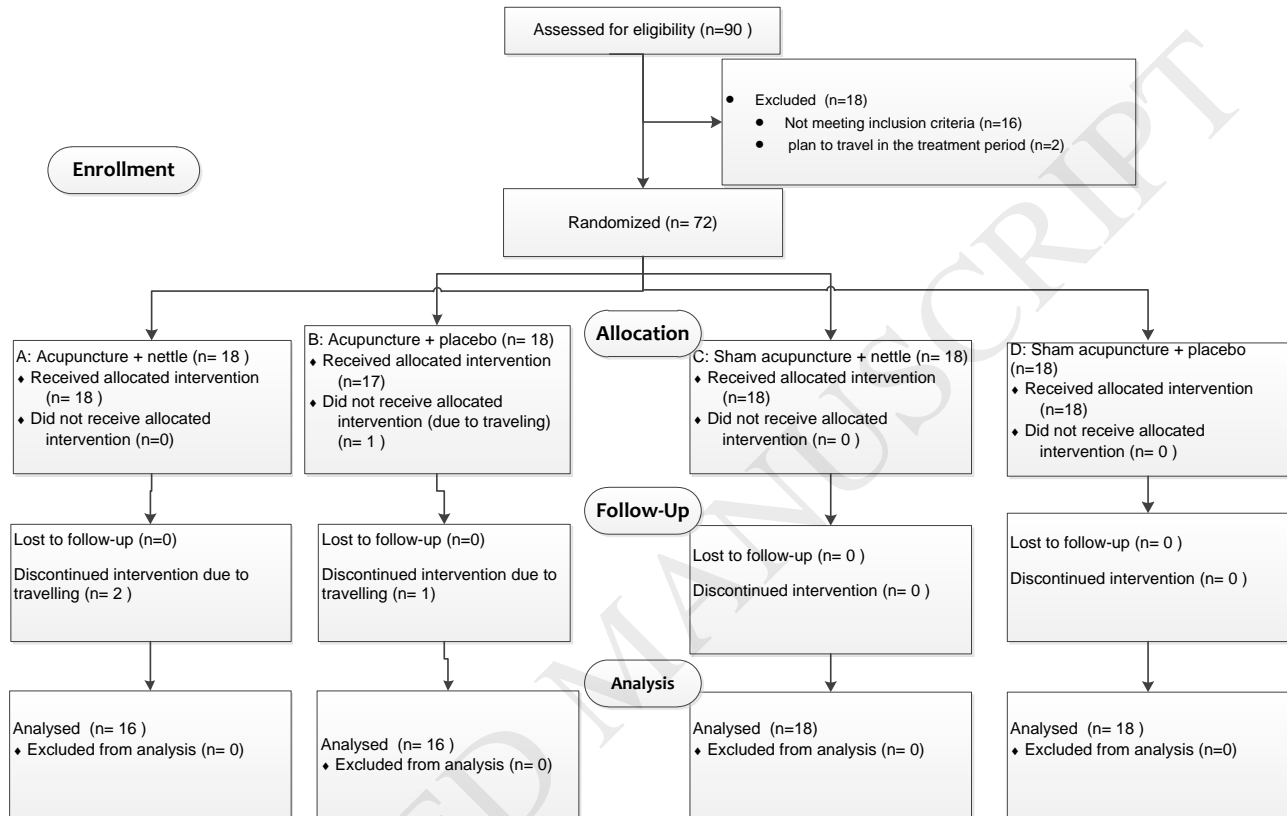


Figure 1: CONSORT flow chart of the study

Table 1. Selected acupoints for the treatment of menopausal hot flashes and other menopausal symptom

Point	location
PC6	2 cun above the transverse crease of the wrist, between the tendon of musculus palmaris longus and musculus flexor radialis.
HT6	The point is on the radial side of the tendon of the flexor carpi ulnaris. 5 cun above the transverse crease of the wrist.
HT7	At the ulnar end of the distal wrist crease when the palm faces upward, on the radial side of flexor carpi ulnaris tendon.
LI4	On the dorsum of the hand, between the first and second metacarpal bones, approximately in the middle of the second metacarpal bone.
LU7	1.5 cun proximal to the most distal skin crease of the wrist, proximal to the styloid of the radius in a depression between the tendons of brachioradialis and abductor pollicis longus.
REN4	On the midline 3 cun below the umbilicus
KI3	In the depression between the medial malleolus and the Achilles tendon, level with the tip of the medial malleolus.
KI6	1 cun inferior to the tip of the medial malleolus, in a depression formed by two ligamentous bundles.
KI7	On the medial aspect of the lower leg, 2 cun directly above KID 3, anterior to tendo calcaneus.
LR3	Just distal to the junction of the bases of the 1st and 2nd metatarsals.
SP6	3 cun directly superior to the tip of the medial malleolus on the posterior border of the tibia.
ST36	On the lateral side of the shank, one finger breadth from the anterior crest of the tibia.
DU20	At the vertex, on the midline, 5 cun posterior to the anterior hairline.

