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Original Research Article

Effect of Alisma plantago-aquatica Linn extract on chronic prostatitis in rats

Xiao-ming Wang, Dong-dong Wang, Yu-zheng Wu, Peng-de Ma, Guang Sun and Yong Xu*

Department of Urology, Tianjin second Hospital of Tianjin Medical University, Tianjin Institute of Urology, Tianjin 300211, China

For correspondence:* ***Email:* *xuyong494@sina.com;* ***Tel:*** *+86 13001309410*

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Abstract

Purpose: *To study the effect of Alisma plantago-aquatica Linn. extract (APLE) on chronic prostatitis in rats.*
Methods: *Experimental chronic non-bacterial prostatitis (CNP) was induced in rats by injecting carrageenan into prostate. Rats in drug-treated groups were administered APLE or cernilton (positive control, i.e., reference standard) for 3 weeks while rats in normal and negative control groups were treated with saline at the same time. After treatment, prostate index (PI) and prostate-specific antigen (PSA) of all the rats were examined by enzyme-linked immunosorbent assay (ELISA). In addition, the relative inflammatory factors, tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), cyclooxygenase-2 (COX-2), prostaglandin E2 (PEG2), transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) of the prostate tissues were measured by ELISA.*
Results: *A high dose of APLE (480 mg/kg) significantly decreased PI (0.7 ± 0.2 mg/g) relative to reference group (2.8 ± 0.3 mg/g, $p < 0.01$), and significantly decreased PSA level (128.6 ± 12.3 pg/mL) relative to reference group (321.3 ± 16.4 pg/mL, $p < 0.01$). Compared with reference group, TNF-α level (109.7 ± 9.3 pg/mL, $p < 0.01$), IL-1β level (98.3 ± 12.5 pg/mL, $p < 0.01$), PEG2 level (81.5 ± 4.2 pg/mL, $p < 0.01$), COX-2 level (10.5 ± 2.6 pg/mL, $p < 0.01$), TGF-β1 level (86.8 ± 7.3 pg/mL, $p < 0.01$) and CTGF level (70.3 ± 4.3 pg/mL, $p < 0.01$) of prostate tissues of high-dose APLE group rats decreased significantly.*
Conclusion: *APLE shows significant anti-chronic prostatitis activity in rats. Further studies are, however, required to ascertain its therapeutic potentials in humans*

Keywords: *Alisma plantago-aquatica, Chronic prostatitis, Inflammation, Prostate index, Morphometric analysis, Interstitial fibrosis*

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INTRODUCTION

Chronic prostatitis is characterized by a variety of symptoms, and has been shown to have a significant impact on quality of life [1,2]. While most typically associated with pain in the pelvic region, patients may have varying degrees of obstructive and/or irritating voiding symptoms, pain with ejaculation, sexual dysfunction, depression and/or psychosocial dysfunction that may be concomitant or related to the other symptoms. Chronic pelvic or genitourinary pain is a primary component of the condition and is typically present for at least three of the preceding 6 months [3]. As much as 10 – 15 % of the male population may be affected at some point in their lives, and affects men of all ages. Prostatitis is responsible for up to 2 million outpatient clinic visits per year, including 8 % of

all male visits to a urologist and 1 % of men presenting to primary care physicians [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.

At present, orthodox treatment of chronic prostatitis is not satisfactory, and a large number of patients are opting for therapy of traditional Chinese medicine. 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 *Alisma plantago-aquatica Linn.*, which is widely distributed in Southwest of China, is the main material of traditional Chinese medicine ''Zexie''. It was used as folk medicine for immune-modulation [8], anti-tumor [9] and anti-bacterial [10,11]. The aim of the present study was to evaluate the therapeutic effects of APLE against carrageenan-induced chronic non-bacterial prostatitis as well as explore its possible mechanism of action.

EXPERIMENTAL

Materials

Samples of *Alisma plantago-aquatica Linn.* were collected from Liuzhou City, Guangxi Province in China in May 2016. Taxonomic identification of the plant was performed by Professor Ping Wang of Tianjin Medical University, in China. A voucher specimen (no. APLE 201605002) was deposited in the herbarium of College of Pharmacy, Tianjin Medical University, China for future reference.

Alisma plantago-aquatica Linn was dried in an oven. APLE was obtained by steeping the dried *Alisma plantago-aquatica Linn.* in water at 60 ^{o}C three times, each for 1 h before first drying in a oven and then freeze-drying the last extract thus obtained. One gram powder was equivalent to about 1.7 g crude samples. The yield was 58.82 %.

Animals

Eight weeks old male Wistar rats (220 – 250 g) were provided by the Experimental Animal Center of Tianjin Municipality (Certificate no. SYXK 2002-0006). 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 Tianjin Medical University (approval ref no. 20100405) 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 six groups of ten rats in each: control group, reference group, positive drug group (cernilton 100 mg/kg) as well as APLE groups, namely, 120, 240 and 480 mg/kg doses. The drugs were dissolved in water, and administered using a 5 mL syringe with a 4-cm long gavage needle through the mouth once daily for 3 weeks.

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

Chronic non-bacterial prostatitis were induced as previously described [13]. Prostates of rats in control group were injected with 0.1 mL saline by an injector, and the same volume of 1 % carrageenan in rats of other groups. Seven days after preparing the model rats of chronic non-bacterial prostatitis, rats in APLE group, they were orally administered APLE, while rats in positive (reference standard) group were administered cernilton, both groups for 3 weeks. Rats of normal and negative control groups were administered 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). A 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 (Nanjing Jiancheng Biological Technology Co Ltd, Najing, China).

