

Original Research Article

Effect of *Phellodendron chinense* Schneid Extract on Chronic Bacterial Prostatitis Induced by Chlamydia in Rats

Limei Sun¹, Li Zhou², Jing Tian¹ and Shuyun Zheng^{1*}

¹Department of Dermatology, The First Affiliated Hospital of Harbin Medical University, Harbin 150001, ²Department of Urology, The Second Affiliated Hospital of Harbin Medical University, Harbin 150081, Heilongjiang Province, China

*For correspondence: **Email:** zhengshuyun133494@163.com; **Tel:** +86 0451-85553842

Received: 15 May 2015

Revised accepted: 6 February 2016

Abstract

Purpose: To explore the effects of *Phellodendron chinense* Schneid (PCS) extract on chlamydia-induced chronic bacterial prostatitis (CBP).

Methods: Sixty 8-week-old male Sprague-Dawley rats were used in this study. Prostate index (PI) and prostate specific antigen (PSA) were determined after 4 weeks of oral administration of PCS extract (80, 160 or 320 mg/kg) or tetracycline (80 mg/kg) and compared to PI and PSA in untreated rats with CBP and healthy control rats ($n = 10$). Chronic inflammatory cell infiltrates, acinar changes, and interstitial fibrosis were evaluated by histopathological examination. In addition, pertinent inflammatory factors, including tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), cyclooxygenase-2 (COX-2), prostaglandin E₂ (PEG₂), transforming growth factor β 1 (TGF- β 1), and connective tissue growth factor (CTGF), were measured in prostate tissues using ELISA kits.

Results: High doses of PCS (160 and 320 mg/kg) significantly decreased PI and PSA relative to model group ($p < 0.01$). PCS treatment also significantly reduced chronic inflammatory cell infiltrates and interstitial fibrosis in prostate tissue of CBP rats. In addition, TNF- α , IL-1 β , COX-2, PEG₂, TGF- β 1, and CTGF decreased in PCS-treated rats compared to untreated control ($p < 0.01$).

Conclusion: PCS extract has significant anti-inflammatory effects on chlamydia-induced CBP.

Keywords: *Phellodendron chinense* Schneid, Bacterial prostatitis, Inflammatory factors, Morphometric analysis, Interstitial fibrosis, Prostate specific antigen, Prostate 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

Epidemiological research in the past decade has revealed that prostatitis is a major urological health problem [1,2]. There are several causes of chronic bacterial prostatitis (CBP); thus, its diagnosis and treatment have not yet been clearly defined [3].

Oral antibiotics are the typical first-line treatment for CBP. However, antibiotics do not efficiently penetrate the plasma-prostate barrier [4]. There

is a need, therefore, to identify alternative treatments for CBP.

Phellodendron chinense Schneid (PCS), which is also known as huang bai and is widely cultivated in southwest China, is one of the 50 fundamental herbs of traditional Chinese medicine. PCS has immune-modulation [5], anti-tumor [6], and anti-bacterial [7,8] properties.

The aim of the present study was to evaluate the therapeutic effects and mechanisms of action of PCS extract on chlamydia-induced CBP.

EXPERIMENTAL

Materials

Herbal samples of *Phellodendron chinense* Schneid were collected from Bozhou City, Anhui Province in China in May 2014. Taxonomic identification of the plant was performed by Professor Wuke Wang of Harbin Medical University in China. A herbarium specimen (no. PCS 201307015) was deposited in the College of Pharmacy, Harbin Medical University, China for future reference.

The same batch of PCS was dried, first in an oven, and then by freeze drying. One gram of powder was equivalent to approximately 1.5 g of crude sample. The yield was 66.67 %. The aqueous extract of PCS was obtained by steeping this dried PCS in water for 1 h at 60 °C for three times.

Animals

Eight week old male Sprague-Dawley rats (250–300 g) were provided by the Experimental Animal Center of Harbin Province (certificate no. SYXK2005-0004). The animals had free access to food and water and were allowed to acclimate to the laboratory environment for at least 1 week prior to the experiments. This study was approved by the Animal Care and Use Committee of Harbin Medical University (approval ref no. 20120805) and was carried out in compliance with Directive 2010/63/EU on the Handling of Animals Used for Scientific Purposes [9].

The rats were randomly divided into 6 groups of 10 rats each: healthy group (not have chlamydia-induced CBP), model group, positive treatment group (80 mg/kg tetracycline), and three PCS-treated groups (80, 160, and 320 mg/kg doses). Tetracycline and PCS extract were both dissolved in water. Treatments were administered orally once per day for 4 weeks.

