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Original Research Article

Effect of *Phellodendron chinense* Extract on Carrageenan-Induced Chronic Prostatitis in Rats

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Abstract

Purpose: *To investigate the effect of Phellodendron chinense Schneid (PCS) extracts on carrageenan-induced chronic prostatitis in rats.*
Methods: *Experimental chronic non-bacterial prostatitis (CNP) was induced in rats by injecting carrageenan into prostatitis. Rats in treated groups were administered either PCS extract or positive control (cernilton) for 3 weeks while those in the control group received saline instead. After treatment, prostate index (PI) and prostate-specific antigen (PSA) of all rats were examined by ELISA. The degree of chronic inflammatory cell infiltrates, acinar changes and interstitial fibrosis were evaluated by histopathological examination. In addition, relative inflammatory factors, viz, tumor necrosis factor-α (TNF-α), interleukin1β (IL-1β), cyclooxygenase-2 (COX-2), prostaglandin E2 (PEG_2), transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) of prostate tissues of all the rats were measured by ELISA.*
Results: *High-dose PCS (400 mg/kg) significantly decreased PI (1.0 ± 0.1 mg/ml) relative to reference group (2.1 ± 0.2, $p < 0.01$) while high-dose PCS (400 mg/kg) significantly decreased PSA level (154.2 ± 14.3 pg/ml) relative to the reference group (312.5 ± 20.5 pg/ml, $p < 0.01$). Morphometric analysis demonstrated the presence of chronic inflammatory cell infiltrates, and interstitial fibrosis decreased significantly compared to the reference group. Compared with reference group (165.4 ± 14.2 pg/ml), TNF-α level in the prostate tissues of high-dose PCS group rats decreased (116.5 ± 9.3) significantly ($p < 0.01$). Similarly, compared with reference group (176.1 ± 12.1 pg/ml), IL-1β level of prostate tissues of high-dose PCS group rats decreased (97.6 ± 11.3) significantly ($p < 0.01$).*
Conclusion: *PCS extract has significant therapeutic effect on carrageenan-induced chronic prostatitis in rats.*

Keywords: *Phellodendron chinense, Chronic prostatitis, Chronic inflammation*

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INTRODUCTION

Epidemiological research of the past decade indicates prostatitis as one of the major medical healthcare problems in urology [1]. It is the most common urological condition in men under 50 years of age. Prostatitis has been classified into three clinical entities: Acute bacterial prostatitis; chronic bacterial prostatitis and chronic prostatitis (CP)/chronic pelvic pain syndrome (CPPS) [2]. Chronic non-bacterial prostatitis (CNP) is the most common form of the prostatitis syndromes, approximately eight times more prevalent than bacterial prostatitis [3]. CNP is characterized by chronic, idiopathic pelvi-perineal pain and an inflammatory sub-type with leukocytes expressed in their prostatic secretions, post prostate massage urine, or semen [4]. Cernilton is one of

the most widely used drugs for treating chronic non-bacterial prostatitis, but has not achieved significant curative effect in clinic.

Owing to an apparent dissatisfaction with the 'standard' medical approach to the treatment of chronic prostatitis, a large number of patients are seeking relief outside of traditional approaches and attention has been paid to phytotherapy and other alternative treatments for improvement in the quality of life for patients with chronic prostatitis. Herbal based therapies are prevalent and popular in urological disease, more so in prostatic disorders. Examples include Chinese herbs, green tea extracts, saw palmetto and bee pollen, but unfortunately not all studies are adequately controlled [5-7].

The plant *Phellodendron chinense* Schneid, which is widely distributed in Southwest of China, is the main material of traditional Chinese medicine ''huangbai''. It was used as folk medicine for immune-modulation [8], anti-tumor [9] and anti-bacterial, etc [10,11]. In the present study, we evaluated the therapeutic effects of PCS extract against carrageenan-induced chronic non-bacterial prostatitis and explored its possible mechanism of action.

