Tropical Journal of Pharmaceutical Research February 2016; 15 (2): 293-297
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v15i2.10

Original Research Article

Radix bupleuri Extract Inhibits Hyperplasia of Mammary Gland in Rats

Bin Liu and Zi-he Guan*
Golden Section, Heilongjiang University of Traditional Chinese Medicine, Haerbin 150010, Heilongjiang Province, China

For correspondence: **Email: liubin133494@163.com; **Tel:** +86 0451 82114400*

Received: 2 June 2015 *Revised accepted: 30 December 2015*

Abstract

Purpose: *To investigate the effect of Radix bupleuri extract (RBE) on hyperplasia of mammary gland (HMG) in rats.*
Methods: *Forty virgin female Wistar rats were randomly divided into control, HMG model, positive control (Rupisanjie capsule, RPSJC), and low-, middle-, and high-dose RBE groups. Estrogen and progestogen were intramuscularly injected into the rats to induce HMG. RPSJC and RBE were administered by intragastric administration. Changes in nipple height were measured; serum estradiol (E2), progesterone (P), prolactin (PRL), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels were evaluated; and uterus and ovary indices were calculated. Morphological changes in mammary glands were observed by light microscopy.*
Results: *Compared with HMG model rats, those treated with RBE had significantly decreased nipple height and uterus index (both $p < 0.01$), and the numbers of mammary gland lobules and secretion were reduced by RBE. The treatment also significantly decreased serum E2, PRL, and FSH levels (all $p < 0.01$), while serum P and LH levels ($p < 0.01$) were significantly increased in RBE-treated HMG rats. Histopathologic observation confirmed that high dose of RBE attenuated HMG.*
Conclusion: *The findings suggest that RBE has significant effect of anti-hyperplasia of mammary gland.*

Keywords: *Radix Bupleuri, Anti-inflammatory, Anti-hyperplasia, Mammary gland, Nipple height, Uterus index*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Hyperplasia of mammary gland (HMG) is a pathologic hyperplasia of the mammary gland lobules induced by an imbalance of estrogen and progesterone [1]. It is related to menstrual cycle, lactation, occupation, sex hormone use, diet, and stress [2,3]. The prevalence of HMG has increased in recent years, which is notable since it is associated with cancer. Indeed, HMG may be confused with early breast cancer. A recent study reported that traditional Chinese medicine (TCM) can significantly attenuate endocrine dyscrasia and inhibit HMG development [4]. *Radix bupleuri*, also named Chaihu in Chinese, is a common TCM herbal drug used to regulate endocrine disorders [5].

In this study, we evaluated the effects of *Radix bupleuri* extract (RPE) in a rat model of HMG.

EXPERIMENTAL

Material

Three batches of *Radix bupleuri* were collected

from Bozhou City, Anhui Province of China in July 2014. Taxonomic identification was performed by Professor Gan Gu of Zhejiang Pharmaceutical College, China. A voucher specimen of herbarium (No. RBE 201405031) was deposited in the College of Pharmacy, Zhejiang Pharmaceutical College, China for future reference. Aqueous RBE was obtained by steeping the dried plant three times in water at 60 °C for 1 h each time before drying in an oven and freeze-drying the last extract obtained. The yield was 66.67 %; 1 g powder was roughly equivalent to 1.5 g crude sample.

Animals

Virgin female Wistar rats weighing 200–240 g and Kunming female mice (18–22 g) were provided by the Heilongjiang University of Traditional Chinese Medicine (certificate No. SYXK 2006-0004). The animals had free access to feed and water and were allowed to acclimatize for at least 1 week before the study. The animal experiment was approved by Animal Care and Use Committee of Heilongjiang University of Traditional Chinese Medicine (approval ref no. 20120907) and was carried out in compliance with the 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) intramuscularly for 25 days, followed by progestogen (5 mg/kg) for another 5 days to induce HMG. The rats were randomly divided into 6 groups of 10 rats each: control (untreated); HMG; HMG + positive control (RPSJC 800 mg/kg); and three HMG + RBE groups (200, 400, or 800 mg/kg doses). From the 31st day, rats in the control and HMG groups received distilled water by gavage, those in the RPSJC group were treated with RPSJC, and the RBE groups received RBE. Treatments were given orally once daily for 4 weeks.

