Abacopteris penangiana exerts testosterone-induced benign prostatic hyperplasia protective effect through regulating inflammatory responses, reducing oxidative stress and anti-proliferative

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Abstract

Ethnopharmacological relevance: Abacopteris penangiana (Hook.) Ching (AP) is a member of parathyeliperis glanduligera and used in folk medicine for the treatment of blood circulation and blood stasis, edema and inflammation as recorded in the “Chinese Materia Medica”.

Aim of the study: The purpose of this study was to investigate the effects of total flavanol glycosides (TFA) from AP and its acid hydrolysate (AHT) on testosterone-induced benign prostatic hyperplasia (BPH) in rats by measuring the levels of inflammatory responses, oxidative stress and prostate cell proliferation.

Materials and methods: BPH was induced in rats by subcutaneous injection of testosterone after castration. Seventy rats were divided into seven groups. After oral administration of AHT and TFA (100 or 200 mg/kg/d) for 4 weeks, the prostate index (PI), 5α-reductase (5α-R) and dihydrotestosterone (DHT) were determined. Then the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were determined. In addition, the relative inflammatory factors, cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8) and interleukin 17 (IL-17) were measured. Finally, the prostatic expression of nuclear transcription factor-κB (NF-κB) and phosphoinositide3-kinase (PI3K)/Akt were determined by immunohistochemistry. The prostatic expression of Bcl-2 was determined by western blot analysis.

Results: The results showed that AHT and TFA decreased serum DHT and 5αR-activities compared with model group, as well as the PI and histopathological examination findings. In addition, oral treatment of AHT and TFA can significantly increase the activities of SOD, GPx and CAT while the level of MDA was significantly decreased compared with the model group. Moreover, AHT and TFA remarkably decreased the levels of inflammatory cytokines in prostatic tissue. Further investigation demonstrated that AHT and TFA treatment down-regulated the protein expressions of p-Akt, NF-κB and Bcl-2.

Conclusions: These results suggest that AHT and TFA have anti-BPH properties via anti-inflammatory, antioxidant and anti-proliferative effects. Hence, AP represents a potential herb for the treatment of BPH.

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1. Introduction

Benign prostatic hyperplasia (BPH) is one of the most common diseases in men (Boyle and Napalkov, 1996). Epidemiological observations indicate that more than 50% of men older than 50 exhibit signs of BPH, and approximately 45 million men will suffer from BPH by 2020 (Napalkov et al., 1995; Flannery et al., 2006). Although aging and androgens are the two established risk factors for the development of BPH, recent novel findings also highlighted the key role of inflammation (Sun et al., 2008; Briganti et al., 2009). Basic science and clinical studies suggest that inflammation may be another central mechanism in prostate enlargement and BPH progression. In fact, repeated tissue damage and oxidative stress related to chronic inflammation may provoke a compensatory cellular proliferation with the risk of hyperplastic growth (Lee and Peehl, 2004; Robert et al., 2009). Clinically, inflammatory infiltrate prevalence in 98% of 162 analyzed BPH specimens (Alberto et al., 2009). The inflammatory infiltrate in BPH is largely composed of activated lymphocytes and macrophages. Activated lymphocytes and macrophages produce proinflammatory cytokines which in turn induce various immune responses such as the release of soluble mediators like prostaglandin E2 (PGE2), leukotrienes, platelet-activating factor (PAF), nitric oxide (NO), and reactive oxygen (ROS) (Wang et al., 2009; Manzarbeitia et al., 2010). As a result, reducing inflammation plays a crucial role in...
the treatment of BPH and finally leads to a better clinical outcome. Recently, many kinds of pharmaceutical are very popular in treating BPH. Although these drugs have great efficacy, their adverse effects should not be overlooked (Foley and Kirby, 2003; Kyprianou, 2003). Thus, alternative herbal-based therapies are prevalent and popular in urologic disease in general and prostatic disorders in particular (Shoskes, 2002). Therefore, it is reasonable to find effective natural products from medicinal plants for treatment of BPH.

