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

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ABSTRACT

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 (PEG_2), 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, PEG_2, 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.

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 pelviperineal pain and an inflammatory subtype with leukocytes expressed in their prostatic secretions,

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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′-methoxy-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 antioxidative, 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-ethhylbenzthiazoline-6-sulfonic acid)) radical cation scavenging activity

The ABTS radical cation was used to measure the antioxidant effect of AHT and TFA. Seven millimolar 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 standard antioxidant is vitamin C (VC). For measurement, the ABTS radical cation ($ABTS^+$) 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 6 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.