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Original Research Article

Effect of Achyranthes bidentata Blume extract on carrageenan-induced chronic prostatitis in rats

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Abstract

Purpose: *To investigate the effect of Achyranthes bidentata Blume. Extract (ABBE) on carrageenan-induced chronic prostatitis in rats.*
Methods: *Experimental chronic non-bacterial prostatitis (CNP) was induced in rats by 1 % carrageenan (0.1 mL). Prostate index (PI) and prostate specific antigen (PSA) were determined after 3 weeks of adiministration. Relative inflammatory factors, viz, tumor necrosis factor-α (TNF-α), interleukin1β (IL-1β), cyclooxygenase-2 (COX-2), prostaglandin E2 (PEG2), transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) in the prostate tissues of all group rats were measured.*
Results: *In CNP model, ABBE decreased PI and PSA compared with untreated control group ($p < 0.01$). Morphometric analysis showed a significant reduction in chronic inflammatory cell infiltrates and interstitial fibrosis compared to control group. Reduced values of TNF-α, IL-1β, COX-2, PEG2 were observed in ABBE-treated groups compared with model group respectively ($p < 0.01$). Moreover, the levels of TGF-β1 and CTGF in ABBE-treated groups were significantly lower, and in addition, there was alleviation of the inflammatory state of the prostate gland ($p < 0.01$).*
Conclusion: *ABBE showed antichronic non-bacterial prostatitis effect by multiple ways in rats, thus indicating its potential suitability for the treatment of chronic prostatitis.*

Keywords: *Achyranthes bidentata Blume., Chronic prostatitis, Inflammatory cell*

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INTRODUCTION

Epidemiological research indicates prostatitis is one of the major medical healthcare problems in urology [1]. Prostatitis has been classified into three clinical entities: (I) acute bacterial prostatitis (II) chronic bacterial prostatitis; (III) chronic prostatitis (CP)/chronic pelvic pain syndrome (CPPS) [2]. Category III is further subdivided into category IIIA or inflammatory, and category IIIB or non-inflammatory. Chronic non-bacterial prostatitis (CNP), which belongs to category IIIA, is the most common form of the prostatitis syndromes, approximately eight times more prevalent than bacterial prostatitis [3]. CNP is characterized by chronic, idiopathic pelviperineal pain and an inflammatory subtype with leukocytes expressed in their prostatic secretions [4].

The plant, *Achyranthes bidentata Blume.*, widely distributed in southwest of China, was the main material of traditional Chinese medicine "niuxi", was used as folk medicine for immune-modulation [5], anti-tumor [6] and anti-bacterial [7,8], etc. In this study, we evaluated the effect of

ABBE against carrageenan-induced chronic non-bacterial prostatitis and its possible mechanisms.

EXPERIMENTAL

Plant material

Achyranthes bidentata Blume was collected from Bozhou City, Anhui Province in China in May 2015. Taxonomic identification of the plant was performed by Professor Hui Wang of Fudan University, in China. A voucher specimen (no. ABBE 20150504) was deposited in the herbarium of College of Pharmacy, Fudan University, China for future reference. The aqueous extract of ABBE was obtained by steeping three batches of dried *Achyranthes bidentata Blume* in water at 60 ^{o}C three times, each for one hour, and then drying the extract in an oven and thereafter freeze-drying it. One gram powder was equivalent to about 1.5 g crude sample, giving a yield of 66.67 %.

Animals

Eight-week old male Wistar rats (300 – 350 g) were provided by the Experimental Animal Center of Shanghai Municipality (certificate no. SYXK 2008-0006). 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 the Animal Care and Use Committee of Huadong hospital affiliated to Fudan University (approval ref no. 20111013) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [9].

Animal groups

The rats were randomly divided into 6 groups of ten rats each: normal group, negative control group, positive control group (cernilton 100 mg/kg) as well as ABBE groups, namely, 100, 200 and 400 mg/kg doses. Treatments were given orally once daily for 3 weeks, dissolved in water. The drugs were given into the rats by intragastric administration.

Preparation of carrageenan-induced chronic non-bacterial prostatitis (CNP) model

CNP model rats were prepared as previously described [10]. Briefly, for sham group, prostates of each rat were injected with 0.1 mL saline, and the same volume of 1 % carrageenan in model and drug administration groups. Seven days later, rats in drug administration and positive groups were kept for oral administration of ABBE extract or cernilton for 3 weeks, the 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 measured. PI = prostate weight (mg) / body weight (g). The blood sample of rats was get by eyeball removal after the prostates were revoming. The collected 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).

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

Quantitative measurement of pro-inflammatory cytokines TNF-α and IL-1β was done in the prostate tissue of both CNP and treated groups using commercial ELISA assay kits (Shenzhen Xin-Bo-Sheng Biological Technology Co Ltd, China), according to manufacturer's instruction. The samples and standards were all run in quadruplicate and the data are averaged. The results are expressed as pg/mL.

Measurement of PGE_2, COX-2, TGF-β1 and CTGF

The effect of CNP on productions of 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 amounts of PGE_2, COX-2, TGF-β1 and CTGF in prostate tissue were expressed as pg/mL.

Statistical analysis

The data are presented as mean ± standard deviation (SD), and were analyzed 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 ABBE on PI and PSA

After 3 weeks of administration, the effects of oral administration of ABBE on the levels of PI and PSA are summarized in Table 1. In the model group, the levels of PI and PSA increased to 2.1 mg/g and 312.5 pg/mL, respectively ($p < 0.01$). By contrast, after treatment with ABBE,

especially 400 mg/kg, the levels of PI and PSA had dropped significantly ($p < 0.01$).