Cotton pellet-induced granuloma test

Sterile cotton pellets (10 mg) were subcutaneously implanted in the groin of anesthetized mice (18–22 g). Ten animals were used for every treatment. The animals received 200, 400, or 800 mg/kg of RBE; RPSJC (800 mg/kg); or saline (10 ml/kg). These treatments were given 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 was determined and used for further calculation.

HMG assessment

The nipple heights of all rats were measured after the treatment. Levels of serum estradiol (E2), progesterone (P), prolactin (PRL), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were determined using enzyme-linked immunosorbent assay kits (Shenzhen Xin-Bo-Sheng Biological Technology Co Ltd, China). The uterus and ovary indices were calculated as organ weight divided by body weight.

Histopathologic assessment

Mammary gland tissues from each group were fixed in 10 % buffered formalin, embedded in paraffin, cut into 4-µm-thick sections, stained with hematoxylin-eosin (H-E) and Masson's trichrome (M-T) and examined under a light microscope. HMG severity was based on four parameters (gland alveolus hyperplasia, lobules, vessel shape and thickness, and secretion).

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 differences. $P < 0.05$ was considered significant in all cases.

RESULTS

Cotton pellet-induced granuloma

RBE significantly and dose-dependently decreased the dried weight of the cotton pellet granuloma. The inhibitory values for 200, 400, and 800 mg/kg of RBE were 17.69, 21.82, and 27.38 %, respectively. Notably, RPSJC had a lower inhibitory effect than the 200 mg/kg RBE dose. These results suggest that RBE effectively suppressed granulomatous tissue formation during chronic inflammation (Table 1).

Effect of RBE on nipple height in rats

As shown in Table 2, rat nipple height was significantly decreased by both RPSJC (800 mg/kg) and RBE (800 mg/kg) compared with the HMG group ($p < 0.01$) (Table 2).

Effect of RBE on serum sex hormone levels in rats

After injection of estrogen and progestogen in

HMG model rats, E2, PRL, and FSH levels significantly increased, while P and LH levels decreased. RBE (800 mg/kg) treatment significantly decreased E2 PRL, and FSH compared with the HMG group (all $p < 0.01$), while it significantly increased P and LH levels ($p < 0.01$) (Table 3).

Effect of RBE on uterus and ovary index in rats

The uterus index of HMG rats was significantly increased compared with untreated control animals ($p < 0.01$). This was significantly attenuated by both RPSJC ($p < 0.01$) and high-dose RBE ($p < 0.05$). The ovary index in the high-dose RBE group was not significantly different compared with untreated control animals (Table 4).

Effect of RBE on mammary gland morphology

Control rats did not have any evidence of histologic abnormalities or proliferative lesions; they also had fewer acinars and no mammary duct secretion (Fig 1A and B). Conversely, HMG rats exhibited intense hyperplasia of the gland alveolus and lobule, secretion, vessel arborization, lymphoplasia, and plasma cells (Fig 1C and D). At week 4, RPSJC-treated group rats showed significantly decreased hyperplasia, less secretion, and fewer lymphocytes (Fig. 1E and F). RBE-H (800 mg/kg) markedly alleviated the degree of HMG by limiting hyperplasia in the gland alveolus and lobules and decreasing secretion and lymphocytes (Fig 1G and H).