Abacopteris penangiana (Hook.) Ching (AP), an ethnomedicinal herbaceous species that grows abundantly in the south of China, India, and Nepal, has been traditionally used to promote blood circulation, remove blood stasis and dampness, edema and inflammation (Administration Bureau of National Chinese Traditional Medicine, 1998). Epithelial hyperplasia is an integral morphological change in BPH and hyperplastic epithelium may predispose to obstruction and stasis (Anim et al., 1998). Furthermore, in the theory of Traditional Chinese Medicine, dampness-heat obstruction and stasis are two of the most important factors for prostatic hyperplasia (Qu et al., 2012). The total flavon glycosides from Abacopteris penangiana rhizomes (TFA) and its acid hydrolysate (AHT) were obtained by our previous study. HPLC–DAD–MS/MS analysis indicated that 15 compounds including 12 flavan-4-ol glycosides existed in TFA, and a 3-deoxyoxygenated anthocyanidin compound of the flavonoids family, 7-hydroxy-4'-methoxy-6,8-dimethylanthocyanin, was the main constituent of AHT (Wei et al., 2012). Previous study indicated that TFA and AHT were able to improve the symptoms of prostatitis (Yang et al., 2014). Nickel et al. (1999) detected inflammation in all specimens of patients undergoing transurethral resection of prostate (TURP) for BPH who had no symptoms of prostatitis. Indeed, the underlying process of BPH is cumulative, involving androgenic stimulation, cytokines such as interleukins, tumor necrosis factor-α (TNF-α) and cyclooxygenase (COX) (Aquila et al., 2009; Agati et al., 2012), and finally relieving the state of the prostate gland in BPH (Li et al., 2013). Therefore, we can safely assume that TFA and AHT might have the effect of treatment of BPH by reducing oxidative stress and inflammation. In this study, we evaluated the therapeutic effects of AHT and TFA against testosterone-induced BPH and explored their possible mechanisms.

2. Materials and methods

2.1. Chemicals and reagents

Finasteride was purchased from Merck Company (Hangzhou, China). Testosterone propionate injection was purchased from General Pharmaceutical Co., LTD. (Shanghai, China). Superoxide dismutase (SOD), glutathione peroxidase (GSH-px), catalase (CAT) and malondialdehyde (MDA) detection kits were obtained from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Sa-reductase (SAR), dihydrotestosterone (DHT), cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8) and interleukin 17 (IL-17) ELISA kits were purchased from R&D Systems. All chemicals were of analytical reagent grade.

2.2. Herbal material and extraction

The rhizome of AP was purchased from Enshi (Hubei, China) and identified by Prof. Ceming Tan (Jiujiang Forest Plants Specimen Mansion). A voucher specimen (PZX0311) was deposited in the Herbarium of College of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology. According to the method described previously, we obtained AHT and TFA (Yang et al., 2014). Briefly, the roots were cut into small pieces and extracted with 80% aqueous ethanol for 3 times at 80 °C. Then the crude extract was dissolved in water which was used for subsequent macroporous resin column chromatography (porous polymer resin HPDS500, Bon Adsorber Technology Company, Hebei, China). The absorbed resins were eluted with distilled water and then with 70% aqueous ethanol. The 70% aqueous ethanol fraction was collected and evaporated to dryness under reduced pressure at 50 °C to produce TFA. Part of TFA was dissolved in 10% HCl, stirred at 95 °C for 6 h and at 45 °C overnight. After being cooled, the reaction mixture was filtered and AHT were collected.

2.3. Animals

Male Sprague-Dawley (SD) rats (250–300 g) were obtained from the Animal Research Center of Tongji Medical Center, Huazhong University of Science and Technology, Wuhan, China (Quality certificate number: SCXK (e) 2010-0009) and allowed to acclimate in quarantine for a week prior to experiments. All animals were housed in a ventilated room at 25 ± 5 °C under a 12 h light/dark cycle 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. All procedures involving animals complied 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. Experimental design

