

THE EFFECTS OF EXTRACTS OF *ATRACTYLODES MACROCEPHALA* KOIDZ COMBINED WITH
TRANSCUTANEOUS ELECTRICAL ACUPOINT STIMULATION IN TREATING THE
OVARIECTOMIZED FEMALE RATS

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Abstract

Background: The present study was to explore the effects of the extracts of *Atractylodes macrocephala* Koidz (EAMK) combined with transcutaneous electrical acupoint stimulation (TEAS) on the ovariectomized female rats.

Materials and Methods: Forty female Sprague-Dawley rats were randomly divided into four groups: sham-operation, model, livial and EAMK+TEAS groups (n=10 in each group). After the individual treatments for 8 weeks ended, the serum levels of estradiol (E2), follicle stimulating hormone (FSH), luteinizing hormone (LH), osteocalcin and interleukin-6 (IL-6) were measured with commercial enzyme-linked immunosorbent assay.

Results: We found that in the sham-operation group, the serum E2 and osteocalcin levels were significantly higher, and the serum levels of FSH, LH and IL-6 were markedly lower than those of other groups (P<0.05). No significant differences existed between the livial and EAMK+TEAS groups on the serum E2 and osteocalcin levels (P>0.05), however, the serum FSH and IL-6 levels of EAMK+TEAS group were significantly lower than those of the livial group (P<0.05).

Conclusion: EAMK combined with TEAS has promises in treating the ovariectomized female rats.

Key words: Extracts of *Atractylodes macrocephala* Koidz (EAMK), transcutaneous electrical acupoint stimulation (TEAS), osteocalcin, interleukin-6 (IL-6).

Introduction

Menopause is the time of life when menstrual cycles cease, which is caused by the reduced secretion of the ovarian hormones (Nelson, 2008). In Asian menopausal women, the physical symptoms were the most menopausal prevalent symptoms compared to psychological, vasomotor and sexual symptoms (Islam et al., 2014). Some menopausal women chose to seek medical advice for management of the menopausal symptoms (Nelson, 2008).

In recent years, more and more menopausal women have chosen complementary and alternative medicine, especially Chinese medicine, for relief from their menopausal symptoms (Posadzki et al., 2013). The phytoestrogens were found to effectively alleviate the frequency of hot flushes in menopausal women without serious side-effects (Chen et al., 2014). The authors' group have also demonstrated that Chinese herbs may be a useful alternative treatment for the women suffering from menopausal symptoms, who are unable or do not want to receive hormone replacement therapy (HRT) (Qu et al., 2009; Zhou et al., 2007). As an important non-pharmaceutical treatment, acupuncture exhibited a significant improvement in the perimenopausal symptoms in women who have had an oophorectomy, although the conclusion may have been influenced by a potential bias and the small sample size (Qu et al., 2007). However, whether Chinese medicinal plant combined with acupuncture possesses more markedly positive effects in alleviating menopausal symptoms is still unknown.

Atractylodes macrocephala Koidz (AMK) is a commonly used Chinese medicinal plant in China and most of the other Asian countries (Lee et al., 2007). Transcutaneous electrical acupoint stimulation (TEAS) is an innovative and non-invasive acupuncture treatment, which is a new development of traditional Chinese acupuncture under the advancement of modern science (Zhang et al., 2014; Zhang et al., 2011). In the present study, we will explore the effects of the extracts of AMK (EAMK) combined with TEAS on the ovariectomized female rats.

Materials and Methods

Materials

The plant materials of *Atractylodes macrocephala* Koidz were obtained from Tiantai, Zhejiang Province, China. **The roots of the plants were used.** Identification of the plant species was verified by a fellow botanist. The plant materials were firstly thoroughly washed and dried at 40°C for 3 days before use. Preparation of the standardized extract, EAMK, was carried out at a Good Manufacturing Practice facility. Briefly, the AMK powder was extracted three times with 70% ethanol by sonication for 0.5 h, followed by rotary evaporation at 4°C under reduced pressure. The ethanol extract was then loaded onto a Diaion HP-20 open column (Sigma Aldrich) and sequentially eluted with a methanol gradient beginning with 100% water and 30%, 65%, and finally 80% methanol.