Table 2. Demographic characteristics of the study groups

characteristic		Group A N=16	Group B N=16	Group C N=16	Group D N=16	P- value
Marital status No. (%)	Married	14(87.5)	14(87.5)	18(100.0)	14(77.8)	0.454
	Single	0(0.0)	1(6.2)	0.0	1(5.6)	
	widow	2(12.5)	1(6.2)	0.0	3(16.7)	
Education No. (%)	<=diplom	13(81.2)	14(87.5)	11(61.1)	16(88.9)	0.148
	daneshgah	3(18.8)	2(12.5)	7(38.9)	2(11.1)	
Job No. (%)	Housewife	16 (81.2)	12(75.0)	14(77.8)	17(94.4)	0.444
	Employee	3(18.8)	4(25.0)	4(22.2)	1(5.6)	
Age of menopause Mean (SD)		48.9±3.6	48.6±3.7	45.8±12.7	47.0±12.6	0.741
Age of menarche Mean (SD)		13±1.8	13.4±1.9	14.0±1.7	13.5±1.2	0.323
Satisfaction with marital life No. (%)	Yes	12(75.0)	15(93.8)	15(83.3)	11(61.1)	0.128
	No	4(25.0)	1(6.2)	3(16.7)	7(38.9)	
Age Mean (SD)		53.6±2.9	52.5±5.5	51.8±4.0	54.1±3.0	0.265
BMI Mean (SD)		27.6±3.8	32.6±3.5	27.3±3.4	27.7±4.1	0.347
Children Number Mean (SD)		2.93±1.2	2.93±1.4	3.22±1.5	4.11±2.3	0.149
Salary No. (%)	low	4(0.25)	3(18.8)	5(27.8)	5(27.8)	0.709
	moderate	12(0.75)	13(81.2)	13(72.2)	13(72.2)	
Exercise No. (%)	No or few	5(31.2)	6(37.5)	12(66.7)	11(61.1)	0.175
	Moderate	4(25.2)	2(12.5)	3(16.7)	4(22.2)	
	Frequent	7(43.8)	8(50.0)	3(16.7)	3(16.7)	

Table 3. Primary and secondary outcomes in the study groups

	Group A N=16	Group B N=16	Group C N=18	Group D N=18	P Value
Hot flashes score at wk1, median (IQR)	33.5 ±61.8	29.6±15.3	24.3±19.7	21.2±21	0.402
Hot flashes score at wk9, median (IQR)	2.9±24.7	11.6±28.3	6.7±14.4	17.1±31.1	0.166
Change in hot flashes score at wk9 from wk1 median (IQR), within group comparison p value	-20.2±31.7 ;0.001	-19±18; 0.013	-14.6±25.4; 0.000	-1.6±11.6;0.381	<0.0001
Mean (SD)	-33.4±35.8	-14.0±19.0	-18.7±12.2	-0.3±13.3	
Hot flashes score at wk12, median (IQR)	0.5±5	8.9±35.4	6.2±8.4	18.9±29.8	0.012
Change in hot flashes score at wk12 from wk1 Mean (SD)	-32.9±35.8	-11.7±24.8	-20.8±13.0	1.0±13.3	
median (IQR), within group comparison p value	-21.1±25.1;0.001	-17.3±27;0.050	-15±24;0.000	-0.2±13.4;0.981	<0.0001
Change in hot flashes score at wk12 from wk9 Mean (SD)	0.5±14.8	2.4±13.4	-2.0±3.7	1.3±7.0	
median (IQR) ,	-0.1±2.5;0.133	0.0±4.3;0.638	-0.6±4.7;0.079	0.0±9.6; 0.433	0.323

