Multitargeted protective effect of *Abacopteris penangiana* against carrageenan-induced chronic prostatitis in rats

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**A B S T R A C T**

*Ethnopharmacological relevance:* *Abacopteris penangiana* (Hook.) Ching (AP) is traditionally used in Chinese medicine to promote blood circulation, remove blood stasis and dampness and for the treatment of edema and inflammation. In order to further support and develop the traditional use of *Abacopteris penangiana* as Chinese folk medicine, the aim of this study is to investigate the protective effect of the total flavanol glycosides (TFA) from AP and its acid hydrolysate (AHT) on chronic non-bacterial prostatitis (CNP) by measuring the levels of oxidative stress and inflammatory responses in rats.

**Materials and methods:** First, the antioxidant and anti-inflammatory activities of AHT and TFA were investigated. Then the experimental chronic non-bacterial prostatitis was induced by carrageenan. The prostate index (PI) and prostate specific antigen (PSA) were determined. The activities of AHT and TFA on inhibiting free radicals and oxidative stress were investigated. Subsequently, the degree of chronic inflammatory cell infiltrates, acinar changes and interstitial fibrosis were evaluated by histopathological examination. 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) were measured. Finally, the prostatic expression of nuclear transcription factor-κB (NF-κB) was determined by immunohistochemistry and western blot analysis.

**Results:** The whole results showed that AHT and TFA had strong antioxidant and anti-inflammatory activities. In CNP model, AHT and TFA successfully decreased PI and PSA. The activities of antioxidant enzymes in AHT or TFA group were enhanced. Additionally, a morphometric analysis of the prostate gland of AHT or TFA treated rats demonstrated a significant reduction in chronic inflammatory cell infiltrates and interstitial fibrosis compared to model group. The reduced values of TNF-α, IL-1β, COX-2, PEG2, inducible nitric oxide synthase (iNOS) and nitric oxide (NO) were observed both in AHT and TFA treated groups. Moreover, the levels of TGF-β1 and CTGF in AHT and TFA treated groups were significantly decreased along with the alleviation of the inflammatory state of the prostate gland. Besides, the prostatic expression of NF-κB was inhibited.

**Conclusions:** These results suggest that AHT and TFA have anti-prostatitis properties via inhibiting oxidative stress, NF-κB dependent pro-inflammatory cytokines, fibrosis-related factors and antinociceptive activity. Hence, AP represents a potential herb for the treatment of prostatitis.

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1. Introduction

Epidemiological research of the past decade indicates "prostatitis" to be one of the major medical healthcare problems in urology (Motrich et al., 2005). 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) (Werner, 2003). Category III is further subdivided into category IIIA or inflammatory, and category IIIB or noninflammatory. 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 (Schaeffer, 1999). CNP is characterized by chronic, idiopathic pelvic pain, lower urinary tract discomfort, frequent urinary tract infection, prostatitis, and prostatic abscess. In Western society, CNP is considered a phenomenon of Western society, and the clinical signs and symptoms of prostatitis are often attributed to bacterial prostatitis. However, in China, prostatitis is considered to be a disease caused by pathogens, and the pathogen is not clear. For a long time, prostatitis is considered to be a disease caused by bacteria, and the pathogen is not clear.
postprostate massage urine, or semen (Krieger et al., 1999). Recently, oxidative stress has been detected in prostatitis patients and it is well accepted that regions of prostatic inflammation will generate free radicals, such as nitric oxide (NO) and various species of oxygen (Palapattu et al., 2005; Turk and Kullisaar, 2011). Free radicals and oxidative stress are considered to be associated with inflammation (Winrow et al., 1993). The excessive production of reactive oxygen species (ROS), radical nitrogen species (RNS) and prostaglandins (PGs) is a hallmark of the inflammatory process (Forman and Torres, 2002). It is well accepted that radical scavengers can suppress the upregulation of ROS, COX-2 and iNOS subsequently reduce PGs and NO, finally reduce inflammation (Feng et al., 1995; Burk et al., 2009). To sum up, enhancing the activities of the antioxidant enzymes is useful to treat CNP. Some natural polyphenols and flavonoids derived from plants are reported to have the potential effect of enhancing the activities of antioxidant enzymes and anti-inflammatory (Shoskes, 1999; Guabiraba et al., 2010; Cho et al., 2013). And it has been reported that 59% patients of chronic prostatitis have a significant improvement in symptoms after treatment with bioflavonoids (Shoskes et al., 1999). Thus, alternative herbal-based therapies are prevalent and popular in urologic disease in general and prostatic disorders in particular (Shoskes, 2002). In this regard, a primary goal of this study is to search for active flavonoids against CNP from natural products.