Animals, Group and Administration

The Laboratory Animal Center of Zhejiang University (Hangzhou, China) provided forty ten-week-old female Sprague-Dawley (SD) rats (weighing 220±20g). The animals were kept in a room under a 12h light - 12h dark cycle and environmentally controlled conditions of 22±2°C. The research was carried out according to the National Research Council's protocol for the care and use of laboratory animals. The rats were randomly divided into four groups: sham-operation group, model group, livial group and EAMK+TEAS group with ten rats in each group. All of the rats were fed with standard pelleted food and plain tap water ad libitum. For each rat in the sham-operation group, after the abdominal fur was shaved and the skin disinfected, the abdominal cavity was opened and then closed. The sham-operation group was used to insure the aseptic conditions. The rats of model group, livial group and EAMK+TEAS group were bilaterally ovariectomized as described by El-Bakri et al (El-Bakri et al., 2004). All of the rats were anesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbital (50 mg/kg). The vaginal smear was taken from each of the rats once daily for six consecutive days from the 3rd day after the ovariectomized operation. All the ovariectomized rats were successfully established, confirmed by predominantly leukocytes with few epithelial cells in the vaginal smears over at least 4 days. The rats in the sham-operation and model groups were orally administrated water (10ml/kg) once daily for eight consecutive weeks. The rats in the livial group were orally administrated livial (Tibolone, made by Nanjing Oujianong Pharmaceutical Co., Ltd, Nanjing, China) at 1.2 mg/kg dissolved in water once daily for eight consecutive weeks. The rats in EAMK+TEAS group received the combined treatments of EAMK and TEAS for eight consecutive weeks. They were orally administrated with the EAMK (0.3g/kg) dissolved in water once a day. In the TEAS treatment, the acupoints: Guanyuan (RN4), Zhongwan (RN12) and Sanyinjiao (SP6) were applied. The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) to minimize any restraint stress before TEAS treatment. TEAS were applied to rats through self-adhesive surface electrodes using a TEAS device (HANS-200A, Nanjing Jisheng Medical Technology Co., Ltd, Nanjing, China). The electrodes were cut into 5-mm squares and applied to the denuded skin. The TEAS treatment lasted for 30 min. The stimulation parameters were set as a frequency of 2/100 Hz and an intensity of 5-25 mA, strong enough to elicit visible muscle contraction. The TEAS treatment was taken once every day for consecutive 8 weeks. One rat of the model group died during the above treatment period.

The Collection and Measurement of the Samples

After the above 8-week treatment ended, each of the rats was anaesthetized with urethane (1.2 g/kg, intraperitoneally) after

fasting for 12 h. The blood samples were taken from the hepatic portal vein into the heparinized injectors, which were then centrifuged at 3000 rpm in 4°C for 10 min. The supernatant serums were transferred to clean EP tubes and stored at -80°C until assay. After the samples were collected, the rats were sacrificed. The serum levels of estradiol (E2), follicle stimulating hormone (FSH), luteinizing hormone (LH), osteocalcin and interleukin-6 (IL-6) were measured with commercial enzyme-linked immunosorbent assay kits (E2, FSH, LH and IL-6 by R&D Systems, MN, USA; osteocalcin by Pu-Zheng Biotech, Shanghai, China). All the measurements were performed in duplicate and conducted according to the manufacturer's instructions. Intra and inter assay coefficients of variation were <10%.

Statistical Analysis

The results were analyzed with Statistical Package for Social Sciences (SPSS 15.0 for Windows). Analysis of variance (ANOVA) was employed for analyzing all the data. A 5% significance level ($P < 0.05$) and two-tailed tests were used for all hypothesis tests.

Results

The Serum Levels of E2, FSH and LH

In the sham-operation group, the serum E2 levels were significantly higher, and the serum FSH and LH levels were markedly lower than those of other groups ($P < 0.05$) which were shown as Figure 1. In the livial and EAMK+TEAS groups, the serum E2 levels were significantly higher, and the serum FSH levels were significantly lower than those of the model group ($P < 0.05$). No significant differences existed between the livial and EAMK+TEAS groups on the serum E2 levels ($P > 0.05$), however, the serum FSH levels of EAMK+TEAS group were significantly lower than those of the livial group ($P < 0.05$). There were no significant differences on the serum LH levels among the model, livial and EAMK+TEAS groups ($P > 0.05$).