Chronic bacterial prostatitis model (CBP)

CBP was induced using previously described procedures [10]. Briefly, a strain of *Chlamydia* (Z17, O2:K1:H-) was grown overnight at 37 °C in a tryptic soy broth (TSB) in a shaker. Cells were spun, washed three times, and re-suspended in TSB to obtain 10⁸ cells/mL.

Rats were anaesthetized with ether, and the genital area was cleaned with 70 % alcohol and subsequently catheterized with a lubricated sterile polyethylene tube (0.9-mm outer diameter,

2.5-cm length). An insulin syringe was attached to the needle, and 0.2 mL of the bacterial suspension containing 1 × 10⁸ colony-forming units per mL of chlamydia was injected into the prostatic urethra. Anesthesia was maintained for 1 h to prevent urinary leakage due to movement of the rat and to allow a sufficient time for bacteria to invade the prostate.

Measurement of prostate index (PI) and prostate specific antigen (PSA)

The prostatic index (PI) of all rats was computed as prostate weight (mg)/body weight (g). Blood was collected by removing the eyeball. The blood sample was allowed to clot, and the serum was separated at 3500 r/min for 15 min. ELISA kits (provided by Shenzhen Xin-Bo-Sheng Biological Technology Co Ltd, Shenzhen, China) then were used to determine the level of prostate specific antigen (PSA).

Measurement of tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β)

Pro-inflammatory cytokines tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) were measured in prostate tissue from CBP group and PCS-treated groups using commercial ELISA assay kits (Shenzhen Xin-Bo-Sheng Biological Technology Co Ltd) according to the manufacturer's instructions. Samples and standards were run in duplicate and resulting data were averaged. Results are expressed in pg/mL.

Measurement of PGE₂, COX-2, TGF- β 1, and CTGF

The effects of CBP on prostaglandin E₂ (PGE₂), cyclooxygenase-2 (COX-2), transforming growth factor β 1 (TGF- β 1), and connective tissue growth factor (CTGF) levels were measured in prostate tissues using commercial ELISA kits (Shenzhen Xin-Bo-Sheng Biological Technology Co Ltd). All assays were performed in 10 % prostate supernatant in accordance with the manufacturer's instructions. Results are expressed in pg/mL.

Histopathological examination

Prostates were excised and fixed in 4 % paraformaldehyde for histopathological studies. Sections of prostates were dehydrated by ethanol, and 4 - 5 mm sections were cut, stained with hematoxylin and eosin, and examined under a light microscope (Beijing Olympus Co. Ltd., Beijing China).

Statistical analysis

Data are presented as mean \pm standard deviation (SD), and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test in Statistical Package for the Social Sciences software (SPSS for Windows, version 16.0, SPSS, Inc., Chicago, IL, USA). Differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of PCS extract on PI and PSA

The effects of 4 weeks of oral administration of PCS extract on PI and PSA levels are summarized in Table 1. Compared with healthy controls, PI and PSA increased to 2.4 mg/g and 327.8 pg/mL, respectively ($p < 0.01$), in the *Chlamydia*-induced CBP group. After treatment with PCS extract, PI and PSA significantly decreased ($p < 0.01$). The largest decrease was observed in response to the highest dose of PCS extract. PI and PSA levels also decreased after 4 weeks of tetracycline administration ($p < 0.01$).

Effect of PCS extract on TNF- α and IL-1 β

The effects of 4 weeks of oral administration of PCS extract on TNF- α and IL-1 β levels are

summarized in Table 2. Compared with healthy controls, TNF- α significantly increased in *Chlamydia*-induced CBP rats ($p < 0.01$). TNF- α was significantly decreased by 160 and 320 mg/kg doses of PCS extract compared with the model group ($p < 0.05$ and $p < 0.01$, respectively). Similarly, IL-1 β was significantly increased in *Chlamydia*-induced CBP rats compared with healthy controls ($p < 0.01$), and significantly decreased by 160 and 320 mg/kg doses of PCS extract ($p < 0.01$). Tetracycline also significantly decreased TNF- α and IL-1 β levels ($p < 0.01$).