EXPERIMENTAL

Materials

Herbal samples of *Phellodendron chinense* Schneid were collected from Bozhou City, Anhui Province in China in July 2014. Taxonomic identification of the plant was performed by Professor Wei-ke Lu of Henan University of Science and Technology, in China. A voucher specimen (No. PCS 201308024) was deposited in, Henan University of Science and Technology, China for future reference.

The herbal samples *Phellodendron chinense* Schneid was dried by an oven. Aqueous extract of PCS was obtained by steeping the dried *Phellodendron chinense* Schneid in water at 60 ^{o}C three times, each for one hour before first drying in an oven and then freeze-drying the last extract thus obtained. One gram powder was equivalent to about 1.8 g crude samples. The yield was 53.28 %.

Animals

Eight week old male Wistar rats (300 – 350 g) were provided by the Experimental Animal Center of Henan Province (Certificate no. SYXK 2004-0003). The animals had free access to feed and water, and were allowed to acclimatize for at least one week before use. The rat experiment was approved by the Animal Care and Use Committee of Henan University of Science and Technology (Approval ref no. 20131016) and was carried out in compliance with the Directive 2010/63/EU on the handling of animals used for scientific purposes [12].

Animal groups

The rats were randomly divided into 6 groups of ten rats: Control group, reference group, positive drug group (cernilton 100 mg/kg) as well as PCS extracts groups, namely, 100, 200 and 400 mg/kg doses. Drugs were dissolved in water, and administered using a 5-ml syringe with a 4-cm long gavage needle through the mouth to the mouth once daily for 3 weeks.

Carrageenan-induced chronic non-bacterial prostatitis model (CNP)

The rats in the CNP groups were induced as previously described [13]. Briefly, for control group, prostates of each rat were injected with 0.1 mL saline by an injector, and the same volume of 1 % carrageenan in reference and drug-treated groups. Seven days later, rats in PCS extract group were kept for oral administration of PCS extract, and rats in positive drug group were given of cernilton for 3 weeks. Rats of sham and model groups were given saline at the same time.

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

The prostatic index (PI) of all rats was assessed as the ratio of prostate weight (mg) to rat body weight (g). The blood sample was taken from eye enucleation, and serum was separated at 3500 r/min for 15 min and used for determination of prostate specific antigen (PSA) by ELISA kits (Shenzhen Xin-Bo-Sheng Biological Technology Co Ltd, China).

Biochemical assays

After sacrificing the rats, the pro-inflammatory cytokines, TNF-α and IL-1β, from prostate tissues in both CNP and drug-treated groups were measured by commercial ELISA assay kits (Shenzhen Xin-Bo-Sheng Biological Technology Co Ltd, China), according to manufacturer's recommendations. The samples and standards were all run in duplicates and the data were then averaged. The results are expressed as pg/ml.

PGE_2, COX-2, TGF-β1 and CTGF were measured in prostate tissues using commercial ELISA kits (Shenzhen Xin-Bo-Sheng Biological Technology Co Ltd, China). All assays were performed in 10 % prostate supernatant in accordance with manufacturer's instructions. The levels of PGE_2, COX-2, TGF-β1 and CTGF in prostate tissue are expressed in pg/mL.

Histopathological examination

After sacrificing the rats, the prostate of rats was excised and fixed in 4 % paraformaldehyde for histopathological research. Prostates sections were dehydrated by series of graded ethanol and sections of 4 – 5 mm were cut and stained with haematoxylin and eosin, and then examined under a light microscope.

Statistical analysis

Values were presented as means ± standard deviation (SD). Results were analyzed statistically by one-way ANOVA followed by Tukey's multiple comparison using SPSS 16.0 software for Windows. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of PCS extract on PI and PSA

The effects of oral administration of PCS extract on the levels of PI and PSA are summarized in Table 1. Compared with control group, PI and PSA levels of reference group rats both increased significantly ($p < 0.01$). Compared with reference group, PI and PSA levels of high-dose of PCS extract decreased significantly ($p < 0.01$).