*Abacopteris penangiana* (Hook.) Ching is widely distributed throughout the south of China as recorded in the ‘Chinese Materia Medica’. It has been traditionally used as one of the primary Chinese herbs to promote blood circulation, remove blood stasis and dampness, edema and inflammation (The Editorial Committee of Chinese Materia Medica, 1999). In the theory of Traditional Chinese Medicine, dampness–heat obstruction and stasis are two of the most important factors for prostatitis and prostatic hyperplasia (Fan, 2009; Yao et al., 2013). In early study, the rhizome of AP has been proven to contain many flavan glycosides, especially novel flavan-4-ol glycosides (Zhao et al., 2006; Zhao et al., 2008; Zhao et al., 2010). Previous study showed that total flavan glycoside from AP (TFA) and its acid hydrolysate (AHT) were able to improve the symptoms of prostatic hyperplasia (BPH) (Wei et al., 2012). Clinically, the symptoms of BPH and prostatitis have many similarities. Besides, a lot of investigators have found that men who have received a diagnosis of BPH are more likely to also receive a diagnosis of prostatitis compared to men who have never been diagnosed with BPH. Thus, medicine treatment BPH alone is insufficiently effective (Jennifer et al., 2008). Hence, we want to find effective Chinese folk medicine to treat both BPH and prostatitis.

In this study, we prepared TFA, as well as AHT. AHT mainly contains 7-hydroxy-4-methox-6,8-dimethylanthocyanidin, which belongs to 3-deoxygenated anthocyanidins of the flavonoids family. It is well known that many flavonoids have received great attentions owing to their biological properties, including anti-oxidative, radical scavenging, immunoregulative, anti-inflammatory, and anticancer effects (Havsteen, 2002). One of the most well known flavonoids is quercetin, which shows a remarkable antioxidant and anti-inflammatory properties in chronic prostatitis (Shoskes, 1999; Cai et al., 2009). Additionally, it is reported that total flavonoids of *Clerodendranthus spicatus* have therapeutic effects on CNP by decreasing the levels of TNF-α and IL-8 in the serum and prostate tissues (Gan et al., 2013). Similarly, bastard speedwell total flavonoids can significantly decrease correlated symptom and significantly improve the prostate tissue change (Zhang et al., 2009). These studies suggest that flavonoids may be a therapeutic candidate for prostatitis. Therefore, we can safely assume that AHT and TFA might have the effect of treatment of prostatitis.

In the present study, we evaluated the therapeutic effects of AHT and TFA against carrageenan-induced chronic non-bacterial prostatitis and explored its possible mechanisms.

## 2. Materials and methods

### 2.1. Plant material

The rhizome of AP was purchased from Enshi (Hubei, China) as a dried herb and identified by Prof. Ceming Tan of Jiujiang Forest Plants Specimen Mansion. The voucher specimen (PZX0311) was deposited in School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology. According to the method described previously (Wei et al., 2012), the root was cut into small pieces (2 kg) and extracted three times with 80% EtOH at 80 °C. The supernatants were combined and vacuum concentrated at 50 °C to obtain the crude extract (787.5 g). The extract was dissolved in water (10 L) and then subjected into chromatography column (10 × 60 cm, porous polymer resins HPD500, Bonherb Technology Company, Hebei, China). At first, the absorbed resins were eluted in a total volume of five times (v/w) distilled water at 2 ml/min, and then with five times 70% EtOH at 1 ml/min. Evaporation of the extract at 50 °C, we got TFA (187 g), TFA (50 g) was dissolved in 10% HCl (986 ml), stirred at 95 °C for 6 h and at 45 °C overnight. After being cooled, the reaction mixture was filtered and AHT (34 g) were collected.