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

Groups	Dosage (mg/kg)	Granuloma weight (mg)	Inhibition rate (%)	*P*
Control	—	73.92 ± 9.45		
RPSJC	800	61.50 ± 11.75	16.8	>0.05
RBE-L	200	60.84 ± 18.48	17.69	>0.05
RBE-M	400	57.79 ± 13.24	21.82	<0.05
RBE-H	800	53.68 ± 12.94	27.38	<0.05

RBE-L: low-dose of RBE; RBE-M: middle-dose of RBE; RBE-H: high-dose RBE; RPSJC: Rupisanjie capsule; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. model group

Table 2: Nipple height in rats

Group	Dosage (mg/kg)	Nipple height (mm)	
		Right 2	Right 3
Control	—	1.56 ± 0.15**	1.62 ± 0.21**
Model	—	2.98 ± 0.27	2.58 ± 0.29
RPSJC	800	2.06 ± 0.24**	2.09 ± 0.09**
RBE-H	800	1.72 ± 0.08**	2.06 ± 0.08**

RBE-H: high-dose RBE; RPSJC: Rupisanjie capsule; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. model group

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

Group	Dosage	E2 (pmol/L)	P (ng/mL)	PRL (pg/mL)	FSH (IU/L)	LH (mIU/mL)
Control	—	2.80 ± 0.34**	1.41 ± 0.16**	286.37 ± 7.08**	0.08 ± 0.02*	1.85 ± 0.10*
Model	—	4.85 ± 0.19	0.63 ± 0.06	411.26 ± 8.91	0.52 ± 0.01	1.08 ± 0.05
RPSJC	800	3.12 ± 0.28*	1.12 ± 0.20*	414.29 ± 15.24	0.48 ± 0.27	1.48 ± 0.10*
RBE-H	800	2.67 ± 0.16**	1.23 ± 0.12**	278.93 ± 8.42**	0.12 ± 0.02**	1.77 ± 0.09*

E2: estradiol; FSH: follicle-stimulating hormone; LH: luteinizing hormone; P: progesterone; PRL: prolactin; RBE-H: high-dose RBE; RPSJC: Rupisanjie capsule; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. model group

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

Group	Dosage	Uterus index (mg/g)	Ovary index (mg/g)
Control	—	2.13 ± 0.41**	0.68 ± 0.07
Model	—	2.87 ± 0.51	0.56 ± 0.14
RPSJC	800	2.15 ± 0.22**	0.48 ± 0.07
RBE-H	800	2.28 ± 0.46*	0.49 ± 0.08

RBE-H: high-dose RBE; RPSJC: Rupisanjie capsule; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. model group

