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Original Research Article

Effect of Prunella vulgaris L extract on hyperplasia of mammary gland in rats

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Abstract

Purpose: *To explore the effects of Prunella vulgaris L extract (PVE) on hyperplasia of mammary gland (HMG) in rats.*
Methods: *Forty virgin female Wistar rats were randomly divided into normal group, control group (HMG model), positive control group (Rupixiao Capsule, RPXC), and low-, medium- and high-dose (150, 300 and 600 mg/kg) of PVE groups. Injections of estrogen and progestogen were given at the same time to prepare rat. Changes in nipple height were measured, while serum estradiol (E2), progesterone (P), prolactin (PRL), follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were evaluated by ELISA; Uterus and ovary indices were determined.*
Results: *Compared with control group, PVE reduced elevated nipple height to 2.25 ± 0.09 mm ($p < 0.01$) and uterus index to 2.29 ± 0.41 mg/g ($p < 0.01$), as well as reduced the number of mammary gland lobules and secretion in HMG rats. Compared with control group, serum E2 (2.81 ± 0.17 pmol/L), PRL (269.38 ± 8.28 pg/mL) and FSH (0.13 ± 0.03 IU/L) levels ($p < 0.01$) were lowered, but serum P (1.31 ± 0.13 ng/mL) and LH (1.73 ± 0.08 mIU/mL) levels were higher ($p < 0.01$) in rats treated with high-dose PVE.*
Conclusion: *These results suggest that PVE exerts anti-HMG effect in rats induced by estrogen and progestogen.*

Keywords: *Prunella vulgaris L; Anti-inflammatory; Anti-hyperplasia of mammary gland*

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INTRODUCTION

Hyperplasia of mammary gland (HMG) is a common disease in middle-aged women. It is a kind of pathological hyperplasia of lobules of mammary gland induced by balance disorder of estrogen and progesterone. The morbidity of HMG is increasing nowadays, with a risk of causing mammary carcinoma. Therefore, it is important to search for more convenient and effective new drugs with few side effects for treating hyperplasia of mammary glands as well as explore the anti-HMG mechanisms of these drugs that would block its development into breast cancer [1].

HMG is related to menstrual cycle, breast-feeding, occupation, abuse of sex hormone drugs, diet and stress [2,3]. HMG patients have increased in number in recent years. Studies have shown that some Traditional Chinese Medicine products may improve regulatory mechanisms of the body that could inhibit HMG [4].

Prunella vulgaris L, is a herb from barley that is widely used in China. It is capable of regulating endocrine disorder and is frequently used in the treatment of HMG [5]. The aim of the present study was to examine the therapeutic effect of *Prunella vulgaris* L extract against hyperplasia of mammary gland in rats.

EXPERIMENTAL

Material

The herbal samples of were collected from Luoyang City, Henan Province in China in May 2015. Taxonomic identification of the plant was performed by Professor Lu Gan of Zhejiang University, in China. A voucher specimen of herbarium (no. PVE 201505027) was deposited in the herbarium of College of Pharmacy, Zhejiang University, China for future reference. The aqueous extract of *Prunella vulgaris* L was obtained by steeping the dried *Prunella vulgaris* L in water at 60 ^{o}C three times, for 1 hour on each occasion. Then it was dried in a oven and freeze-dried until obtained. The yield was 50.0 %.

Animals

Virgin female Wistar rats weighing 200 – 240 g and Kunming female mice (18 - 22g) were provided by the Experimental Animal Center of Zhejiang Province (Certificate no. SYXK 2005-0002). The animals had free access to food and water, and were allowed to acclimatize for at least one week before use. The rat experiment was approved by Animal Care and Use Committee of Ningbo No. 9 Hospital (approval ref no. 20110605) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [6].

Animal groups

Rats were treated with estrogen (0.5 mg/kg) intra-muscularly for 25 days, followed by progestogen (5 mg/kg) for another 5 days to induce HMG model. The rats were randomly divided into 6 groups of ten rats: normal group, model group, positive group (Rupixiao Capsule, RPXC 400 mg/kg) as well as PVE groups (150, 300 and 600 mg/kg doses). From the 31st day, the rats in the normal group and model group received distilled water by gavage, the rats in RPXC group were treated with RPXC, and the rats in the PVE group were respectively administered PVE by intragastric administration. Treatments were given orally once daily for 4 weeks.

Cotton pellet-induced granuloma

Sterile cotton pellets (10 mg) were implanted subcutaneously in groin of anesthetized mice (18 – 22 g). Ten animals were used for every treatment. The animals received 150, 300 and 600 mg/kg of PVE, RPXC (400 mg/kg) or saline (10 ml/kg) orally, once a day through an oral cannula over 7 consecutive days. On the 8th day, the mice were sacrificed and the cotton pellet removed, dried overnight at 60 °C and weighed. The increase in weight of cotton pellet was determined and used for further calculation.

Determination of nipple height and biochemical assay

The nipple height of all rats were measured after the treatment. The contents of sex hormones - E2, P, PRL, FSH and LH - were determined using ELISA kits (Nanjing Jiancheng Co Ltd, China). Uterus index was calculated as uterus weight divided by body weight, while ovary index derived as the ratio of ovary weight to body weight.

Statistical analysis

Values are expressed as mean ± SEM. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett's test to detect intergroup differences. $P < 0.05$ was considered statistically significant in all cases.

RESULTS

Cotton pellet-induced granuloma

The results revealed that PVE significantly inhibited the dried weight of the cotton pellet granuloma in a dose-dependent manner**.** The values for doses of 150, 300 and 600 mg/kg PVE were 18.15, 22.24 and 28.14 %, respectively. RPXC inhibited granuloma tissue formation by 17.21 %, which is lower than that observed for 100 mg/kg dose of PVE. Thus, PVE significantly suppressed granulomatous tissue formation during chronic inflammation (Table 1).