BPH was induced in the rats by subcutaneous injection of testosterone after castration (Atawia et al., 2013). Briefly, rats were divided into seven groups, after anaesthetization with chloral hydrate (i.p., 350 mg/kg body weight), rats in sham group (Group I) were cut open and then sewed up without cutting off the testicles; rats in the other groups were castrated. After 7 days' recovery, Group I received only olive oil (i.h.) and six other groups were injected testosterone subcutaneously (10 mg/kg/d) to induce BPH. These six groups were further divided into model group (Group II), finasteride: 5 mg/kg/d (i.g., Group III), AHT: 200 mg/kg/d (i.g., Group IV), AHT: 100 mg/kg/d (i.g., Group V), TFA: 200 mg/kg/d (i.g., Group VI), TFA: 100 mg/kg/d (i.g., Group VII). The dosage of AHT and TFA was fixed based on the literature (Wei et al., 2012). The drugs were administered for 4 weeks and 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 sacrificed. Blood was collected, allowed to clot and serum was separated at 3000 g for 15 min and stored at −80 °C. One section of each sample was stored at −80 °C until the analyses of western blot. One section was prepared to make 1:9 (w/v) homogenates with ice-cold physiological saline using a homogenizer. The homogenate was centrifuged at 3000g for 15 min at 4 °C and the supernatant was then stored at −80 °C to the subsequent determinations. The rest section of each prostate tissue was fixed in 4% paraformaldehyde for histopathological research. Tissue slices of 4–5 μm thickness were made from the 4% paraformaldehyde-fixed specimens, and were stained with hematoxylin and eosin (HE) for histopathological examination. Furthermore, immunohistochemical analysis was performed using deparaffinized sections. After 10 min immersed in freshly prepared 3% H2O2 at 37 °C, the sections were blocked with 5% goat serum for 10 min. Afterward, the sections were washed with phosphate buffered solution (PBS) for 3 times, then incubated with a primary antibody (NF-κB and p-Akt) at 37 °C for 1 h, next treated with...
secondary antibody for 10 min at 37 °C, and immersed in diaminobenzidine for 3 min. The hematoxylin-stained sections were dehydrated by ethanol and visualized using an optical microscope.

2.5. The prostate index

The prostate index (PI) of each rat was the ratio of prostate weight to body weight. PI = prostate weight (mg)/body weight (g).

2.6. Serum biochemistry

The assay for the levels of 5α-reductase (5AR) and dihydrotestosterone (DHT) was performed using specific ELISA kits (R&D Systems, Minneapolis, MN). All procedures were performed according to manufacturer’s instructions. The results for 5AR and DHT were expressed in U/L and nmol/L, respectively.

2.7. Assay for antioxidant markers in prostate tissue

Superoxide dismutase (SOD), glutathione peroxidase (GSH-px) and catalase (CAT) were determined using commercial kits purchased from the Institute of Biological Engineering of Nanjing Jianchen (Nanjing, China). The results for SOD, GSH-px and CAT were expressed in U/mg.

2.8. Measurement of lipid peroxidation products in prostate tissue

The lipid peroxidation was measured by the level of malondialdehyde (MDA) (nmol/mg). The MDA concentration in prostate homogenate was measured at 532 nm by its reaction with thiobarbituric acid to form a stable chromophoric product, which was detected according to the instruction of thiobarbituric acid (TBA) assay kit (Biological Engineering of Nanjing Jianchen, Nanjing, China).

2.9. Measurement of cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8) and interleukin 17 (IL-17)

The assay for the levels of COX-2, TNF-α, IL-1β, IL-6, IL-8 and IL-17 was performed in the prostate tissue of BPH and treated groups using specific ELISA kits (R&D Systems, Minneapolis, MN). All procedures were performed according to manufacturer’s recommendations. The results for COX-2, TNF-α, IL-1β, IL-6, IL-8 and IL-17 were expressed in pg/ml.

2.10. Western blotting assay for Bcl-2 and NF-κB

Total protein was isolated using the tissue protein extraction kit and the content was determined (Biossci Biotechnology, China). The samples were boiled in 5 x SDS sample loading buffer for 3 min and then loaded into a 10% sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Beyotime). The protein were separated by electrophoresis and transferred into polyvinylidene difluoride (PVDF) membrane (BioRad). Then the membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) at 4 °C overnight. After that the blocked membranes were incubated with specific primary antibodies for anti-Bcl-2, anti-NF-κB-P65 and β-actin for 2 h at room temperature. The membranes were washed four times with TBST, followed by incubation with a secondary antibody overnight at 4 °C. After four washes with TBST, positive bands were detected using enhanced chemiluminescence following the manufacturer’s instructions (Super Signal West Pico; Pierce Biotechnology, Rockford, IL, USA).

