Efficacy of moxibustion by stimulating acupoints of Danzhong (CV 17) and Ganshu (BL 18) on hyperplasia of mammary gland in rats

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Abstract

OBJECTIVE: To evaluate the efficacy of moxibustion, through stimulating acupoints of Danzhong (CV 17) and Ganshu (BL 18) in rats with hyperplasia of mammary gland (HMG) which induced by estrogen and progestogen.

METHODS: Thirty female Sprague-Dawley rats were randomly divided into saline control group, HMG model group, and HMG moxibustion group with 10 in each group. Saline control was the group injected by saline. HMG model were created by injection of estrogen and progestogen. Moxibustion group was also injected of estrogen and progestogen with moxibustion at the same time. The Changes of nipple diameter and height were measured. The rats’ skin temperature was recorded by an infrared thermal camera at the nipples, mammary areas, Danzhong (CV 17) and Ganshu (BL 18). Pathological changes of mammary gland in rats were also observed under light microscope.

RESULTS: The diameter and height of the nipples in model group were prominently bigger and higher than that in control group ($P < 0.01$). The diameter and height in moxibustion group were prominently smaller and lower than that in model group ($P < 0.01$), and there was no significant difference between moxibustion group and control group. Compared with control group, skin temperature of the nipples, mammary area, and acupoints Danzhong (CV 17) and Ganshu (BL 18) decreased prominently in model group ($P < 0.01$-$0.05$). Compared with model group, skin temperature of that in moxibustion group increased prominently ($P < 0.05$).

CONCLUSION: Treatment with moxibustion can effectively decrease the HMG rats' nipple diameter and height, and increase the skin temperature in HMG model rats at the nipples, mammary areas, Danzhong (CV 17) and Ganshu (BL 18). This study convinces the therapeutic effect of moxibustion on mammary gland hyperplasia.

Keywords: Moxibustion; Fibrocystic disease of breast; Skin temperature; Thermography; Point CV17 (Tanzhong); Point BL18 (Ganshu)
Hyperplasia of mammary gland (HMG) is one of the most common benign breast diseases for middle-aged women. The morbidity of HMG is increasing in recent years, and it has a high tendency to develop into mammary carcinoma. However, HMG is easily to be neglected because more attention has been payed to breast cancer. Nowadays, main treatments for HMG are surgery and hormone drug therapies. But the side effects of these therapies severely affect the life quality of HMG patients such as menstrual disorder, neuropsychiatric symptom, headache or vertigo. Therefore, it is important to find more convenient therapies with prolonged benefits and less side effects for HMG patients.

Reports have suggested that Traditional Chinese Medicine (TCM) could improve the regulatory mechanism in the body to inhibit the HMG. Moxibustion is a famous Chinese traditional medical therapy, which applies the ignited mugwort (Artemisia vulgaris from Traditional Chinese Medicine) directly or indirectly at acupuncture points or other specific parts of the body to cure disease. Previous clinical and experimental studies have shown that moxibustion has been used for various conditions including stroke rehabilitation, rheumatoid arthritis, chronic fatigue syndrome, Crohn’s disease, ulcerative colitis, irritable bowel syndrome, and HMG. Moxibustion is used in the management of those diseases by involving the nervous system, endocrine system, and immune system.

In this study, we select the main points for treating HMG-Danzhong (CV 17) and Ganshu (BL 18). The anti-hyperplasia effect of moxibustion on HMG rats induced by estrogen and progestogen was evaluated. The changes of nipple diameter and height were measured. The rats’ skin temperature was recorded by an infrared thermal camera at the nipples, mammary areas, Danzhong (CV 17) and Ganshu (BL 18). Pathologic changes of mammary gland were also observed under light microscope.

All our researches have been finished in the Level-III Acupuncture Biological Laboratory under State Administration of Traditional Chinese Medicine of China and Key Research Laboratory for Assessment of Therapeutic Characteristics of Acupuncture under State Administration of Traditional Chinese Medicine of China.

MATERIALS AND METHODS

Moxibustion apparatus

Moxibustion apparatus was bought from Beijing Zhongyan Taihe Medicine Co., Ltd. The indirect moxibustion apparatus consists of a cardboard tube (height 3 mm, diameter 12 mm), the top of which is a moxa cylinder (height 8 mm, diameter 7 mm, dried leaves of Artemis vulgaris). The bottom of the tube is adhesive and can be attached firmly to the skin surface.
ternate day (a total of 15 moxibustion treatments), three moxibustion apparatus for one acupoint every time, moxibustion started on the day following estrogen injection.

![Figure 2 Moxibustion at Danzhong (CV 17) and Ganshu (BL 18) A: the location of Danzhong (CV 17); B: the location of Ganshu (BL 18).](image)

**Statistical analysis**
All values were expressed as the mean ± standard division (̅x ± s). Significant differences between the groups were statistically analyzed using a one-way analysis of variance, followed by least significant difference method test. SPSS 17.0 software (SPSS, Inc, Chicago, USA) was used for statistical analysis. *P* < 0.05 was considered statistically significant.

**RESULTS**

**Nipple diameter and height**
The diameter and height of the nipples were significantly increased in HMG model group compared to the saline control group (*P* < 0.01). After moxibustion treatment, the diameter and height of the nipples were significantly decreased in moxibustion group compared with the HMG model group (*P* < 0.01) (Figure 3).

**Skin temperature of the second and third pair nipples**
The skin temperature of the second and third pair nipples was significantly decreased in model group compared to the saline control group (*P* < 0.01). After moxibustion treatment, skin temperature of the second and third pair nipples was significantly increased in moxibustion group compared with the HMG model group (*P* < 0.05-0.01) (Figures 4A, 6).