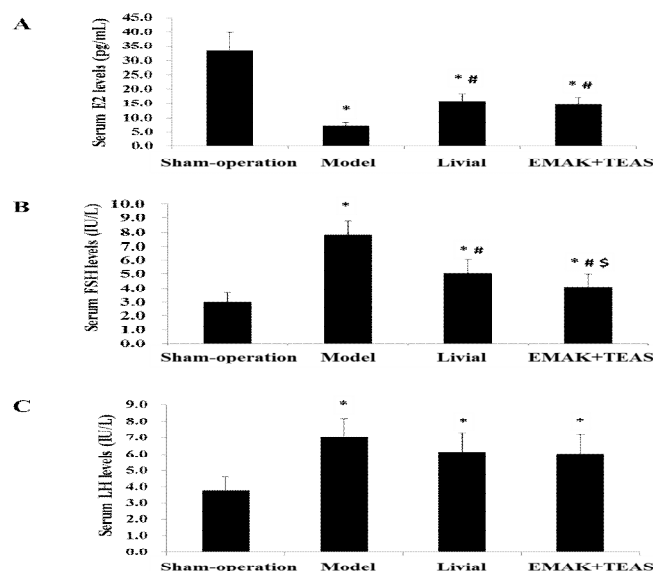


Figure 1: The serum levels of sex hormones. (A) The serum levels of estradiol (E2); (B) the serum levels of follicle stimulating hormone (FSH); (C) the serum levels of luteinizing hormone (LH). Sham-operation, sham-operation group; Model, the ovariectomized model group; Livial, livial group; EAMK+TEAS, the combined treatment of the extracts of *Atractylodes macrocephala* Koidz (EAMK) and transcutaneous electrical acupoint stimulation (TEAS) group. Data are presented as the mean \pm

standard deviation (n=10, 9, 10 and 10 in the sham-operation, model, livial and EAMK+TEAS groups respectively). P<0.05 was considered to indicate a statistically significant difference. *P<0.05, compared with the sham-operation group (analysis of variance); #P<0.05, compared with the model group (analysis of variance); §P<0.05, compared with the livial group (analysis of variance).

The Serum Levels of Osteocalcin

As shown in Figure 2, the serum osteocalcin levels of sham-operation group were significantly higher than those of the other groups (P<0.05). The serum osteocalcin levels of the livial and EAMK+TEAS groups were significantly higher than those of the model group (P<0.05) and no significant differences existed between the livial and EAMK+TEAS groups (P>0.05).

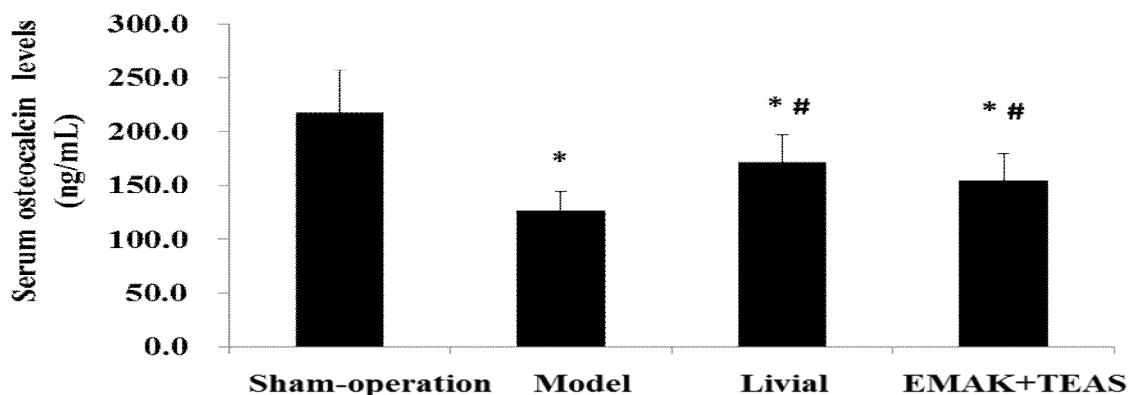


Figure 2: The serum levels of osteocalcin. Sham-operation, sham-operation group; Model, the ovariectomized model group; Livial, livial group; EAMK+TEAS, the combined treatment of the extracts of *Atractylodes macrocephala* Koidz (EAMK) and transcutaneous electrical acupoint stimulation (TEAS) group. Data are presented as the mean ± standard deviation (n=10, 9, 10 and 10 in the sham-operation, model, livial and EAMK+TEAS groups respectively). P<0.05 was considered to indicate a statistically significant difference. *P<0.05 compared with the sham-operation group (analysis of variance); #P<0.05, compared with the model group (analysis of variance); §P<0.05, compared with the livial group (analysis of variance).

The Serum IL-6 Levels

As shown in Figure 3, the serum IL-6 levels of sham-operation group were significantly lower than those of the other groups (P<0.05). The serum IL-6 levels of the livial and EAMK+TEAS groups were significantly lower than those of the model group (P<0.05). The serum IL-6 levels of EAMK+TEAS group were significantly lower than those of the livial group (P<0.05).