Effect of PCS extract on PGE₂, COX-2, TGF- β 1, and CTGF

The effects of 4 weeks of oral administration of PCS extract on PGE₂, COX-2, TGF- β 1, and CTGF levels are summarized in Table 3. PGE₂, COX-2, TGF- β 1, and CTGF were all significantly increased in *Chlamydia*-induced CBP rats ($p < 0.01$). After treatment with PCS, both PGE₂ and TGF- β 1 significantly decreased in a dose-dependent manner ($p < 0.05$). Similarly, CTGF and COX-2 were both significantly decreased by 160 and 320 mg/kg doses of PCS extract ($p < 0.01$). Tetracycline also significantly decreased PGE₂ ($p < 0.01$), COX-2 ($p < 0.05$), TGF- β 1 ($p < 0.05$), and CTGF ($p < 0.01$) levels.

Table 1: Effect of PCS extract on PI and PSA levels

Group	Dose (mg/kg)	PI (mg/g)	PSA (pg/mL)
Healthy	—	0.8 \pm 0.2	119.3 \pm 10.8
Model	—	2.4 \pm 0.2	327.8 \pm 20.5
Tetracycline	80	1.5 \pm 0.4	179.4 \pm 15.6**
PCS-L	80	1.6 \pm 0.3	271.6 \pm 30.4
PCS-M	160	1.3 \pm 0.2*	178.7 \pm 20.8*
PCS-H	320	1.0 \pm 0.1**	149.2 \pm 13.5**

Data are expressed as the mean \pm SD; n = 10 in each group; PCS-L = low dose of PCS extract; PCS-M = moderate dose of PCS extract; PCS-H = high dose of PCS extract; * $p < 0.05$, ** $p < 0.01$ vs. model group

Table 2: Effect of PCS extract on TNF- α and IL-1 β levels

Group	Dose (mg/kg)	TNF- α (pg/mL)	IL-1 β (pg/mL)
Healthy	—	99.6 \pm 8.5**	73.6 \pm 6.4**
Model	—	158.4 \pm 13.2	174.1 \pm 12.1
Tetracycline	80	119.5 \pm 7.4**	120.4 \pm 11.7**
PCS-L	80	146.3 \pm 15.8	149.7 \pm 14.8
PCS-M	160	131.7 \pm 8.2*	122.5 \pm 8.7**
PCS-H	320	122.5 \pm 9.3**	98.6 \pm 10.3**

Data are expressed as the mean \pm SD (n = 10); PCS-L = low dose of PCS extract; PCS-M = moderate dose of PCS extract; PCS-H = high dose of PCS extract; * $p < 0.05$, ** $p < 0.01$ vs. model group

Table 3: Effect of PCS extract on PGE₂, COX-2, TGF-β1, and CTGF levels

Group	Dose (mg/kg)	PGE ₂ (pg/mL)	COX-2 (pg/mL)	TGF-β1 (pg/mL)	CTGF (pg/mL)
Healthy		66.5±3.6	11.5±1.3	70.7±4.1	57.2±3.4
Model		126.6±6.7	33.5±3.4	146.2±12.7	117.4±5.8
Tetracycline	80	85.4±5.9 [*]	15.2±2.8 [*]	110.4±8.6 [*]	89.4±6.1 ^{**}
PCS-L	80	102.6±4.8 [*]	26.1±4.3 [*]	126.4±9.6 [*]	115.6±7.1 ^{**}
PCS-M	160	93.4±6.4 [*]	17.1±3.2 ^{**}	111.6±7.6 [*]	93.3±8.4 ^{**}
PCS-H	320	81.4±4.5 [*]	14.5±2.5 ^{**}	87.5±8.1 ^{**}	72.4±6.3 ^{**}

Data are expressed as the mean ±SD (n = 10); PCS-L = low dose of PCS extract; PCS-M = moderate dose of PCS extract; PCS-H = high dose of PCS extract; *p < 0.05, **p < 0.01 vs. model group

