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Study on the Anti-Inflammatory and Analgesic Mechanisms of Breast Lump Resolution Detergent in a Rat Model of Breast Hyperplasia

Chunying Huang, Yun He, Di Ouyang, Xuhui Zhao

Traditional Chinese Medicine Hospital of Yulin, Yulin, China
Email: panda_1988@126.com

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Abstract

Objective: This study aimed to investigate the anti-inflammatory and analgesic mechanisms of a breast lump resolution detergent in a rat model of breast hyperplasia. **Methods:** A rat model of breast hyperplasia was established using injections of estradiol benzoate combined with progesterone. The effects of the breast lump resolution detergent on nipple height and diameter in the rat model were observed, along with its impact on serum levels of estradiol (E2), prolactin (PRL), and progesterone (P). Additionally, the study examined the morphological changes in breast tissue. The impact of the breast nodule detergent on blood rheology parameters was also observed. Furthermore, the anti-inflammatory effect of the breast nodule detergent was assessed using the cotton ball granuloma experiment, and the analgesic effect was observed using the writhing test. **Results:** The breast lump resolution detergent reduced nipple height and diameter in the rat model, decreased serum levels of E2, PRL, and P, and alleviated pathological changes in breast tissue. It also lowered hemorheological parameters including whole blood high, medium, and low shear viscosity, plasma viscosity, red blood cell hematocrit, red blood cell aggregation index, red blood cell deformability index, red blood cell electrophoresis time, and erythrocyte sedimentation rate in the acute "blood stasis" rat model. The detergent reduced the weight of cotton ball granulomas in mice and decreased the number of writhing episodes caused by acetic acid. **Conclusion:** The breast lump resolution detergent demonstrates favorable therapeutic effects in treating breast hyperplasia, promoting blood circulation and removing blood stasis, exerting anti-inflammatory properties, and providing analgesic effects. The downregulation of serum E2 and PRL levels and the upregulation of P levels may be critical mechanisms underlying its efficacy.

Keywords

Breast Hyperplasia, Breast Lump Resolution Detergent, Estradiol (E2),

Prolactin (PRL), Progesterone (P), Anti-Inflammatory, Analgesic

1. Introduction

Breast hyperplasia refers to the proliferation of breast epithelial and fibrous tissue caused by endocrine disorders and degeneration of mammary ducts and lobules. It commonly occurs in women of reproductive age and is characterized by unilateral or bilateral breast lumps and pain [1]. In recent years, the incidence of breast hyperplasia has been increasing. The probability of breast cancer in patients with breast hyperplasia is 1.4:1, which is five times higher than that of healthy women. Early diagnosis and treatment of breast hyperplasia are effective measures for preventing the occurrence of breast cancer and reducing mortality rates [2]. Currently, modern medicine lacks effective strategies for the prevention and treatment of this disease, while traditional Chinese medicine (TCM) has unique advantages and plays an important role in the prevention and treatment of breast hyperplasia [3]. Basic research has also demonstrated that a large number of Chinese herbs and their active components can significantly improve the progression of breast hyperplasia [4]. Our hospital has developed a breast lump resolution detergent, which has been clinically proven to be effective in treating patients with breast hyperplasia. However, its anti-inflammatory and analgesic mechanisms are still to be explored.

2. Materials

2.1. Animals

Sprague-Dawley (SD) rats, SPF grade, female, weighing between 180 - 220 g. All animals were provided by Changsha Tianqin Biotechnology Co., Ltd., with the animal license number: SCXK (Xiang) 2019-0014. The animals were housed in a SPF laboratory under the following conditions: room temperature of 24˚C ± 2˚C, humidity of (65 ± 5) %, and a 12-hour light/24-hour dark cycle.

2.2. Drugs and Reagents

Breast nodule washout agent, Batch number: 170809, containing 450 mg of total herbal ingredients per mL, provided by Yulin Traditional Chinese Medicine Hospital. Tamoxifen Citrate Tablets, produced by Yangtze River Pharmaceutical Group Co., Ltd., with production batch number: 19031812. Estradiol Benzoate, Progesterone Injection, both products of Shanghai Quanyu Biotechnology Animal Pharmaceutical Co., Ltd., with batch numbers: Veterinary Drug Z163232511, Veterinary Drug Z163231439, respectively. Glacial Acetic Acid, Chloral Hydrate, Formaldehyde, products of China National Pharmaceutical Group Chemical Reagent Co., Ltd., with batch numbers: 20150612, 20161019, 20160904, respectively. Hematoxylin-Eosin staining solution, product of Zhuhai Beisuo Biotechnology Co., Ltd., with batch number: 20211120. Estradiol (E2) Enzyme-linked

Immunosorbent Assay (ELISA) Kit, Progesterone (P) ELISA Kit, Prolactin (PRL) ELISA Kit, all products of Shanghai Fanko Technology Co., Ltd., with batch numbers: 20190115, 20190321, 20190202, respectively.