Table 1: Effect of ABBE on PI and PSA levels (mean ± SD, n = 10)

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

*$p < 0.05$, **$p < 0.01$ vs. model group*

Effect of ABBE on TNF-α and IL-1β

The TNF-α level of the sham group rats was 100.8 pg/ml. Carrageenan-treatment caused significant increase in the level of TNF-α compared with the sham group ($p < 0.01$). Oral treatment of ABBE extract at doses 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 1, 17.5 or 97.6 pg/mL at the dose of 200 and 400 mg/kg groups respectively ($p < 0.01$, Table 2).

Table 2: Effect of ABBE on TNF-α and IL-1β levels (mean ± SD, n = 10)

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

*$p < 0.05$, **$p < 0.01$ vs. model group*

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

ABBE were assessed for their inhibitory activity on TGF-β1 and CTGF production. 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 (< 0.01). After three weeks treatment of ABBE, the TGF-β1 level was dose-dependently decreased ($p < 0.01$). Similarly, the level of CTGF was notably elevated in model group when compared with the sham group ($p < 0.01$). However, in ABBE extract treated group, the elevation was suppressed compared with the model group ($p < 0.01$). Carrageenan treatment stimulated the level of COX-2 compared to sham group ($p < 0.01$). However, treatment of ABBE 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 ABBE extract at 200 and 400 mg/kg resulted in significant decrease of PEG_2 content when compared with model group ($p < 0.01$).

DISCUSSION

Achyranthes bidentata Blume. is traditionally used in Chinese medicine for treatment of prostatitis in China. In our study, the experimental chronic non-bacterial prostatitis was induced by carrageenan. The PI and PSA levels of model group rats were increased proved that prostatitis was established successfully by carrageenan. Then the potential therapeutic effect of ABBE in rats with carrageenan-induced prostatitis was evaluated. The whole results showed that ABBE reduced COX-2 and PEG_2 in model group rats. The TGF-β1 and CTGF levels of ABBE group rats were significantly decreased while the inflammatory state of the prostate gland were alleviated. Thus, the administration of ABBE for 3 weeks significantly inhibited the development of chronic inflammation and fibrosis in prostatic tissue.

It is well accepted that the progression of CNP related to the complex network of cytokines, such as IL-1β and TNF-α [11,12]. IL-1β is a pro-inflammatory cytokine that induces the production of other inflammatory mediators involved with cellular recruitment, fever, acute

Table 3: Effect of ABBE on PGE_2, COX-2, TGF-β1 and CTGF levels (mean ± SD, n = 10)

Group	Dose (mg/kg)	PGE_2 (pg/mL)	COX-2 (pg/mL)	TGF-β1 (pg/mL)	CTGF (pg/mL)
Normal		67.8±3.6**	12.5±1.2**	72.6±4.1**	56.7±3.4**
-ve control		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**
PSA-L	100	105.6±4.8*	25.6±4.3	125.3±9.6*	114.4±7.1
PSA-M	200	92.3±6.4**	16.4±3.2**	110.5±7.6**	92.4±8.4**
PSA-H	400	79.2±4.5**	13.3±2.5**	86.7±8.1**	71.2±5.3**

*-ve, negative; *$P < 0.05$, **$p < 0.01$ vs. model group*

phase protein release, increase of vascular permeability, and hyperalgesia [13]. TNF-α, a pleiotropic pro-inflammatory cytokine, is rapidly produced by macrophages in response to tissue damage [14].

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 [15]. Cytokine - based therapies have been found to be useful in preventing progression of chronic prostatitis [16]. In the present study, the levels of TNF-α and IL-1β were increased in model group rats, whereas on treatment with ABBE extract at 200 or 400 mg/kg, there were significant decreases in the cytokine levels. ABBE could suppress the release of pro-inflammatory mediators, which possess its anti-inflammatory activities. In this study, the COX-2 and PEG_2 levels of rats were examined. It was found that in model group, the levels of those factors were enhanced. However, the increased levels of COX-2 and PEG_2 were reversed in treatment group of ABBE extract. In addition, it was found that ABBE extract at the dose of 400 mg/kg significantly decreased COX-2 and PEG_2 levels. Therefore, the anti-CNP effect of ABBE 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 activate TGF-β and mediate many of the fibrogenic effects of TGF-β [17]. TGF-β1 is known to induce fibroblast differentiation of into myofibroblast/smooth muscle cell in the human prostate [18]. In addition, other evidence suggests that pro-fibrotic effects of TGF-β may be partly mediated by CTGF [19]. As another potent profibrotic factor, CTGF is implicated in fibroblast proliferation, cellular adhesion, angiogenesis, and extracellular matrix (ECM) synthesis [20]. Previous studies showed that CTGF promotes inflammatory response [21]. 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 [22]. Summarily, another possible hypothesis could be given that the TGF-β1/CTGF pathway may also be involved in the CNP. The results indicate that ABBE could suppress the enhancement of the TGF-β1 expression compared with model group rats. And 400 mg/kg of ABBE could decrease the TGF-β1 level of model rats significantly, as well as CTGF level. ABBE regulated the CTGF signaling pathway following the TGF-β1 stimulation. Thus, the anti-CNP effects of ABBE were also associated with the activity of anti-fibrotic.

CONCLUSION

The results of this study demonstrate that ABBE has a significant anti- inflammatory effect on chronic prostatitis in rats, and may thus be suitable for the treatment of chronic prostatitis. There is, however, a need for further investigation of its effectiveness.

DECLARATIONS

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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