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

As shown in Table 2, the TNF-α level was 100.8 pg/mL in sham group. Carrageenan-treatment caused significant increase in the level of TNF-α compared with the sham group ($p < 0.01$). Oral treatment of PCS extract at dose of 400 mg/kg resulted in decrease of TNF-α level when compared to model group ($p < 0.01$). The level of IL-1β was significantly increased in model group compared to control group ($p < 0.01$). However, the IL-1β level was significantly decreased to 117.5 or 97.6 pg/mL at the dose of 200 and 400 mg/kg groups respectively ($p < 0.01$).

Effect of PCS extract on PGE_2, COX-2, TGF-β1 and CTGF

As shown in Table 3, the level of TGF-β1 was 72.6 pg/mL in sham group. Carrageenan caused significant increase in the level of TGF-β1 in model group ($p < 0.01$). After PCS extract has been treated for three weeks, the level of TGF-β1 was dose-dependently decreased ($p < 0.01$). Similarly, the level of CTGF was elevated in model group when compared with the sham group ($p < 0.01$). However, in PCS extract treated group, the elevation was suppressed compared with the model group ($p < 0.01$).

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

Group	Dose (mg/kg)	PI (mg/g)	PSA (pg/ml)
Control	—	0.8±0.2**	123.2±10.8**
Reference	—	2.1±0.2	312.5±20.5
Cernilton	100	1.5±0.4	182.7±16.4**
PCS-L	100	1.6±0.3	274.6±33.5
PCS-M	200	1.3±0.2*	180.5±21.9*
PCS-H	400	1.0±0.1**	154.2±14.3**

*$p < 0.05$, **$p < 0.01$ vs. reference_group; values are mean ± SD (n = 10); PCS-L = low-dose PCS extract; PCS-M = middle-dose PCS extract; PCS-H = high-dose PCS extract*

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

Group	Dose (mg/kg)	TNF-α (pg/ml)	IL-1β (pg/mL)
Control	—	100.8±9.7**	73.6±6.4**
Reference	—	165.4±14.2	176.1±12.1
Cernilton	100	121.5±8.4**	114.5±11.7**
PCS-L	100	148.3±16.8	152.7±15.8
PCS-M	200	134.7±10.2*	117.5±10.7**
PCS-H	400	116.5±9.3**	97.6±11.3**

*$p < 0.05$, **$p < 0.01$ vs. reference group; values are mean ± SD (n = 10)*

Carrageenan treatment stimulated the level of COX-2 compared to sham group ($p < 0.01$). However, treatment of PCS extract decreased the level of COX-2 ($p < 0.01$). The level of PEG_2 was increased in model group compared to sham group ($p < 0.01$). Oral treatment of PCS extract at 200 and 400 mg/kg resulted in significant decrease of PEG_2 content when compared with model group ($p < 0.01$).

Histopathological features

Histological analysis showed that there was no change in the morphological structure of the prostate gland of rats in sham group (Fig 1A), while severe diffuse chronic inflammation characterized by leukocyte infiltration and papillary fronds protruded into the gland cavities, the prostatic epithelial height was increased notably in the lateral lobe of the prostate from rats in the model group (Fig 1B). However, these changes were significantly suppressed in the rats administered cernilton or PCS extract, especially those given a dose of 200 and 400 mg/kg PCS extract per day (Fig 1E–F).