### 2.2. ABTS (2, 2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid)) radical cation scavenging activity

The ABTS radical cation was used to measure the antioxidant effect of AHT and TFA. Seven millimol ABTS was added to 2.45 mM potassium phosphate 16 h before starting the experiment and stored the solution in darkness at room temperature. Test samples were dissolved separately in methanol to get different concentrations (0.1, 0.2, 0.5, 1, 1.5, 2, 2.5 and 3 mg/ml). The ABTS radical cation solution was diluted to an absorbance of 0.7 + 0.02 at 752 nm. 3.9 ml of ABTS solution and 0.1 ml AHT or TFA were mixed for 5 min and then measured.

### 2.3. Animals

Eight weeks old male SD rats (180–220 g) were obtained from the Animal Research Center of Tongji Medical Center, Huazhong University of Science and Technology (Wuhan, China) and housed in a ventilated room at 25 ± 5 °C under a 12 h light/dark cycle. The animals were acclimatized for 1 week before surgery and had free access to standard food and water ad libitum. We compared survival in all groups throughout the treatment and all animals were carefully monitored. Experimental protocols were performed in accordance with the European Community guidelines for the use and care of laboratory animals and approved by Animal Ethical Committee of Tongji Medical College, Huazhong University of Science and Technology (HUST), China.

### 2.4. Carrageenan-induced paw edema model

The carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity (Huang et al., 2012). To groups of rats, AHT (200 mg/kg), TFA (200 mg/kg), Aspirin (200 mg/kg) or normal saline was administered orally, and 7 days later, 1% of carrageenan was injected into the plantar side of left hind paws of the rats. After 2 h, the paw volume was measured immediately.
2.5. Carrageenan-induced chronic non-bacterial prostatitis model (CNP)

Eight-week-old male Sprague-Dawley (SD) rats were divided into seven groups with animal number of 10 which were used for sham group, model group, positive group (cernilton, 100 mg/kg) and four drug administration groups (AHT or TFA, 200 or 100 mg/kg), respectively. The dosage of AHT and TFA was fixed based on the literature (Wei et al., 2012). The rats in the CNP groups were induced as previously described (Chen et al., 2011b). 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 AHT, TFA or cernilton for 3 weeks, the sham and model groups were given saline at the same time. The general physical condition of each rat was observed throughout the test period. Food consumption and body weight were measured once per week. After final administration, the SD rats were deprived of food for 12 h, then weighed and blood samples were collected. Finally, the rats were sacrificed and the prostates were excised. One section of each sample was stored at −80°C until the analyses of western blot. Another section was fixed in 4% paraformaldehyde for histopathological research. Small pieces of prostates were dehydrated by series of graded ethanol and sections of 4–5 μm were cut and stained with haematoxylin and eosin, and then examined under a light microscope. Furthermore, immunohistochemical analysis was performed using deparaffinized sections. The sections were immersed in freshly prepared 3% H2O2 at 37°C for 10 min, washed with phosphate buffered solution (PBS) for three times and blocked with 5% goat serum for 10 min. Then a primary antibody (anti-NF-κB p65) was added and incubated at 37°C for 1 h, and next the secondary antibody was incubated for 10 min at 37°C. After that, the sections were immersed in diaminobenzidine for 3 min. Besides, in order to make sure that the rat models of carrageenan-induced chronic non-bacterial prostatitis model were successfully established, the prostatic index (PI) was tested. PI=prostate weight (mg)/body weight (g). The collected blood sample was allowed to clot and serum was separated at 3500 r/min for 15 min and used for determination of prostate specific antigen (PSA) by ELISA kits (R&D Systems, Minneapolis, MN).