2.11. 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. Prostate weight and prostate indices (PI) changes

Prostate weight and PI are commonly used to evaluate the development of BPH. As shown in Fig. 1, the levels of prostate weight and PI in rats injected with testosterone had significantly increased compared with rats in sham group, which suggested that testosterone successfully induced BPH in the castrated rats. However, testosterone-treated rats given TFA (200 mg/kg/d) and AHT...
(200 mg/kg/d) had remarkably lower prostate weight and PI (P < 0.05).

3.2. 5α-R and DHT changes

The main prostatic androgen is DHT, which is formed by the steroid enzyme 5α-R from testosterone. The levels of 5α-R and DHT were determined via ELISA kits. Data in Fig. 2 indicate that the prostates of rats in model group had increased 5α-R and DHT levels. However, daily administration of AHT and TFA for 4 weeks decreased the levels of 5α-R and DHT in serum.

3.3. Histological and immunohistochemical analysis

The histoarchitecture of the prostate gland was showed clearly in Fig. 3A. Normal histological features of prostate gland are visible in sham group: the tissues were tightly packed; epithelium was cuboidal and regular in size (Fig. 3Aa). In model group, the prostate gland showed the typical patterns of glandular hyperplasia: glandular hyperplasia with epithelial proliferation and nuclear stratification, papillary fronds protruded into the gland cavities, the prostatic epithelial height was increased notably, and decreased glandular luminal area (Fig. 3Ab). However, AHT and TFA treatments showed mild glandular hyperplasia compared with model group. The luminal...
volume was increased and the glandular epithelial height was significantly reduced (Fig. 3Ad–g).

PI3K/Akt pathway is well known to inhibit the cell death signaling through phosphorylation of several components of the apoptotic machinery, including Bcl-2. Akt is known to be a downstream effector of PI3 K that mediates survival signaling. As shown in Fig. 3Ba, the expression of phospho-Akt remained high in model group. Oral administration of AHT and TFA at 100 or 200 mg/kg/d reduced the expressions of phospho-Akt in a dose dependent manner (Fig. 3Bd–g).

The expression of NF-κB in the rat prostates was examined by immunohistochemical analysis. The immunostaining showed that the expression of NF-κB in model group was enhanced when compared with that in sham group (Fig. 3Ca and b). However, oral treatment of AHT or TFA at 100 or 200 mg/kg/d depressed the NF-κB enhancement (Fig. 3Cd–g).

3.4. Lipid peroxidation products changes

Testosterone caused a significant increase of up to 2.76 ± 0.15 nmol/mg in the level of prostatic MDA from 1.12 ± 0.09 nmol/mg of the sham group. Interestingly, the administration of AHT and TFA decreased the level of MDA, and both were more effective at 200 mg/kg/d than 100 mg/kg/d (Fig. 4A).

3.5. Antioxidant enzymes changes

It is well known that the increased lipid peroxidation is attributed to the decreased radical-scavenging antioxidant system, and therefore, the levels of SOD, GPx and CAT in experimental rats were further verified. The effects of AHT and TFA on modulating the activities of SOD, GPx and CAT in experimental rats were described in Fig. 4. The rat treated with testosterone in model group showed significantly decrease of SOD levels compared with the sham group. However, in AHT and TFA treated groups, the activities of SOD were increased (Fig. 4B). In model group, the activities of GPx were reduced compared with sham group. Oral treatment of AHT and TFA at 100 or 200 mg/kg/d resulted in increase of GPx content compared with model group (Fig. 4C). The activities of CAT were decreased when treated with testosterone in model group. In Fig. 4D, the results showed that AHT and TFA treatment increased the activities of CAT and the rate of rise showed a significant dose–response relationship.