Biochemical assays

After the rats were sacrificed by cervical dislocation, the pro-inflammatorycytokines TNF-α and IL-1β of prostate tissues of all rats were measured by commercial ELISA assay kits (Nanjing Jiancheng Biological Technology Co Ltd, Najing, China), according to manufacturer's instruction. The samples and standards were all run in duplicates and the data were then averaged. The results were 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.

Statistical analysis

Data are presented as mean ± standard deviation (SD) and 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 APLE on PI and PSA

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

Effect of APLE on TNF-α and IL-1β

As shown in Table 2, TNF-α level was 88.6 pg/mL in control group. Carrageenan-treatment caused significant increase in the level of TNF-α compared with the control group ($p < 0.01$). TNF-α level decreased when treated with 480 mg/kg of APLE compared to control group ($p < 0.01$). The level of IL-1β was significantly increased in control group compared to control group ($p < 0.01$). However, the IL-1β level was significantly decreased to 111.6 and 94.1 pg/mL at the dose of 240 and 480 mg/kg groups respectively ($p < 0.01$).

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

As shown in Table 3, the level of TGF-β1 was 81.7 pg/mL in control group. Carrageenan caused significant increase in the level of TGF-β1 in reference group ($p < 0.01$). After rats was treated with APLE 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 control group ($p < 0.01$). However, in APLE treated group, the elevation was suppressed compared with the reference group ($p < 0.01$).

COX-2 level was increased significantly compared to control group ($p < 0.01$). However, treatment of APLE decreased the level of COX-2 ($p < 0.01$). The level of PEG_2 was increased in negative control group compared to control group ($p < 0.01$). Oral treatment of APLE at 240 and 480 mg/kg resulted in significant decrease of PEG_2 content when compared with negative control group ($p < 0.01$).

Table 1: Effect of APLE on PI and PSA levels

Group	Dose (mg/kg)	PI (mg/g)	PSA (pg/ml)
Control	—	0.7±0.2**	106.4±11.8**
Negative control	—	2.8±0.3	321.3±16.4
Cernilton	100	1.6±0.4	141.7±13.5**
APLE-L	120	1.7±0.3	268.5±28.3
APLE-M	240	1.5±0.4*	159.2±17.5*
APLE-H	480	0.8±0.3**	128.6±12.3**

*$P < 0.05$, **$p < 0.01$ vs. negative control group; values are mean ± SD (n = 10); APLE-L: low-dose of APLE; APLE-M: middle-dose of APLE; APLE-H: high-dose of APLE

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

Group	Dose (mg/kg)	TNF-α (pg/ml)	IL-1β (pg/mL)
Control	—	88.6±7.7**	84.5±6.2**
Negative control	—	169.4±13.5	154.2±9.8
Cernilton	100	128.3±9.5**	127.2±11.4**
APLE-L	120	146.6±12.4	137.3±12.2
APLE-M	240	134.1±9.3*	122.5±9.2**
APLE-H	480	109.7±9.3**	98.3±12.5*

*$P < 0.05$, **$p < 0.01$ vs. negative control group. Values are mean ± SD (n = 10)

Table 3: Effect of APLE 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		53.5±3.8**	12.3±1.8**	79.4±4.2**	71.6±4.5**
Negative control		124.1±4.9	38.6±2.9	129.6±12.1	121.4±5.8
Cernilton	100	76.3±4.2**	18.2±2.6*	115.3±8.7*	96.4±4.4**
APLE-L	120	117.2±5.1*	22.3±4.7	123.6±8.4*	112.2±6.2
APLE-M	240	90.2±5.4**	16.5±3.7**	111.4±7.2**	92.5±5.8**
APLE-H	480	81.5±4.2**	10.5±2.6**	86.8±7.3**	70.3±4.3**

*$P < 0.05$, **$p < 0.01$ vs. negative control group. Values are mean ± SD (n = 10)

DISCUSSION

Antibiotics are largely ineffective in the treatment of CNP as the chronic nature of the syndrome is thought not to be completely attributable to an ongoing active or latent bacterial infection. Despite this, up to almost 80 % of CNP patients receive antibiotics as treatment at some point during their disease course, more than 7 times that of non-CNP patients, and many receive multiple rounds of antibiotics despite lack of efficacy [14]. It is well accepted that the progression of CNP is related to the complex network of cytokines, including IL-1β and TNF-α [14,15]. IL-1β is a pro-inflammatory cytokine that induces the production of other inflammatory mediators involved in 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 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 APLE at 240 or 480 mg/kg, there was a significant decrease in the cytokine levels. APLE suppressed the release of pro-inflammatory mediators due to its 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 APLE. In addition, it was found that APLE at the dose of 480 mg/kg significantly decreased COX-2 and PEG_2 levels. Therefore, the anti-CNP effect of APLE 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 activates TGF-β and mediate many of the fibrogenic effects of TGF-β [22]. TGF-β1 is known to induce fibroblast differentiation into myofibroblast/smooth muscle cell in the human prostate [23]. In addition, other evidence suggests 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].

CONCLUSION

The findings of this study reveal that APLE has anti-inflammatory activities and exerts anti-chronic prostatitis effect in rats. However, further investigations are required determines its therapeutic action in humans.

DECLARATIONS

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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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