2.6. Measurements of NO, iNOS, malondialdehyde (MDA) and antioxidant enzymes

Prostate tissues were washed three times in cold isotonic saline (0.9%). Tissues were homogenized with cold Tris–HCl buffer (pH 7.4) to obtain 10% homogenate (w/v) and centrifuged at 4°C during 30 min at 3000 g. Supernatants were stored at −20°C. NO, iNOS, MDA, GSH-px and SOD in the supernatant were measured according to the assay kits (Nanjing Jiancheng Bioengineering institute, Nanjing, China). The NO and MDA levels were expressed as μM/g, nmol/mg protein and the activities of iNOS, GSH-px and SOD enzymes were expressed as U/mg protein.

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

The quantitative measurement of pro-inflammatory cytokines TNF-α and IL-1β were done in the prostate tissue of both CNP and treated groups using commercial ELISA assay kits (Neobioscience Technology Co., Ltd), 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.

2.8. Measurement of PGE2, COX-2, TGF-β1 and CTGF

The effect of CNP on productions of PGE2, COX-2, TGF-β1 and CTGF was measured in prostate tissues using commercial ELISA kits (Uscn Life Science Inc. Wuhan). All assays were performed in 10% prostate supernatant in accordance with manufacturer’s instructions. The amounts of PGE2, COX-2, TGF-β1 and CTGF in prostate tissue were expressed as pg/ml.

2.9. Western blot analysis

The expressions of COX-2, iNOS, TGF-β1 and NF-κB in prostates were further evaluated using western blot analysis. Briefly, after grindened in liquid nitrogen, the total protein concentrations were determined using a western blot lysis kit (Dingguo Biotechnology, China). The 50 μg protein lysate was separated by 10% SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 1 h, and then incubated with specific primary antibodies for anti-COX-2 (1:500), anti-iNOS (1:500), anti-TGF-β1 (1:500), anti-NF-κB (1:500) and β-actin (1:500) overnight at 4°C. After four washes with TBST, the blots were incubated with the secondary antibody conjugated with horseradish (1:3000) were added into the membrane and incubated for 1 h. Immunocomplexes were visualized by using enhanced chemiluminescence following manufacturer’s instructions (Super Signal West Pico; Pierce Biotechnology, Rockford, IL, USA).

2.10. Statistical analyses

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

3. Results

3.1. AHT and TFA inhibited the activity of ABTS cations

AHT and TFA showed strong ABTS radical scavenging activities. In Fig. 1, AHT and TFA significantly inhibited the activity of ABTS radical cations, similar to the vitamin C (VC).
3.2. Effect of AHT and TFA on paw edema

Paw edema were investigated in AHT and TFA groups. As can be seen from Fig. 2, carrageenan treatment resulted in significant rise in the levels of paw edema. AHT or TFA at the dose of 200 mg/kg showed a significant reduction of paw edema compared to model group. Besides, AHT or TFA significantly reduced paw edema, similar to the aspirin ($P < 0.05$).

3.3. Effect of AHT and TFA on PI and PSA

In order to make sure that the rat models of carrageenan-induced chronic non-bacterial prostatitis model were successfully established, we compared the prostatic index. After 3 weeks administration, the effects of oral administration of AHT or TFA on the levels of PI and PSA were summarized in Table 1. In the model group, the levels of PI and PSA increased to 1.7 ± 0.1 mg/g and 298.4 ± 19.2 pg/ml, respectively. By contrast, after treatment with AHT, especially 200 mg/kg, the levels of PI and PSA had dropped significantly. The levels of PI and PSA in TH group remained at a significantly lower level compared with model group ($P < 0.05$).