3.6. Effect of AHT and TFA on inflammatory factors

Inflammatory factors play a crucial role in proliferation in prostatic cells in BPH. As shown in Fig. 5A–E, compared with the sham group, treatment with testosterone significantly increased the levels of TNF-α, IL-1β, IL-6, IL-8 and IL-17. However, AHT and
TFA at 100 or 200 mg/kg/d reduced the levels of inflammatory factors in a concentration dependent manner. COX-2 has been detected in all inflammatory cells in the epithelium and interstitial space and it is increased in proliferative inflammatory lesions, generating pro-inflammatory prostaglandins (Sciarra et al., 2008). As shown in Fig. 5F, testosterone treatment stimulates the level of COX-2 compared to sham group. However, treatment of AHT and TFA decreased the level of COX-2. The level of COX-2 was 108.7 ± 7.2 pg/ml in model group. However, the COX-2 level was significantly decreased to 62.1 ± 6.3 pg/ml and 71.3 ± 6.1 pg/ml at the dose of 200 mg/kg/d in AHT and TFA groups, respectively.

3.7. Effect of AHT and TFA on Bcl-2 and NF-κB expressions

Anti-apoptotic factor Bcl-2 was analyzed by a western blotting method. As demonstrated in Fig. 6A and B, treatment with testosterone significantly increased the expression of Bcl-2 compared to the sham group. When the rats were treated with AHT and TFA at 100 or 200 mg/kg/d, the expressions of Bcl-2 decreased respectively. The effects of AHT and TFA on the changes of the activation of NF-κB were further determined by western blot analysis. In Fig. 6, results showed that the testosterone stimulates activation of NF-κB compared to the sham group. However, the treatment of AHT and TFA at 100 or 200 mg/kg/d decreased the NF-κB expression in testosterone-induced rats (Fig. 7).

4. Discussion

Benign prostatic hyperplasia (BPH) is the most common urologic disease among elderly males and predominantly affects men aged > 50 yr (Garraway et al., 1991). Testosterone-induced chronic inflammation is frequently associated with BPH (Anim et al., 1998). Chronic inflammation produces simple epithelial hyperplasia, increased epithelial cell proliferation, through oxidative stress, can also produce repeated tissue damage, post-translational DNA modifications (Naber and Weidner, 2000). Thus, in the present study, the potential benefits of AHT and TFA in ameliorating testosterone-induced BPH might be correlated with their anti-inflammatory responses, reducing oxidative stress and then anti-proliferative effects.

Testosterone has been demonstrated to be involved in BPH (Atawia et al., 2013). In our study, the animals were injected testosterone subcutaneously for 4 weeks to induce BPH. After 4 weeks, PI in model group were increased accompanied with the DHT and 5α-R levels were increased when compared to the sham group, indicating testosterone successfully established BPH. However,
oral administration of AHT and TFA at an appropriate dose for 4 weeks was useful in the attenuation of BPH. At 200 mg/kg/d, the effects of AHT and TFA were comparable to that of finasteride. 