2.3. Instruments

Enzyme reader 1510 was a product of Thermo Fisher Scientific Oy, USA; SA-6000 fully automatic hemorheological testing analyzer was a product of Beijing Saikexide Technology Development Co., Ltd.; RM2255 fully automatic rotary microtome, EG1150H paraffin embedding machine, DM2500 + DFC495 pathological microscope, and EG1150C cooling table were all products of Leica Microsystems, Germany; Excelsior fully enclosed water machine was a product of Thermo Fisher Scientific Inc., USA. TDL-5-A low-speed centrifuge was a product of Shanghai Anting Scientific Instrument Factory; SQP electronic balance was a product of Sartorius Scientific Instrument (Beijing) Co., Ltd. 0 - 150 vernier caliper was a product of Shanghai Hengliang Measuring Tools Co., Ltd. SPX-250BIII biochemical incubator was a product of Tianjin Testda Technology Co., Ltd.

2.4. Experimental Modeling

Each rat was injected intramuscularly in the inner side of the hind leg with 0.5 mg/kg estradiol benzoate, once a day for 25 days, followed by intramuscular injection of 5 mg/kg progesterone, once a day for 5 days, to establish a mammary hyperplasia model [5]. After 24 hours from the last administration, except for the blank control group, all rats were subcutaneously injected with 0.8 mg/kg adrenaline, a total of 2 times, with an interval of 4 hours. The first administration of adrenaline at 0.8 mg/kg was followed by immersing the rats in 0˚C ice water for 5 minutes, and the second administration of adrenaline at 0.8 mg/kg was given 2 hours later, resulting in acute "blood stasis" syndrome in the rats.

2.5. Experimental Grouping and Administration

The rats were randomly divided into five groups: blank control group, model control group, tamoxifen group, high-dose breast nodule washout group (9.00 mg total herbal ingredients/mL), and low-dose breast nodule washout group (2.25 mg total herbal ingredients/mL), with 10 rats in each group. Starting from day 31, the breast nodule washout groups were treated with soaking administration. The volume of administration was 200 mL per rat, and the temperature of the solution was maintained at 37˚C. The blank control group and model control group rats were soaked with an equal volume of warm water (37˚C) once a day for 30 minutes continuously for 45 days. The rats in the tamoxifen group were orally administered with the corresponding medication at a dose of 10 mL/kg body weight once a day for 45 days. This study was conducted under the ethical approval of the Guangxi Institute of Traditional Chinese Medicine (Ethics Review Number: 20190506).

2.6. Observation Indicators

24 hours after the last administration, the height and diameter of the second pair of nipples of the rats were measured using a precision caliper. Blood was collected from the rat's eyeball, centrifuged at 4˚C and 3500 r/min for 10 minutes to obtain serum, which was stored at −20˚C. The levels of estradiol (E2), prolactin (PRL), and progesterone (P) in rat serum were determined according to the instructions of the ELISA kit. Rats were anesthetized with 35 mg/kg pentobarbital sodium via intraperitoneal injection, placed supine and fixed on a mouse board, and the mammary glands of the second pair of breasts were obtained. They were fixed in 10% formaldehyde solution, stained with routine H&E staining, and observed under a light microscope to evaluate the proliferation of mammary gland tissues in each group. The rheological indicators of rat blood, such as whole blood high, medium, and low shear viscosity, plasma viscosity, red blood cell hematocrit, and erythrocyte sedimentation rate were determined using an automated hemorheological analyzer.

2.7. Statistical Analysis

Statistical analysis was performed using SPSS 13.0 software. Continuous data were presented as "mean ± standard deviation." One-way analysis of variance (ANOVA) was used for statistical analysis, and a p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Intervention Effect of Milk-Duct-Flushing Detergent on Mammary Hyperplasia in Rats

Compared with the blank control group, rats in the model control group showed an increase in nipple diameter and nipple height, elevated levels of serum estradiol and prolactin, and decreased progesterone levels, indicating the successful establishment of a rat model of hormone-induced mammary hyperplasia.