3.4. Histopathological examination and immunohistochemical analysis

The degree of chronic inflammatory cell infiltrates, acinar changes and interstitial fibrosis were evaluated after 3 weeks of treatment. Histological analysis showed that there was no change in the morphological structure of the prostate gland of rats in sham group (Fig. 3IA), while severe diffuse chronic inflammation characterized by leukocyte infiltration and papillary fronds protruded into the gland cavities, the prostatic epithelial height was increased notably, and the gland lumen diameter was significantly smaller in the lateral lobe of the prostate from rats in the model group (Fig. 3IB). However, these changes were significantly suppressed in the rats administered with AHT or TFA, especially those given a dose of 200 mg/kg per day (Fig. 3ID–IG).

The expression of NF-κB in the rat prostates was examined by immunohistochemical analysis. The immunostaining in Fig. 3IA and 3IB showed that the levels of NF-κB in model group were enhanced in prostatic glandular epithelial cells and inflammatory cells when compared with that in sham group. However, the expression was reduced in the rats administered with AHT or TFA (Fig. 3ID–IG).

3.5. Effect of AHT and TFA on MDA, SOD and GSH-px levels

The effects of AHT and TFA on modulating the activities of antioxidant enzymes (SOD and GSH-px) and the level of MDA are described in Table 1. In model group, the activities of SOD and GSH-px were markedly reduced, the contents of MDA increased compared with the sham group. Oral treatment of AHT or TFA can significantly increase the activities of SOD and GSH-px while the level of MDA was significantly decreased compared with the model group. There was no significant difference between the sham group, positive group and high-dose groups.

3.6. Effect of AHT and TFA on TNF-α and IL-1β

As shown in Fig. 4A, the TNF-α level was 99.2 ± 8.3 pg/ml in sham group. Carrageenan-treatment caused significant increase in the level of TNF-α compared with the sham group. Oral treatment of AHT or TFA at doses of 100 and 200 mg/kg resulted in decrease of TNF-α level when compared to model group. In Fig. 4B, the level of IL-1β was significantly increased in model group compared to control group. However, IL-1β level was reduced in AHT or TFA group. The level of IL-1β was 154.6 ± 10.9 pg/ml in model group. However, the IL-1β level was significantly decreased to 106.8 ± 10.7 or 113.3 ± 8.6 pg/ml at the dose of 200 mg/kg in AHT or TFA group, respectively.

3.7. Effect of AHT and TFA on iNOS, NO, COX-2 and PEG2

The changes of the levels of COX-2, PEG2, iNOS and NO were investigated. The results showed that carrageenan treatment stimulates the level of COX-2 compared to sham group. However, treatment of AHT or TFA decreased the level of COX-2 (Fig. 5A). In Fig. 5B, the level of PEG2 was increased in model group. Oral treatment of AHT or TFA at 100 and 200 mg/kg resulted in decrease of PEG2 content when compared with model group. In Fig. 5C, the level of iNOS was increased by carrageenan in model group. The result indicated that the treatment of AHT or TFA (100 and 200 mg/kg) decreased the iNOS level when compared with model group. The level of NO was significantly increased in model group compared to sham group. Rat treated with AHT or TFA at 100 and 200 mg/kg showed decrease in NO level when compared with model group (Fig. 5D). Namely, the levels of COX-2, PEG2, iNOS and NO were increased in model group compared with sham group. However, AHT or TFA promoted a significant reduction of COX-2, PEG2, iNOS and NO productions when compared with model group.
3.8. Effect of AHT and TFA on TGF-β1 and CTGF

AHT and TFA were assessed for their inhibitory activity on TGF-β1 and CTGF production. As shown in Fig. 6A, the level of TGF-β1 was 67.3 ± 7.8 pg/ml in sham group. Carrageenan caused significant increase in the level of TGF-β1 in model group. After AHT or TFA-treated for three weeks, the level of TGF-β1 was dose-dependently decreased (P < 0.05). Similarly, the level of CTGF was notably elevated
in model group when compared with the sham group (Fig. 6B). However, in AHT or TFA treated group the elevation was suppressed compared with the model group.