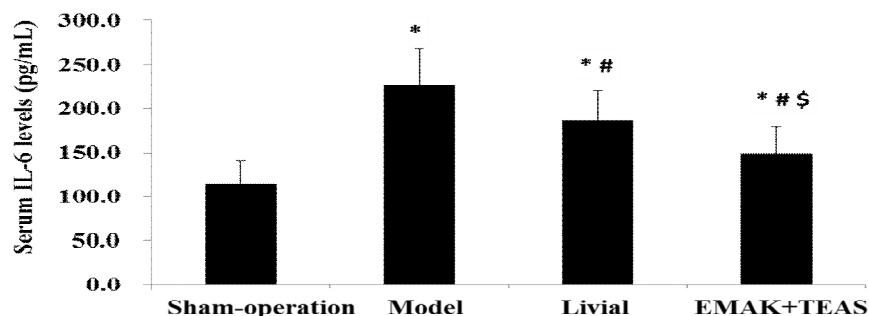


Figure 3: The serum levels of interleukin-6 (IL-6). Sham-operation, sham-operation group; Model, the ovariectomized model group; Livial, livial group; EAMK+TEAS, the combined treatment of the extracts of *Atractylodes macrocephala* Koidz (EAMK) and

transcutaneous electrical acupoint stimulation (TEAS) group. Data are presented as the mean \pm standard deviation (n=10, 9, 10 and 10 in the sham-operation, model, livial and EAMK+TEAS groups respectively). $P < 0.05$ was considered to indicate a statistically significant difference. * $P < 0.05$ compared with the sham-operation group (analysis of variance); # $P < 0.05$, compared with the model group (analysis of variance); $^{\S}P < 0.05$, compared with the livial group (analysis of variance).

Discussion

As we know, estrogen has been used to alleviate the menopausal symptoms in clinical practice for more than six decades. However, some menopausal women were often confused by the different opinions of the risks and benefits of hormone replacement therapy (HRT) (Pardini, 2014). The risk-benefit balance of HRT needs to be individualized for each woman (Pardini, 2014). In a study to explore the use and attitude toward HRT in Chinese women, the researchers found, for most of Chinese women, the awareness of HRT was poor, which was influenced by menopausal, working and marital status (Jin et al., 2015). Chinese medicine, including acupuncture, has significant advantages in treating menopausal symptoms (Baccetti et al., 2014; Qu et al., 2009; Qu et al., 2007; Scheid et al., 2015; Zhou et al., 2007). A pilot study to investigate the effects of Chinese medicine on London women, who suffered from menopausal symptoms, also found that the patients showed significant improvement across all domains measured by the menopause specific quality of life questionnaire and Greene climacteric scales, and no adverse events were found (Scheid et al., 2015). In a recent trial, acupuncture was found to significantly alleviate the occurrence of hot flushes, sudden sweating, sleep disorders, tightness in the chest, irritability, bone pain and depression of the menopausal women (Baccetti et al., 2014).

AMK is a widely prescribed tonic Chinese medicinal plant and the authors' group has ever demonstrated that the bioactive ingredients of AMK were closely related to the ecological factors (Zhou et al., 2011). The EAMK can inhibit the movement of uterus smooth muscle through inhibiting the cholinergic system and the Ca^{2+} movement (Zhang et al., 2000). AMK and its derivate can also be used as potential aromatase inhibitors and the oral administration of AMK protein sample was found to suppress the ovalbumin-mediated allergic diarrhea by preferential stimulation of the Th1-type immune responses (Jiang et al., 2011; Kim et al., 2005).

In the study, besides the female hormones, IL-6 and osteocalcin were chosen as the parameters to evaluate the effects of EAMK combined with TEAS treatment on the ovariectomized female rats. As a pleiotropic cytokine, IL-6 is produced by the immune as well as the nonimmune cells and the mechanisms underlying the effects of HRT can be attributed to the immunomodulation properties, which seems to restore the cytokine homeostasis in the postmenopausal women (Rachon, 2005). The circulating IL-6 levels were interactively affected by menopause, and the estrogen deprivation after menopause may increase the cytokine production of the peripheral blood mononuclear cells, which leads to the higher IL-6 levels (Kim et al., 2012). The serum level of IL-6 is a predictor of postmenopausal bone loss, which appears to be the most relevant through the first postmenopausal decade (Scheidt-Nave et al., 2001). The serum levels of IL-6, sIL-6R and sgp130 were found to exhibit different patterns of age- and menopause-related changes, and the IL-6 activity may be increased with the progress of the age-related osteoporosis (Giuliani et al., 2001). Osteocalcin is a specific bone marker of the early post-menopause in healthy women (Lukacs et al., 2006). We found in the present study that EAMK combined with TEAS treatment and the livial treatment had similar effects in significantly increasing the serum levels of E2 and osteocalcin, however, the EAMK combined with TEAS treatment exhibited more significant effects in regulating the serum FSH and IL-6 levels of the ovariectomized female rats. The present study showed that EAMK combined with TEAS has eminently positive effects in treating the ovariectomized female rats. Further studies should be focused on the underlying transcriptional regulation mechanism.

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