Compared with the model control group, continuous soaking administration of milk-duct-flushing detergent at high and low doses (9.00 mg total herbal medicine/mL, 2.25 mg total herbal medicine/mL) in rats significantly suppressed the nipple diameter and nipple height in mammary hyperplasia rats ($P < 0.05$, $P < 0.01$), as shown in Table 1.

Compared with the model control group, continuous soaking administration of milk-duct-flushing detergent at high and low doses (9.00 mg total herbal medicine/mL, 2.25 mg total herbal medicine/mL) in rats reduced the levels of serum estradiol and prolactin in mammary hyperplasia rats and increased progesterone levels, with statistically significant differences ($P < 0.05$, $P < 0.01$), as shown in Table 2.

Histopathological results of rat mammary gland tissue showed that in the normal group, the number of lobular acini in rat mammary glands was low, and there was no secretion in the acini and ducts. In the model group, the volume of

lobular acini in the mammary glands increased, the number of acini in the lobules increased, the ducts dilated, and there was a large amount of secretion in the lumen. In the tamoxifen group, the number of lobular acini and the amount of secretion in the acini decreased compared to the model group, and the number of dilated ducts and secretion also decreased. In the milk-duct-flushing detergent groups at clinical concentrations of 2.25 mg total herbal medicine/mL and 4 times the clinical concentration of 9.00 mg total herbal medicine/mL, the number of lobular acini in the mammary glands decreased significantly compared to the model group, and the number of dilated ducts and secretion also decreased, as shown in **Figure 1**.

These results indicate that milk-duct-flushing detergent at clinical concentrations of 2.25 mg total herbal medicine/mL and 4 times the clinical concentration of 9.00 mg total herbal medicine/mL can effectively inhibit mammary hyperplasia in rats and improve the hormonal levels and pathological characteristics of mammary hyperplasia.

Table 1. Effects of milk-duct-flushing detergent on nipple diameter and nipple height in rats with mammary hyperplasia ($\overline{x} \pm s$, n = 10).

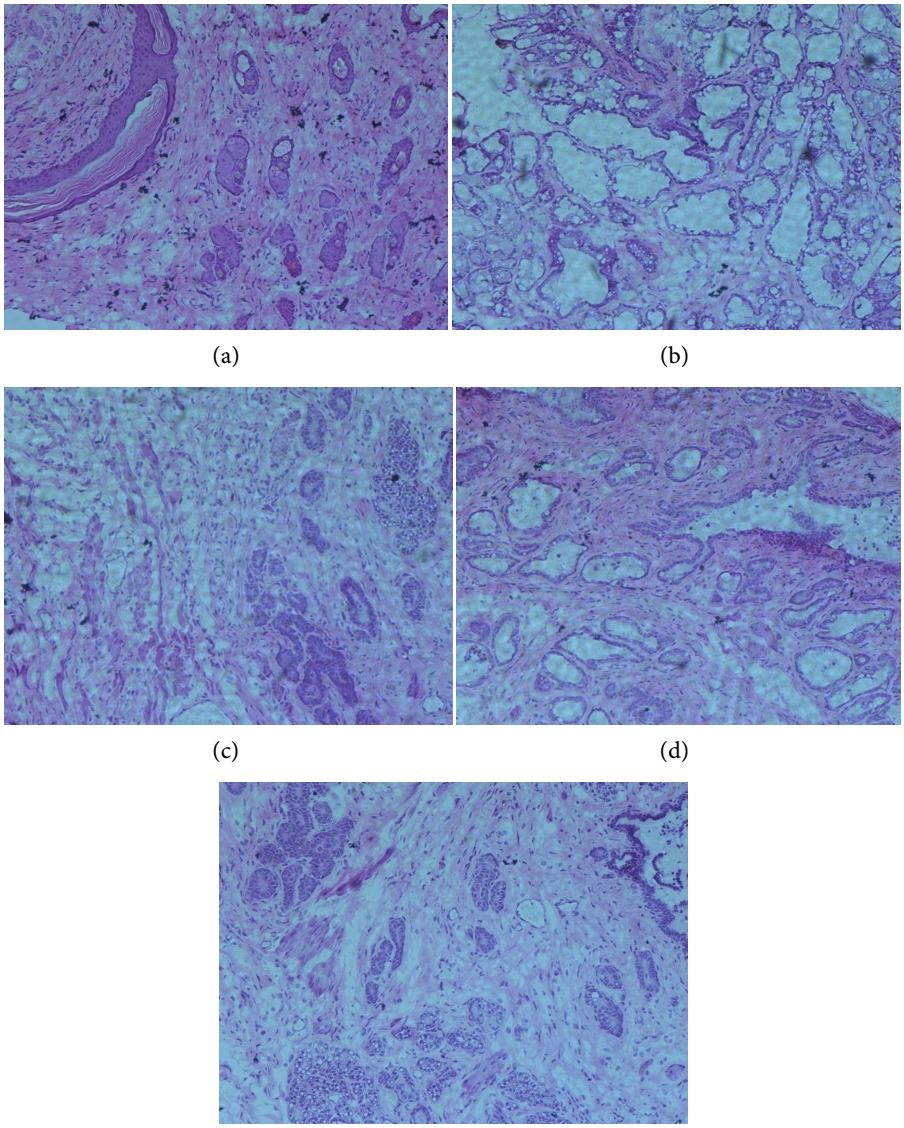
Groups	Dose (mg/kg)	Diameter of nipple (mm)	Nipple height (mm)
Blank control group	water	0.99 ± 0.06	1.06 ± 0.06
Model control group	water	1.28 ± 1.30#	1.11 ± 0.06#
Tamoxifen group	1.8	1.11 ± 0.08**	1.04 ± 0.03**
Breast lump resolution detergent	2.25 (mg/mL)	1.17 ± 0.08*	1.06 ± 0.04*
Breast lump resolution detergent	9.00 (mg/mL)	1.14 ± 0.09*	1.05 ± 0.04*