3.9. Western blot for COX-2, iNOS, TGF-β1 and NF-κB

The effects of AHT and TFA on the changes of the activation of COX-2, iNOS, TGF-β1 and NF-κB were further determined by western blot analysis. In Figs. 7A and B, results showed that the carrageenan stimulates activation of COX-2, iNOS and TGF-β1 compared to the sham group. However, the treatment of AHT and TFA decreased the COX-2, iNOS and TGF-β1 expression in carrageenan-induced rats. Namely, iNOS and COX-2 expressions were reduced at 200 mg/kg of AHT and TFA, respectively, compared to model group. NF-κB is an important transcription factor in inflammation. Therefore, the suppression of NF-κB could result in reducing inflammation. As can be seen from Fig. 7C, the expression of NF-κB was nobly decreased along with the increased time of administration. The result indicates that the treatment of AHT and TFA decreased NF-κB expression in carrageenan-induced rats.
Abacopteris penangiana (Hook.) Ching (AP) is a fern plant and widely distributed in the south of China. Previous phytochemical investigation showed that rhizomes of AP were rich in antioxidant constituents and various pharmacological activities have been reported in recent years (Chen et al., 2011a; Lei et al., 2011; Wei et al., 2012). The effect of AP on prostatitis has not been reported until now. In this study, the antioxidant and anti-inflammatory activities of AHT and TFA from AP were investigated. And 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 AHT and TFA in rats with carrageenan-induced prostatitis was evaluated. The whole results showed that AHT and TFA successfully enhanced the activities of antioxidant enzymes and attenuated the levels of various pro-inflammatory cytokines. Moreover, reduced COX-2, PEG2, iNOS and NO were observed both in AHT and TFA treated groups. The levels of TGF-β1 and CTGF in AHT and TFA treated groups were significantly decreased along with the inflammatory state of the prostate gland were alleviated. Furthermore, the anti-CNP effect of AHT and TFA may be associated with the decreased prostatic expression of NF-κB. Thus, the administration of AHT or TFA for 3 weeks significantly inhibited the development of oxidative stress, chronic inflammation and fibrosis in prostatic tissue and some underlying mechanisms might be correlated with these properties.

During carrageenan-induced inflammatory response, leukocytes and mast cell are recruited first to produce free radical and various mediators like chemokines and cytokines which further recruit inflammatory cells to produce free radical and free radical attack plasmamembrane result in MDA production (Sheu et al., 2009). Uncontrolled production of ROS or change in intracellular antioxidants level causes damage or modification of cellular macromolecules (Waris and Ahsan, 2006). It is becoming increasingly apparent that inflammatory injury may be mediated by ROS or its metabolites, and antioxidant therapy has been shown to prevent in vivo tissue injury during inflammation (Anette et al., 2007). Therefore, the anti-CNP effects of AHT and TFA seemed to relate to their antioxidant activity. In the present study, the changes of the activities of antioxidant enzymes were investigated. The activities of SOD and GSH-px in model group were decreased and the level of MDA was elevated. Daily treatment of AHT or TFA for 3 weeks significantly increased the activities of antioxidant enzymes. Simultaneously, the increased level of MDA was reversed. Thus, it indicated that AHT and TFA prevented the oxidative damage due to ROS overproduction from CNP response.

Accumulating studies have been reported that ROS might initiate and/or amplify inflammation via activation in gene expression of several redox sensitive transcription factors, such as NF-κB (Schreck et al., 1991; Conner and Grisham, 1996). In the oxidative status, ROS can promote the rapid translocation of active NF-κB from the cytosol into the nucleus, resulting in the formation of inflammatory enzymes, including COX-2 and iNOS (Khan et al., 2013). Moreover, activation of NF-κB produces an amplification of the inflammatory response by upregulating the production of various pro-inflammatory cytokines and pro-fibrogenic cytokines such as IL-1β, TNF-α and TGF-β1 (Kim et al., 2006; Castello et al., 2010). Hence, NF-κB plays an important role in inflammatory responses and inhibiting NF-κB activation may be an effective method for CNP. In this study, the immunohistochemical and WB analysis demonstrated that the NF-κB expression was strongly enhanced in model group. This may be due to the fact that oxidative stress in the prostate tissues was severe. However, it was found that AHT or TFA at the dose of 200 mg/kg significantly decreased the expression of NF-κB compared with the model group. Thus, all these effects data strongly indicate that NF-κB plays a central role in CNP and AHT or TFA could against CNP possibly through a decreasing activation of NF-κB.