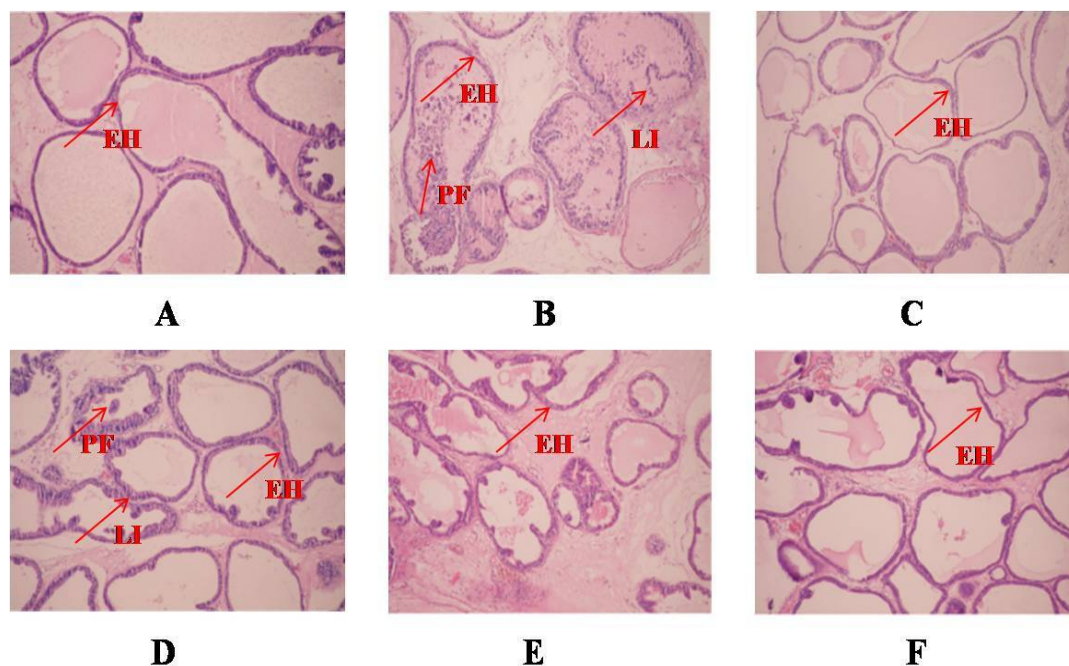


Figure 1: Effect of PCS extract on the histomorphology of prostate tissues in rats. Hematoxylin and eosin stained (x 200) prostate cells in the A: healthy group; B: model group; C: positive group (80 mg/kg tetracycline); D: 80 mg/kg PCS extract group; E: 160 mg/kg PCS extract group; F: 320 mg/kg PCS extract group; EH = epithelial height; PF = papillary fronds; LI = leukocyte infiltration

Histopathological characteristics

No change in the morphological structure of the prostate gland was found in healthy controls (Figure 1A). In contrast, severe diffuse chronic inflammation characterized by leukocyte infiltration and papillary frond protrusion into the gland cavities, as well as an increase in prostatic epithelial height, were observed in the lateral lobe of the prostate in chlamydia-induced CBP rats (Figure 1B). However, these changes were significantly suppressed in rats administered tetracycline or PCS extract, especially at doses of 160 and 320 mg/kg of PCS extract per day (Figure 1E–F).

DISCUSSION

PCS is a traditional Chinese medicine used for the treatment of prostatitis in China. In our study,

experimental CBP was induced by *Chlamydia*, which was confirmed by increased levels of PI and PSA. Four weeks of oral administration of PCS extract significantly decreased PI and PSA, as well as several inflammatory markers, including COX-2, PGE₂, TGF-β1, and CTGF. Thus, the administration of PCS extract for 4 weeks significantly inhibited the development of chronic inflammation and fibrosis in prostatic tissue [11,12].

IL-1β is a pro-inflammatory cytokine [13], and TNF-α is rapidly produced by macrophages in response to tissue damage [14]. Studies have shown that the activation of transcription factor NF-κB by TNF-α is one of several actions of TNF-α [15]. Cytokine-based therapies have been found to be useful in preventing the progression of chronic prostatitis [16]. In the present study, increased levels of TNF-α and IL-1β in chlamydia-induced CBP rats were suppressed by

160 and 320 mg/kg of PCS extract. Similarly, increased levels of COX-2 and PGE₂ in chlamydia-induced CBP rats were reversed by PCS treatment. Therefore, the anti-CBP effect of PCS extract may be related to its anti-inflammatory properties.

TGF- β , which is the most extensively studied molecule in fibrosis, stimulates the production of reactive oxygen species in various types of cells [17-19]. In addition, CTGF has been implicated in fibroblast proliferation, cellular adhesion, angiogenesis, and extracellular matrix synthesis [20-22]. Our results indicate that PCS extract suppresses CBP-enhanced TGF- β 1 expression. Our results also suggest that PCS extract regulates the CTGF signaling pathway following TGF- β 1 stimulation.

CONCLUSION

The results of this study demonstrate that PCS has a significant anti-inflammatory effect on chronic bacterial prostatitis in rats. Further development *in vivo* studies are required to its therapeutic potentials.