Table 3: Effect of PCS extract on PGE_2, 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)
Control		67.8±3.6**	12.5±1.2**	72.6±4.1**	56.7±3.4**
Reference		128.6±6.7	31.5±3.4	149.2±12.7	121.4±5.8
cernilton	100	87.4±5.9**	16.2±2.8*	108.4±8.6*	87.4±6.1**
PCS-L	100	105.6±4.8*	25.6±4.3	125.3±9.6*	114.4±7.1
PCS-M	200	92.3±6.4**	16.4±3.2**	110.5±7.6**	92.4±8.4**
PCS-H	400	79.2±4.5**	13.3±2.5**	86.7±8.1**	71.2±5.3**

*$P < 0.05$; **$p < 0.01$ vs. reference group; values are mean ± SD ($n = 10$)*

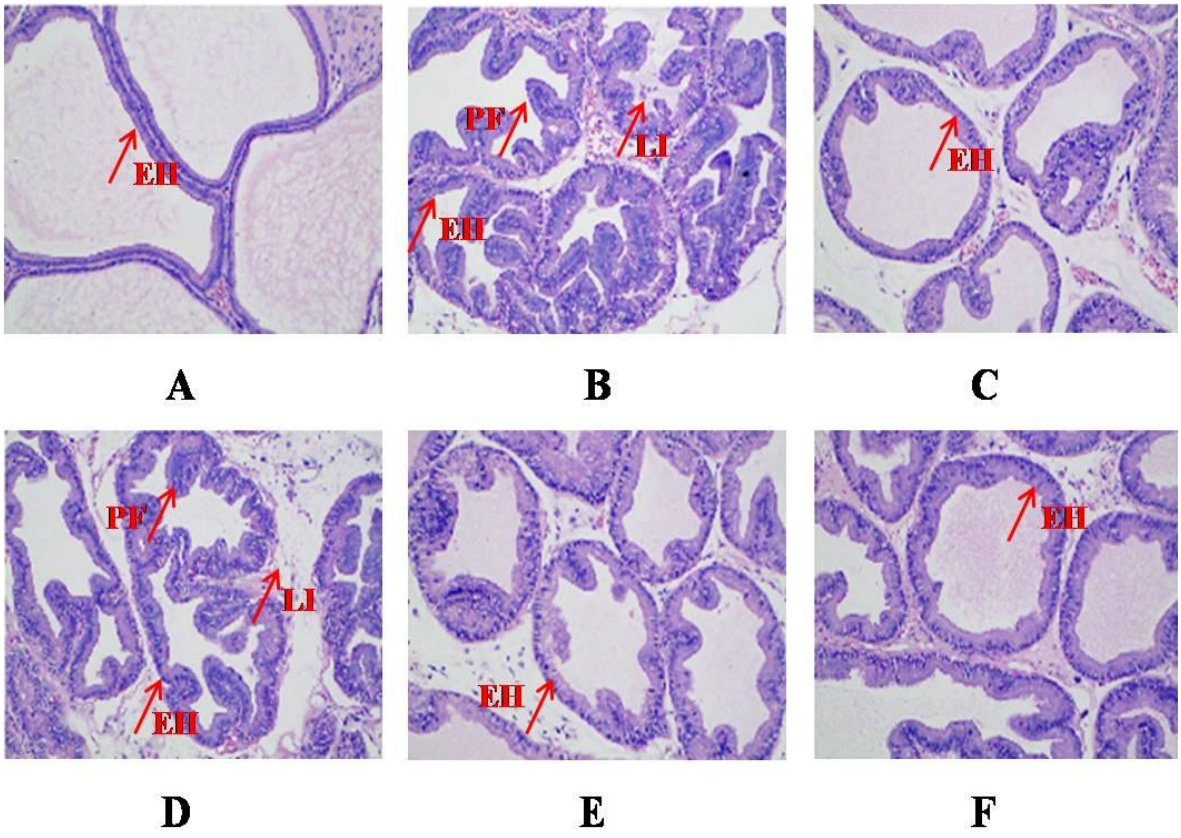

Fig 1: Effect of PCS extract on the histomorphology of prostate tissues in rats. EH: epithelial height; PF: papillary fronds; LI: leukocyte infiltration; A: control group; B: reference group; C: positive drug group (cernilton 100 mg/kg); D: PCS extract group (100 mg/kg); E: PCS extract group (200 mg/kg) F: PCS extract group (400 mg/kg); HE: 200×)