Currently, it is well accepted that the progression of CNP related to the complex network of cytokines, such as IL-1β and TNF-α (Nadler et al., 2000; Tsunemori et al., 2011). These cytokines also upregulate the expression of COX-2 and iNOS, that lead to increased synthesis of PGE2 and NO (Au et al., 2007; Su et al., 2012a, 2012b). 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 (Dinarello, 1998). TNF-α, a pleiotropic pro-inflammatory cytokine, is rapidly produced by macrophages in response to tissue damage (Beutler and Cerami, 1989). Previous studies have shown that activation of transcription factor NF-κB by TNF-α is one of the myriad actions of TNF-α that causes genes to generate potentially cell damaging oxidative enzymes, as well as further release of TNF-α, IL-1β and other pro-inflammatory cytokines (Tahir et al., 2013). Cytokine based therapies have been found to be useful in preventing progression of chronic prostatitis (Lu et al., 2011). In the present study, the levels of TNF-α and IL-1β were increased in model group rats, whereas on treatment with AHT or TFA at 200 mg/kg, there was a significant decrease in the cytokine levels. Several natural anti-oxidant polyphenol compounds, such as quercetin, resveratrol, suppressing the release of pro-inflammatory factors could possess anti-inflammatory activities. In this study, the levels of iNOS, COX-2, NO and PEG2 were examined. And it was found that in model group, the levels of those factors were enhanced. However, the increased levels of iNOS, COX-2, NO and PEG2 were reversed in treatment group of AHT or TFA. In addition, it was found that AHT and TFA at the dose of 200 mg/kg significantly decreased iNOS, COX-2, NO and PEG2 levels. Therefore, the anti-CNP effect of AHT and TFA 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 (Liu and Gaston Pravia, 2010). TGF-β1 is known to induce fibroblast differentiation of myofibroblast/smooth muscle cell in the human prostate (Untergasser et al., 2005). In addition, other evidence suggested that pro-fibrotic effects of TGF-β may be partly mediated by CTGF (Lexisk and Abraham, 2004). As another potent profibrotic factor, CTGF is implicated in fibroblast proliferation, cellular adhesion, angiogenesis, and extracellular matrix (ECM) synthesis (Lau and Lam, 1999). Previous studies showed that CTGF promotes inflammatory response (Kular et al., 2011). 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 (Borthwick et al., 2013). Summarily, another possible hypothesis could be given that the TGF-β1/CTGF pathway may also be involved in the CNP. The results indicated that AHT and TFA could suppress the enhancement of the TGF-β1 expression compared with model group rats. And at 200 mg/kg, the decreased level of TGF-β1 was observed significantly, as well as the level of CTGF was decreased in AHT or TFA treated groups. AHT and TFA regulated the CTGF signaling pathway following the TGF-β1 stimulation. Thus, the anti-CNP effects of AHT and TFA were also associated with the activity of anti-fibrotic.

5. Conclusion

In summary, AHT and TFA obtained from AP showed the activities of attenuating CNP. On the one hand, AHT or TFA had the activities of modulating the activities of SOD and GSH-px. On the other hand, AHT or TFA participated in the NF-κB signaling pathway, reduced the expression of NF-κB and subsequently decreased the levels of TNF-α, IL-1β, iNOS, COX-2 and TGF-β1, further reduced inflammation and fibrotic. Based on this, our further work should be conducted to determine the anti-CNP potential of 7-hydroxy-4′-methoxy-6, 8-dimethylnaphthyridin and flavan-4-ol glycosides, which are the main constituents of AHT and TFA, respectively.

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