Effect of PVE on rat nipple height

The height of nipples (right 2 and right 3) of rats was significantly decreased by RPXC (400 mg/kg) and PVE (600 mg/kg) treatments compared with HMG control group ($p < 0.01$, Table 2).

Table 1: Effect of PVE in mice cotton pellet-induced inflammation

Group	Dose (mg/kg)	Granuloma weight (mg)	Inhibition rate (%)	*P*-value
Normal	—	72.36 ± 8.41		
RPXC	400	64.24 ± 11.56	16.68	> 0.05
PVE-L	150	62.12 ± 15.78	19.26	> 0.05
PVE-M	300	57.28 ± 13.35	24.35	< 0.05
PVE-H	600	55.76 ± 12.29	27.29	< 0.05

*$P < 0.05$, **$p < 0.01$ vs model group; PVE-L: low-dose of PVE; PVE-M: middle-dose of PVE; PVE-H: high-dose of PVE; RPXC: Rupixiao Capsule*

Table 2: Effect of PVE on nipple height in rats

Group	Dosage (mg/kg)	Nipple height (mm)	
		Right 2	Right 3
Normal	—	1.62 ± 0.15**	1.63 ± 0.16**
Control	—	2.79 ± 0.27	2.64 ± 0.21
RPXC	400	2.24 ± 0.23**	2.34 ± 0.12**
PVE-H	600	1.93 ± 0.08**	2.25 ± 0.09**

*$P < 0.05$, **$p < 0.01$ vs model group; PVE-H: high-dose of PVE; RPXC: Rupixiao Capsule

Table 3: Effect of PVE on serum sex hormone levels in rats

Group	Dosage (mg/kg)	E2 (pmol/L)	P (ng/mL)	PRL (pg/mL)	FSH (IU/L)	LH (mIU/mL)
Normal	—	2.85±0.38**	1.45±0.17**	275.46±6.48**	0.12±0.02*	1.92±0.09*
Model	—	4.65±0.16	0.72±0.08	398.54±8.26	0.58±0.02	1.12±0.05
RPXC	400	3.36±0.24*	1.22±0.15*	423.23±12.34	0.47±0.19	1.42±0.10*
PVE-H	600	2.81±0.17**	1.31±0.13**	269.38±8.28**	0.13±0.03**	1.73±0.08*

*$P < 0.05$, **$p < 0.01$ vs. model group; PVE-H: high-dose of PVE; RPXC: Rupixiao Capsule*

Effect of PVE on rat serum sex hormone levels

After injection of estrogen and progestogen, E2, PRL and FSH contents were significantly increased, while P and LH levels decreased in HMG control rats. E2, PRL and FSH were decreased by PVE (600 mg/kg) treatment compared with model group ($p < 0.01$), P and LH contents was remarkably increased compared to HMG control group ($p < 0.01$) (Table 3).

Effect of PVE on uterus and ovary index in rats

Uterus index in HMG model rats were significantly increased compared with normal group ($p < 0.01$). Uterus index was significantly decreased by RPXC (400 mg/kg) ($p < 0.01$) and PVE (600 mg/kg) ($p < 0.05$) treatment as compared with HMG model group. Ovary index was not shown distinct difference compared with control group (Table 4).

DISCUSSION

There is evidence that chronic inflammation brought about by persistent chemical, bacterial or viral agents is a risk factor for cancer [7-10]. Inflammation by innate immunity, which is required to fight microbial infections, heal wounds and maintain tissue homeostasis, can lead to cancer [11-13].

Table 4: Effects of PVE on uterus and ovary index in rats

Group	Dosage (mg/kg)	Uterus index (mg/g)	Ovary index (mg/g)
Normal	—	2.23 ± 0.36**	0.82 ± 0.08
Model	—	2.87 ± 0.41	0.52 ± 0.12
RPXC	400	2.35 ± 0.24**	0.47 ± 0.06
PVE-H	600	2.29 ± 0.41*	0.45 ± 0.06

*$P < 0.05$, **$p < 0.01$ vs model group; PVE-H: high-dose of PVE; RPXC: Rupixiao Capsule*

Several recent studies have suggested that inflammation has an important role in all phases of tumor development, including tumor initiation, tumor promotion, invasion, metastatic dissemination, and evasion of immune system [14]. The development of many types of cancer including breast cancer is related to inflammation.

Epidemiological studies have revealed that the use of non-steroidal anti-inflammatory drugs can decrease the risk of developing breast cancer [15,16]. The study revealed that PVE-H had strong anti-inflammatory activities in chronic inflammation model mice. The inflammatory

granuloma is a typical feature of a chronic inflammatory process. The dried weight of the pellets correlates with the amount of granulomatous tissue. Therefore, cotton pellet granuloma method has been widely used to evaluate the proliferative components of chronic inflammation.

In the present study, PVE inhibited chronic proliferative inflammation processes with a dose-dependent inhibition of granuloma formation in mice, and also has ameliorative effects in HMG rats induced by estrogen and progestogen. The heights of nipples of rats were significantly decreased by PVE-H treatment compared with control. Results showed that uterus index were remarkably decreased by PVE-H treatment compared with HMG model group. Ovary index was not shown distinct difference compared with control group. After administration of PVE-H in HMG rats, E2, PRL and FSH were remarkably decreased, while P and LH significantly increased in relation to control.

CONCLUSION

The findings of this study demonstrate that PVE significantly inhibits mammary gland hyperplasia in rats. Therefore, the plant has the potential to be developed as a treatment of HMG in patients.

DECLARATIONS

Acknowledgement

None declared

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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