DISCUSSION

PCS is traditionally used in Chinese medicine for treatment of prostatitis. In our study, the experimental chronic non-bacterial prostatitis was induced by carrageenan. The increased levels of PI and PSA were detected in model group which proved that carrageenan successfully established prostatitis. Then the potential therapeutic effect of PCS in rats with carrageenan-induced prostatitis was evaluated. The whole results showed that PCS extract successfully reduced COX-2 and PEG_2 in model group. The levels of TGF-β1 and CTGF in PCS extract treated groups were significantly decreased along with the inflammatory state of the prostate gland were alleviated. Thus, the administration of PCS extract for 3 weeks significantly inhibited the development of chronic inflammation and fibrosis in prostatic tissue and some underlying mechanisms might be correlated with these properties.

Currently, it is well accepted that the progression of CNP related to the complex network of cytokines, such as IL-1β and TNF-α [14,15]. IL-1β is a pro-inflammatory cytokine that induces the production of other inflammatory mediators involved with cellular recruitment, fever, acute phase protein release, increase of vascular permeability, and hyperalgesia [16]. TNF-α, a pleiotropic pro-inflammatory cytokine, is rapidly produced by macrophages in response to tissue damage [17]. Previous studies have shown that activation of transcription factor NF-κB by TNF-α is one of the myriad actions of TNF-α that cause genes to generate potentially cell damaging oxidative enzymes, as well as further release of TNF-α, IL-1β and other pro-inflammatory cytokines [18-20]. Cytokine based therapies have been found to be useful in preventing progression of chronic prostatitis [21]. In the present study, the levels of TNF-α and IL-1β were increased in model group rats, whereas on treatment with PCS extract at 200 or 400 mg/kg, there was a significant decrease in the cytokine levels. PCS extract capable of suppressing the release of pro-inflammatory mediators could possess anti-inflammatory activities. In this study, the levels of COX-2 and PEG_2 in the model group, were enhanced. However, the increased levels of COX-2 and PEG_2 were reversed in treatment group of PCS extract. In addition, it was found that PCS extract at the dose of 400 mg/kg significantly decreased COX-2 and PEG_2 levels. Therefore, the anti-CNP effect of PCS extract may be related to its anti-inflammatory properties.

TGF-β is the most extensively studied molecule in fibrosis and stimulates the production of reactive oxygen species (ROS) in various types of cells, whereas ROS activated TGF-β and mediate many of the fibrogenic effects of TGF-β [22]. TGF-β1 is known to induce fibroblast differentiation of into myofibroblast/smooth muscle cell in the human prostate [23]. In addition, other evidence suggested that pro-fibrotic effects of TGF-β may be partly mediated by CTGF [24]. As another potent profibrotic factor, CTGF is implicated in fibroblast proliferation, cellular adhesion, angiogenesis, and extracellular matrix (ECM) synthesis [25]. Previous studies showed that CTGF promotes inflammatory response [26]. Chronic inflammatory response can result in pathological wound repair and the accumulation of permanent fibrotic scar tissue at the site of injury and this fibrosis may lead to a decrease in organ function and, in some cases, organ failure and death [27]. Summarily, another possible hypothesis could be given that the TGF-β1/CTGF pathway may also be involved in the CNP. The results indicated that PCS extract could suppress the enhancement of the TGF-β1 expression compared with model group rats. At 400 mg/kg, the decreased level of TGF-β1 was observed, as well as the level of CTGF was decreased in PCS extract treated groups. PCS extract regulated the CTGF signaling pathway following the TGF-β1 stimulation.

CONCLUSION

The findings of this study demonstrate that PCS has a significant anti-inflammatory and anti-fibrosis effect on chronic prostatitis in rats.

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