within group comparison p value					
MENQOL score at wk1, Mean (SD)	82.7±28.0	90.6±36.3	94.4±32.0	91.9±32.3	0.763
MENQOL score at wk9, Mean (SD)	40.0±20.2	49.9±26.5	56.7±28.8	77.7±37.5	0.038
Change in MENQOL score at wk9 from wk1 Mean (SD), within group comparison p value	-42.6±21.1;0.000	-40.7±29.8;0.000	-37.8±26.8;0.000	-9.8±14.3;0.029	0.001
FSH at wk1, Mean (SD)	56.9±27.9	55.4±25.0	65.4±36.9	53.2±24.9	0.851
FSH at wk9, Mean (SD)	71.4±31.5	69.6±27.1	75.0±32.5	53.7±19.4	0.147
Change in FSH at wk9 from wk1 Mean (SD), within group comparison p value	14.5±20.5;0.13	14.3±25.0;0.37	9.6 ±29.1;0.18	0.5±10.8;0.854	0.297
LH at wk1, median (IQR)	41.2±11.2	36.3±21.8	42.1±20.7	32.8±22.2	0.107
LH at wk9, median (IQR)	44.0±18.0	36.2±13.8	42.3±14.4	34.1±11.6	0.266
Change in LH at wk9 from wk1 Mean (SD), within group	1.4±9.6;0.572	3.7±7.0;0.5	0.5±13.0;0.876	1.4 ±6.5;0.368	0.955

comparison p value					
Estradiol at wk1, median (IQR)	13.0±15.5;0.660	11.0±13.8;0.035	8.5±8.7;0.044	1.0±4.9;0.188	0.057
Estradiol at wk9, median (IQR)	8.0±23.0	3.0±23.0	1.0±4.7	1.0±8.0	0.033
Change in Estradiol at wk9 from wk1					
Mean (SD)	-1.0±10.7	-14.4±29.1	-5.6±14.9	-5.7±22.7	
median (IQR) , within group comparison p value	-1.5±14.0;0.660	-5.5±16.6;0.035	-4.7±12.0;0.044	-4.7±12.0;0.188	0.461
Hot flashes reduction session, Median, (IQR)	4.5 ±2.0	4.0±4.0	4.0±2.0	4.0±4.3	0.49

MENQOL denotes Menopausal Quality of Life

Table 4. Hot flashes frequency reduction, as percentage of baseline at 9 and 12 weeks among study groups

	Group A N=16	Group B N=16	Group C N=18	Group D N=18	P Value
Hot flashes reduction percent at Wk 9,% (IQR)	82.2(37.3)	62.7(90.7)	68.5(38.4)	8.2(73.6)	0.001
Hot flashes reduction percentage at Wk12,% (IQR)	97.4(16.4)	74(89.4)	70(15.2)	0.5(65.0)	<0.0001

Table 5. Adverse events, patients self-report of experiences and satisfaction rate

	Group A	Group B	Group C	Group D	P value
Ecchymosis, No (%)	6(37.5)	7(43.8)	0(0.0)	0(0.0)	0.001
Bleeding at acupoint, No (%)	2(12.5)	3(18.8)	0(0.0)	0(0.0)	0.088
Pain at acupoint, No (%)	5(31.3)	5(31.3)	0(0.0)	1(5.6)	0.016
Dizziness, No (%)	1(6.3)	2(12.5)	1(5.6)	1(5.6)	0.844
Light-headedness, No (%)	1(6.3)	0(0.0)	0(0.0)	0(0.0)	0.348
Worsening of hot flashes, No (%)	0(0.0)	0(0.0)	0(0.0)	2(11.1)	0.126
Mood recovery	14(87.5)	13(81.3)	13(72.2)	8(44.4)	0.108
Appetite improvement, No (%)	6(37.5)	5(31.3)	8(44.4)	2(11.1)	0.159
Relaxation, No (%)	14(87.5)	14(87.5)	13(72.2)	9(50.0)	0.135
Increase in energy, No (%)	13(81.3)	12(75.0)	11(61.1)	5(27.8)	0.032
Vaginal Bleeding, No (%)	0(0.0)	0(0.0)	2(11.1)	1(5.6)	0.32
Patient	7.8±2.8	7.9±2.5	7.16±2.5	6.9±2.7	0.505
Satisfaction					
VAS^a, mean (SD)					

^aPatient Satisfaction VAS rated from 1 to 10.