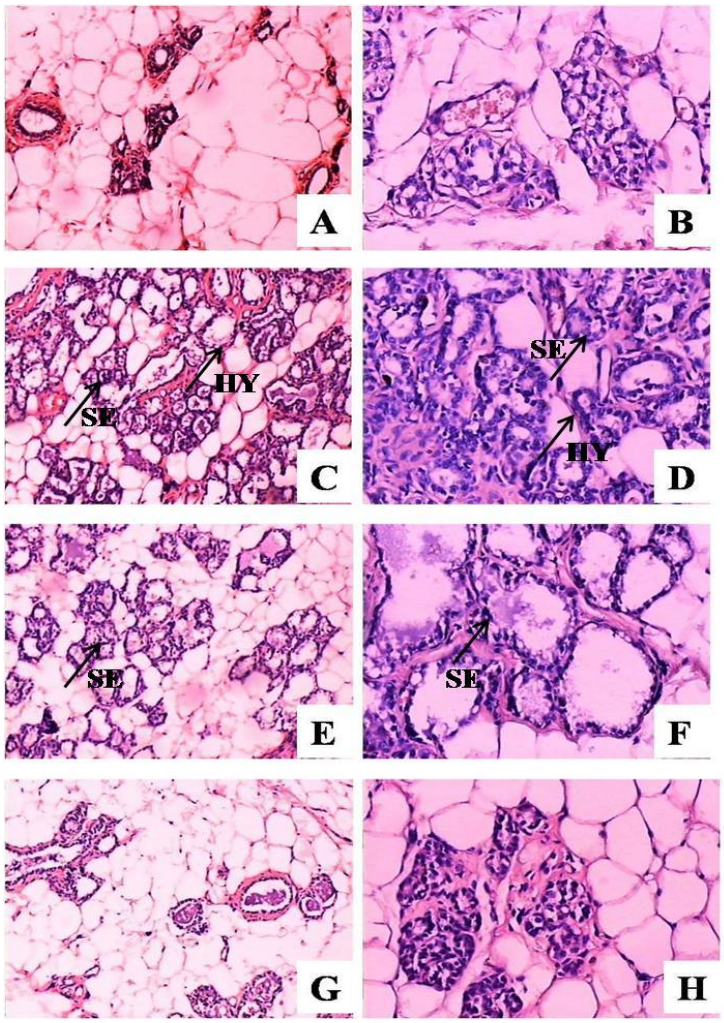

Figure 1: Histologic images of mammary gland tissues. A-B, Control rats; C-D, HMG rats; E-F, HMG + RPSJC; G-H, RBE-H (800mg/kg). The left and right columns show ×100 and ×200 magnification, respectively. HY: hyperplasia; SE: secretion

DISCUSSION

There is evidence that chronic inflammation induced by persistent chemical, bacterial, or viral agents is a risk factor for cancer [7-10]. Inflammation is an important process required to fight microbial infections, heal wounds, and maintain tissue homeostasis, but it can also lead to cancer [11-13]. Several recent studies suggested that inflammation has an important role in all phases of tumor development, including tumor initiation, tumor promotion, invasion, metastatic dissemination, and immune system evasion [14]. Specifically, the development of breast cancer is related to inflammation. Epidemiological studies have revealed that non-steroidal anti-inflammatory drug use can decrease the risk of developing breast cancer [15,16].

The inflammatory granuloma is a characteristic feature of chronic inflammatory processes. Dried weight of pellets correlates with the amount of granulomatous tissue; therefore, the results of the present study show that RBE-H exerts strong anti-inflammatory activity in a mouse model of chronic inflammation.

In the present study, RBE effectively inhibited chronic proliferative inflammatory processes and in a rat model of HMG. The mechanism might involve regulating endocrine and immune functions to balance hormone levels and attenuate pathologic mammary gland proliferation.

CONCLUSION

RBE is a potentially useful treatment for HMG. Therefore, further studies are required to develop *Radix bupleuri* for clinical use.

REFERENCES

1. Bennett IC. Serum oestradiol in women with and without breast disease. Br J Cancer 1990; 61: 142–147.
2. Xu KY. The etiology and clinical intervention of hyperplasia of mammary glands. China Prac Med. 2011; 6: 99.
3. Jia CM. The cause analysis of hyperplasia of mammary glands. Zhongcaoyao 2011; 4: 157-158.
4. Qian LQ. Clinical observation on treatment of hyperplasia of mammary gland by Lirukang Granule. Chin J Integ Med. 2007; 13: 120-124.
5. Yang HZ, Lin BS. The curative effect observation of Malt Shuyu Tangon 85 hyperplasia of mammary gland patients. Guide of China Med. 2012; 10: 309-310.
6. European Commission [homepage on the internet]. Directive 2010/63/EU on the protection of animals used for scientific purposes [cited 2013 Jan 16]. Available from:http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm.
7. Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer 2007; 121: 2373-2380.
8. Mantovani A. Cancer-related inflammation. Nature 2008; 454: 436-444.
9. Mantovani A. Molecular pathways linking inflammation and cancer. Curr Mol Med 2010; 10: 369-373.
10. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest 2007; 117: 1175-1183.
11. Trinchieri G. Inflammation in cancer: a therapeutic target? Oncology (WillistonPark) 2011; 25: 418-420.
12. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
13. Chang ZL. Important aspects of Toll-like receptors, ligands and their signaling Pathways. Inflamm Res. 2010; 59: 791-808.
14. Trinchieri G. Cancer and inflammation: an old intuition with rapidly evolving new concepts. Annu Rev Immunol. 2012; 30: 677–706.
15. Howe, LR. HER2/neu-induced mammary tumorigenesis and angiogenesis are reduced in cyclooxygenase-2 knockout mice. Cancer Res. 2005; 65: 10113-10119.
16. Reed JR. Interleukin-1 beta and fibroblast growth factor receptor 1 cooperate to induce cyclooxygenase-2 during early mammary tumourigenesis. Breast Cancer Res. 2009; 11: r21.