Comparison with blank control group: #$P < 0.01$; Compared with the model control group: *$P < 0.05$, **$P < 0.01$.

Table 2. Effects of milk-duct-flushing detergent on serum levels of E_2, PRL, and P in rats with mammary hyperplasia. ($\overline{x} \pm s$, n = 10).

Group	Dose (mg/kg)	E_2 (ρ/ng·L^{-1})	PRL (ρ/μg·L^{-1})	P (ng/ml)
Blank control group	water	2.81 ± 0.58	219.7 ± 22.4	1.53 ± 0.40
Model control group	water	11.10 ± 2.44#	569.3 ± 44.3#	0.58 ± 0.10#
Tamoxifen tablets	1.8	6.68 ± 1.44**	378.7 ± 113.9**	0.90 ± 0.08**
Breast lump resolution detergent	2.25 (mg/mL)	9.12 ± 1.48*	508.7 ± 66.0*	0.69 ± 0.09*
Breast lump resolution detergent	9.00 (mg/mL)	9.09 ± 0.78*	483.2 ± 88.1*	0.70 ± 0.06*

Comparison with blank control group: #$P < 0.01$; Compared with the model control group: *$P < 0.05$, **$P < 0.01$.



(a) (b) (c) (d) (e)

Figure 1. Light microscopy images of routine histological sections of rat mammary tissue in each group. (a) Blank control group (×200); (b) Model control group (×200); (c) Tamoxifen group (×200); (d) Breast duct lavage solution group with a total herbal medicine concentration of 2.25 mg/mL (×200); (e) Breast duct lavage solution group with a total herbal medicine concentration of 9.00 mg/mL (×200).

3.2. Effects of Breast Nodule Washout Agent on Cotton Ball Granuloma

Compared to the blank control group, the model control group exhibited a significant decrease in the mass of cotton ball granulomas, with a statistically significant difference ($P < 0.01$). When compared to the model control group, both the high-dose (9.00 mg total herbal ingredients/mL) and low-dose (2.25 mg total herbal ingredients/mL) breast nodule washout groups showed an increase in the mass of cotton ball granulomas, with statistically significant differences ($P < 0.05$, $P < 0.01$). The results are presented in **Table 3**.

Table 3. Effects of breast nodule washout agent on cotton ball granuloma ($\overline{x} \pm s$, n = 10).

Group	Dose (g/kg)	Cotton Ball Granuloma Mass (mg/10 g)	Inhibition Rate (%)
Blank control group	water	7.00 ± 0.46	—
Model control group	water	2.16 ± 1.30**	—
Tamoxifen tablets	1.8	4.42 ± 0.83**	36.9
Breast lump resolution detergent	2.25 (mg/mL)	4.96 ± 0.81*	29.1
Breast lump resolution detergent	9.00 (mg/mL)	4.89 ± 1.58*	30.1

Comparison with blank control group: *$P < 0.05$, **$P < 0.01$.