**Skin temperature of the second and third pair mammary areas**
Skin temperature of the second and third pair mammary areas was significantly decreased in HMG model group compared to the saline control group (*P* < 0.05). After moxibustion treatment, skin temperature of the second and third pair mammary areas was significantly increased in moxibustion group compared with the HMG model group (*P* < 0.01) (Figure 4B).

**Skin temperature of acupoints Danzhong (CV 17) and Ganshu (BL 18)**
Skin temperature of acupoint Danzhong (CV 17) and bilateral Ganshu (BL 18) was decreased significantly in HMG model group compared to the saline control group (*P* < 0.01). After moxibustion treatment, skin temperature of acupoint CV17 was significantly increased in moxibustion group compared with the HMG model group (*P* < 0.01) (Figure 5).

**Pathological examination**
Histopathological examination of mammary gland in saline control group showed tremendous loose connective tissue, less acinar, less enclosed mammary ducts, and without mammary ducts secretion (Figure 7A, B). The pathological manifestation in HMG model group was prominent mammary gland hyperplasia, enlarged mammary ducts, less loose connective tissues, mammary ducts ectasia with secretion, expansion of mammary

**Histopathological observations**
At the end of experiments, all the rats were sacrificed. Subsequently, the second pair mammary glands were immediately removed, and then fixed in 4% paraformaldehyde for pathological examination. The specimens were embedded in paraffin. The paraffin embedded mammary gland tissues were sliced into 5 micron thick, and stained with Hematoxylin-Eosin (HE).
lumens, and cuboidal or squamous epithelium in different sizes (Figure 7C, D). Histopathological manifestation of mammary gland in moxibustion group showed less hyperplasia acinaries, enlarged mammary ducts, and mammary lumens in different sizes with less mammary secretion (Figure 7E, F).

DISCUSSION

In this study, we found that moxibustion has therapeutic and preventive effects on HMG rats. In HMG model group, rats’ nipple diameter and height showed significantly increased comparing to the saline control group. In moxibustion group with treatment, rats’ nipple diameter and height show significantly decreased comparing to HMG model group without treatment. Previous study showed that one of the most prominent characteristic of HMG rats was the increased nipple diameter and height, which support with our data found in this study.\(^\text{16}\) Moxibustion treatment show significantly anti-hyperplasia effect in HMG rats.

Infrared thermal imaging technique is a non-touching, visual, and widely used technology to detect the body skin temperature. It has been used for breast disease diagnosis for decades.\(^\text{17}\) Previous clinical research showed that thermography of hyperplasia of mammary gland is manifested as a cluster and sheet-shaped high temperature zone, and the infrared radiation temperature of the acupoints Rugen (ST 18) was higher than that of healthy controls.\(^\text{18}\) Whereas, in this experimental study, skin temperature significantly decreased in HMG model group at the nipples, mammary areas, Danzhong (CV 17) and Ganshu (BL 18) as compared with the saline control group. Therefore, the skin temperature manifestation between human and rats are different. HMG patients manifest as increased skin temperature of mammary areas and corresponding acupoints. HMG rats manifest as decreased skin temperature of mammary areas, Danzhong (CV 17) and Ganshu (BL 18). Many researchers declared that moxibustion therapy affects the blood flow in cutaneous tissues and muscles. It may also have an effect on the local vasodilatory response by increasing the temperature of the skin.\(^\text{19}\) In this study, after moxibustion treatment, skin temperature significantly increased at the nipples, mammary areas, Danzhong (CV 17), and Ganshu (BL 18) as compared with the HMG model group. This result indicates moxibustion has a good thermal effect by regulating the blood circulation.

The meridians and collaterals are pathways in which the Qi and blood of the human body are circulated. They pertain to the Zang-Fu organs interiorly and extend over the body exteriorly. While acupoints are the specific sites through which the Qi of Zang-Fu organs

![Figure 3 Diameter and height of the second and third pair nipples after the 30 day moxibustion treatment](image_url)
and meridians is transported to the body surface. They are also the loci of response to diseases. In acupuncture and moxibustion treatment, proper techniques are applied on the acupoints to prevent and treat diseases by regulating the functional activities of the body and strengthening body immune resistance. When there is an illness in the body, acupoints on the body may have corresponding reactions, such as decreased skin temperature or increased skin temperature. The results in Figure 5 showed that skin temperature of Danzhong (CV 17) and Ganshu (BL 18) decreased in HMG model group. After moxibustion treatment, skin temperature of Danzhong (CV 17) and Ganshu (BL 18) increased significantly. The results indicate that skin temperature changes of Danzhong (CV 17) and Ganshu (BL 18) can not only diagnose HMG, but also reflect the efficacy of moxibustion treatment.

Histopathological examination of mammary gland in saline control group showed tremendous loose connective tissue, less acinar, less enclosed mammary ducts, and without mammary ducts secretion. The pathological manifestation in HMG model group was prominent mammary gland hyperplasia, enlarged mammary ducts, less loose connective tissues, mammary ducts ectasia with secretion, expansion of mammary lumens, and cuboidal or squamous epithelium in different sizes. Histopathological manifestation of mammary gland in moxibustion group showed less hyperplasia acinar, enlarged mammary ducts, and mammary lumens in different size with less mammary secretion.

In conclusion, treatment with moxibustion can effectively decrease the HMG rats’ nipple diameter and...
height, and increase the skin temperature in HMG model rats at the nipples, mammary areas, Danzhong (CV 17) and Ganshu (BL 18). Skin temperature of Danzhong (CV 17) and Ganshu (BL 18) can also be used as a diagnosis index for HMG disease. Therefore, this study proved that moxibustion can be used as an alternative treatment of HMG. Although the exact mechanism of anti-hyperplasia effect of moxibustion remains unclear, further study about the mechanism will be conducted in the future.

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