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

Yang Jiamin[a], Wu Jihui[b], Shen Xiaoyu[c], Zhang Mei[c], Liu Zhenzhen[c], Qi Dandan[c], Zhou Jin[c], Cui Xiao[c], Zhang Lufen[c,*], Li Yuhang[a]

[a] School of Basic Medical Science, Beijing University of Chinese Medicine, Beijing 100029, China
[b] School of Fashion Art and Engineering, Beijing Institute of Fashion Technology, Beijing 100029, China
[c] School of Acupuncture-Moxibustion and Tuina, Beijing University of Chinese Medicine, Beijing 100029, China

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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 ($P < 0.05$).

Conclusions: 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.

1. Introduction

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. [1] 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. [2] Therefore, it is important to find more convenient therapies with prolong 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. [3] Moxibustion is a famous Chinese traditional medical therapy, which applies the ignited mugwort (*Artemisia vulgaris* from Traditional Chinese Medicine) directly or indirectly at acupoints or other specific parts of the body to cure disease. [4,5] Previous clinical and experimental studies have shown

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* Correspondence author:
E-mail address: zhlufen@aliyun.com (Z. Lufen).

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that moxibustion has been used for various conditions including stroke rehabilitation, [6] rheumatoid arthritis, [7] chronic fatigue syndrome, [8] Crohn's disease, [9] ulcerative colitis, [10] irritable bowel syndrome, [11] and HMG. [12] Moxibustion is used in the management of those diseases by involving the nervous system, endocrine system, and immune system. [13]

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.

2. Materials and methods

2.1. 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.

2.2. Chemicals

Estrogen injection was obtained from Ningbo second hormone factory; Progestogen injection was bought from Tianjin Jinyao Amino acids Co., Ltd., Ethylcarbamate was bought from Beijing Honghu Union Chemical Products Co., Ltd.

2.3. Apparatus

FLIR SC640 Infrared thermal camera (Stockholm, Sweden), YD-6L tissue embedding machine (Jinhua, China), Shandon Thermo Fisher Scientific slicer (Waltham, USA), and BX53 microphotography system (Tokyo, Japan).

2.4. Animals

Thirty female Sprague-Dawley rats with average weight of (240 ± 20) g and aged 3 months, were mature and unmated (purchased from the Academy of Military Medical Science, Beijing, China). All rats were caged under a 12/12 h light/dark cycle. Room temperature [(23 ± 1) °C] and humidity (45% ± 5%) were controlled to be constant. Food and water were available *ad libitum*.

Experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC No. BUCM-3-2,013,052,001-1001) of Beijing University of Chinese Medicine. They were allowed for acclimation under climate-controlled conditions for a week before use.

2.5. Animal model and moxibustion treatment

The rats were randomly divided into three groups ($n = 10$), rats in the saline control group were administered with normal saline intramuscularly, rats in other groups were treated with estrogen (0.5 mg/kg) intramuscularly for 25 d, and followed with progestogen (4 mg/kg) for another 5 d to induce HMG model, [14] rats in the moxibustion group were treated with a single moxibustion apparatus on the skin of Danzhong (CV17 - on the anterior median line, at the same level as the 4th intercostal space) and Ganshu (BL 18 - in the back region, at the same level as the inferior border of the spinous process of the ninth thoracic vertebra, lateral to the posterior median line) [15] for 30 d every alternate day (a total of 15 moxibustion treatments), three moxibustion apparatus for one acupoint every time, moxibustion started on the day following estrogen injection. (Figs. 1 and 2).

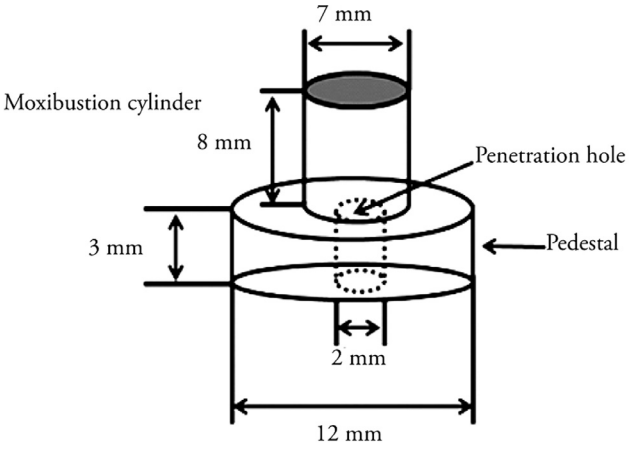

Fig. 1. Schematic view of the moxibustion cylinder and pedestal.

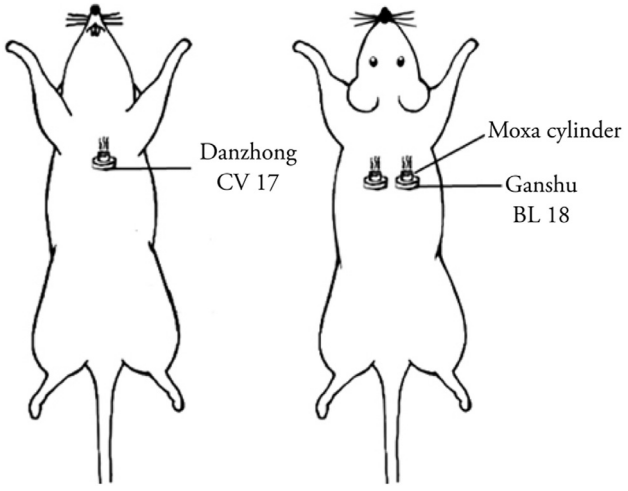

Fig. 2. Moxibustion at Danzhong (CV 17) and Ganshu (BL 18).
A: the location of Danzhong (CV 17); B: the location of Ganshu (BL 18).

2.6. Nipple size measurement

Hyperplasia of mammary gland is a hormone dependent disease, after the injection of Estrogen and progesterone, the hormone balance was disturbed. One of the manifestations is the increased nipple diameter and height. In this study, vernier slide caliper was used to measure the diameter and height of the second and third pair nipples.

2.7. Infrared thermal imaging for skin temperature measurement

Infrared thermal camera was used in this study. All animals were anesthetized with 10% urethane intramuscular injection 24 h after establishment of hyperplasia of mammary gland model. The front and back fur was removed by an electric hair clipper. Rats

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