Table 4. The Effects of Breast Nodule Washout Agent on Acetic Acid-Induced Pain ($\overline{x} \pm s$, n = 10).

Group	Dose (g/kg)	Wristing time (sec)	Wristing frequency	Rate of Inhibition (%)
Blank control group	water	230.52 ± 14.28	32.46 ± 4.76	—
Model control group	water	198.26 ± 13.66**	11.26 ± 3.12**	—
Tamoxifen tablets	1.8	277.80 ± 12.63**	21.67 ± 4.90**	33.24
Breast lump resolution detergent	2.25 (mg/mL)	251.50 ± 11.77**	25.34 ± 3.32**	21.93
Breast lump resolution detergent	9.00 (mg/mL)	236.91 ± 20.32**	26.95 ± 2.66**	16.97

Comparison with blank control group: **$P < 0.01$.

3.3. Analgesic Effect

Compared to the blank control group, the model control group exhibited a significant decrease in the initiation time of torsion and the number of pain-induced torsion caused by acetic acid, with a statistically significant difference ($P < 0.01$). When compared to the model control group, both the high-dose (9.00 mg total herbal ingredients/mL) and low-dose (2.25 mg total herbal ingredients/mL) breast nodule washout groups showed a significant increase in the initiation time of torsion and a reduction in the number of pain-induced torsion caused by acetic acid, with statistically significant differences ($P < 0.01$). The results are presented in Table 4.

4. Conclusions

Breast hyperplasia is a common benign proliferative disease in women, characterized by cyclic breast pain in one or both breasts [2]. Traditional Chinese medicine does not have a specific disease name for "breast hyperplasia," but based on its clinical symptoms, it can be classified under categories such as "breast distention," "breast lump," "breast mass," or "tuberculosis of the breast" [6]. In ancient medical texts, breast distention is described as "pain occurring between the sides intermittently". Sui Dynasty physician Yuánfāng Cháo's "General Discussion of Disease Manifestations". Similarly, "breast distention" is described in Ming Dynasty physician Jūzhōng Gōng's "Definitive Text on Surgery" as "this condition arises above the normal breast…what is referred to as distention is when it is hard but not painful, similar to a stubborn mass." These descriptions closely

align with the clinical symptoms of modern medicine's understanding of breast hyperplasia.

This study successfully replicated a rat model of breast hyperplasia. The model group was evaluated based on general survival conditions and observations of nipple morphology and mammary gland thickness. The results showed that compared to the normal control group, the model group of rats exhibited restlessness, irritability, huddling, significant nipple swelling, and increased mammary gland thickness. Compared to the model group, the various treatment groups showed varying degrees of relief in general survival conditions and nipple swelling, as well as a reduction in mammary tissue thickness.

Additionally, the mammary gland is a target organ for sex hormones, with the tissue primarily regulated by E2, P, and PRL [7]. E2 promotes the growth of mammary ducts and periductal fibrous tissue, P promotes the development of mammary lobules and acinar tissue, and PRL promotes mammary gland development, growth, lactation, and the maintenance of lactation [8]. Elevated PRL levels directly stimulate mammary gland tissue and further inhibit the secretion of P during the luteal phase, while also stimulating E2 synthesis, contributing to elevated E2 levels and continued stimulation of mammary gland tissue, leading to breast hyperplasia [9] [10] [11]. The experimental results showed that compared to the model control group, both the blank control group and the high-dose Xiaozheng Pill treatment group exhibited significant reductions in serum E2 and PRL levels, while P levels were significantly elevated. This suggests that rats in the breast hyperplasia model exhibited abnormal serum hormone levels, and milk-duct-flushing detergent has a regulatory effect on hormone levels. Furthermore, milk-duct-flushing detergent reduced whole blood high, medium, and low shear viscosity, plasma viscosity, red blood cell hematocrit, erythrocyte aggregation index, erythrocyte deformability index, erythrocyte electrophoretic time, and erythrocyte sedimentation rate in rats with acute "blood stasis" syndrome. The detergent also reduced the mass of cotton pellet granuloma in mice and decreased the number of writhing episodes induced by acetic acid. These findings indicate that milk-duct-flushing detergent exhibits favorable effects in treating breast hyperplasia, promoting blood circulation, resolving stasis, reducing inflammation, and providing analgesic effects. The downregulation of serum E2 and PRL levels and the upregulation of P levels may be key mechanisms underlying these therapeutic effects.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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