REFERENCES

- Motrich RD, Maccioni M, Molina R, Tissera A, Olmedo J, Riera CM, Rivero VE. Presence of INF gamma-secreting lymphocytes specific to prostate antigens in a group of chronic prostatitis patients. *Clin. Immunol* 2005; 116: 149-157.
- Werner WH. Anti-inflammatory therapies for chronic prostatitis. *Eur. Urol. Suppl* 2003; 2: 30-33.
- Nickel JC, Olson ME, Costerton JW. Rat model of experimental bacterial prostatitis. *Infection*. 1991; 19: 126-130.
- Nickel JC. Prostatitis and related conditions. In: Walsh PC, Reik AB, Vaughan ED Jr, Wein AJ, editors. *Campbell's urology*. 8th ed. Philadelphia: Saunders; 2002. p. 604-630.
- Park SD, Lai YSH, Kim CHH. Immunopotentiating and antitumor activities of the purified polysaccharides from *Phellodendron chinese* Schneid. *Life Sci* 2004; 75: 2621-2632.
- Jung HW, Jin GZ, Kim SY. Neuroprotective effect of methanol extract of *Phellodendri Cortex* against 1-methyl-4-phenylpyridinium (MPP⁺)-induced apoptosis in PC-12 cells. *Cell Biology International*. 2009; 33: 957-963.
- Chen L, Di DL. Study on Antibacterial Effect *in vitro* of *Phellodendron amurense* Rupr (PAR). *Lishizhen Med. and Mate Medical Res*. 2006; 17: 759-760.
- Xian YF, Mao QQ. Comparison on the anti-inflammatory effect of Cortex *Phellodendri Chinensis* and Cortex *Phellodendri Amurensis* 12-O-tetradecanoyl-phorbol-13-acetate-induced ear edema in mice. *J Ethnopharmacol* 2011; 137: 1425-1430.
- European Commission. 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.
- Dong WS, Chang HH, Yun SJ. Anti-inflammatory and antimicrobial effects of garlic and synergistic effect between garlic and ciprofloxacin in a chronic bacterial prostatitis rat model. *Int J Antimicrob Agent* 2009; 34: 215-219.
- Nadler RB, Koch AE, Calhoun EA, Campbell PL, Pruden DL, Bennett CL, Yarnold PR, Schaeffer AJ. IL-1beta and TNF-alpha in prostatic secretions are indicators in the evaluation of men with chronic prostatitis. *J. Urol.* 2000; 164: 214-218.
- Tsunemori H, Sugimoto M, Xia Z, Taoka R, Oka M, Kakehi Y. Effect of the phytotherapeutic agent Evi prostat on inflammatory changes and cytokine production in a rat model of nonbacterial prostatitis. *Urology* 2011; 77: e1515.
- Dinarello CA. Interleukin-1, interleukin-1 receptors and interleukin-1receptor antagonist. *Int. Rev. Immunol.* 1998; 16: 457-499.
- Beutler B, Cerami A. The biology of cachectin/TNF-a primary mediator of the host response. *Annu. Rev. Immunol.* 1989; 7: 625-655.
- Tahir M, Rehman MU, Lateef A, Khan R, Khan AQ, Qamar W, Ali F, O'Hamiza O, Sultana S. Diosmin protects against ethanol-induced hepatic injury via alleviation of inflammation and regulation of TNF-alpha and NF-kappa B activation. *Alcohol*. 2013; 47: 131-139.
- Lu BY, Cai HF, Huang, WS, Wu XQ, Luo YX, Liu L, Zhang, Y. Protective effect of bamboo shoot oil on experimental nonbacterial prostatitis in rats. *Food. Chem.* 2011; 124: 1017-1023.
- Liu RM, Gaston KA. Oxidative stress and glutathione in TGF-beta-mediated fibrogenesis. *Free. Radic. Biol. Med.* 2010; 48: 1-15.
- Untergasser G, Gander R, Lilg C, Lepperdinger G, Plas E, Berger P. Profiling molecular targets of TGF-beta1 in prostate fibroblast-to-myofibroblast trans differentiation. *Mech. Ageing. Dev.* 2005; 126: 59-69.
- Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. *FASEB J.* 2004; 18: 816-827.
- Lau LF, Lam SC. The CCN family of angiogenic regulators: the integrin connection. *Exp. Cell. Res.* 1999; 248: 44-57.
- Kular L, Pakradouni J, Kitabgi P, Laurent M, Martinerie C. The CCN family: a new class of inflammation modulators? *Biochimie.* 2011; 93: 377-388.
- Borthwick LA, Wynn TA, Fisher AJ. Cytokine mediated tissue fibrosis. *Biochim. Biophys. Acta.* 2013; 1832: 1049-1060.