Hormonal imbalance has been proposed to trigger intraprostatic inflammation. Recent evidences in several epidemiological studies indicate that inflammation may have a prominent role in the pathogenesis of BPH and in the aggravation of its clinical symptoms (Sciarra et al., 2007). Nodules of BPH patients contain infiltrates of T-lymphocytes, macrophages and B-lymphocytes that are chronically activated. These infiltrating cells are responsible for the production of cytokines, such as interleukins, TNF-α, which may mediate prostate enlargement under inflammatory conditions in BPH (Kim et al., 2013). Cytokines signaling has an important growth-promoting role in prostate development (Wang et al., 2008; McLaren et al., 2011). TNF-α and IL-1β are two major pro-inflammatory cytokines. TNF-α is multifunctional cytokine that is believed to mediate inflammation and immune responses by activating nuclear factor-κB (NF-κB) and phosphoinositide3-kinase (PI3K)/Akt signaling pathways and leads to increased expression of other cytokines (such as IL-1β), including TNF-α itself (Jeon et al., 2013). IL-1β is a pro-inflammatory cytokine that induces the production of other inflammatory mediators (Dinarello, 1998). IL-6, IL-8 and IL-17 may perpetuate chronic immune response in BPH and induce fibromuscular growth by an autocrine or paracrine loop (Steiner et al., 2003). IL-6 and IL-8, key executors of stromal growth in BPH, are produced by prostatic stromal cells in response to IL-17 (Lotti and Maggi, 2013). IL-17, exerting synergistic effects with TNF-α and IL-1β, is thought to be involved in local fine tuning of inflammatory signals. Epidemiologic studies have found elevated levels of TNF-α, IL-1β, IL-6, IL-8 and IL-17 in men with BPH as compared to normal prostate (Steiner et al., 2002; Schenk et al., 2010; McLaren et al., 2011). This is consistent with our study. In the present study, testosterone treatment increased the level of TNF-α, IL-1β, IL-6 and IL-17 in model group, whereas treatment with AHT and TFA, there was a significant decrease in the cytokine levels. Moreover, pro-inflammatory cytokines released by inflammatory cells may also induce cyclooxygenase-2 (COX-2) expression in the epithelial cells in BPH (Palapattu et al., 2005). COX-2 plays an important role in the proliferation of prostate cells. The effect of COX-2 on prostate cell proliferation may be obtained either by modulating the inflammatory process or by directly upregulation of Bcl-2 expression to decrease apoptosis (Silverioa et al., 2005). In this study, the level of COX-2 was tested. It was found that in model group, the level of COX-2 was...
increased. However, the increased level was reversed in treatment groups of AHT and TFA. Therefore, anti-BPH effect of AHT and TFA may be related to their anti-inflammatory properties.

Inflammation has been demonstrated to be a source of oxidative stress due to overproduction of reactive oxygen species (ROS) that leads to tissue injury in infiltrated areas (De Marzo et al., 2007). Previous studies have shown that activation of TNF-α increases intracellular reactive oxygen species, which may exacerbate inflammatory processes (Zhou et al., 2009). Moreover, several natural antioxidant polyphenol compounds, such as Quercetin, resveratrol, sesquiterpene lactone, and theaflavin have been shown to directly inhibit the expression of NF-κB-dependent cytokines and COX-2 genes (Hehner et al., 1998; Lin et al., 1999). The suppressive effects of these antioxidant compounds on the production of these inflammatory mediators are associated with their antioxidant activities. Therefore, the changes of the activities of prostatic antioxidant enzymes were investigated. In the present study, activities of SOD, Gpx and CAT were decreased in model group, while daily treatment of AHT and TFA significantly increased the activities of antioxidant enzymes. Furthermore, regions of prostatic inflammation will produce free radical and free radical attack plasmamembrane result in MDA production (Gao et al., 2012). In model group, the level of MDA was elevated compared with sham group. However, treatment with AHT and TFA reversed the increased level of MDA. Thus, AHT and TFA could successfully reverse the changes of antioxidant enzymes and MDA suggesting their anti-BPH effects were related to their antioxidant activities.

Chronic inflammation can induce proliferative events and post-translational DNA modifications in prostate tissue through oxidative stress (Naber and Weidner, 2000). It is also suggested that pro-inflammatory cytokines-induced oxidant production may be mediated by the activation of phosphatidylinositol 3-kinase (PI3K)/Akt and NF-κB pathways (Lee et al., 2011). Simultaneously, ROS formation in turn induces the activation of PI3K/Akt pathway and NF-κB pathway (Taylor et al., 2004). Nuclear factor-κB (NF-κB) has been recognized as one of the most important targets of PI3K/Akt pathway. The activated NF-κB is involved in several stages of the inflammatory-proliferative process by inflammatory cytokines (Baeuerle and Henkel, 1994). Therefore, it may be a promising strategy to inhibit the Akt and NF-κB activation in order to reduce inflammation in BPH. In this study, the expressions of p-Akt and NF-κB in model group were significantly enhanced which may be due to the oxidative stress in the prostate tissues was severe. Treatment with AHT and TFA decreased the expressions of p-Akt and NF-κB compared with the model group. Thus, all these data strongly indicate that PI3K/Akt pathway and NF-κB pathway play central roles in BPH and AHT and TFA could against BPH possibly through a decreasing activation of p-Akt and NF-κB.

BPH is a multifactorial disease characterized by noncancerous form of abnormal prostate cell growth (Gossel and Wuest, 2004). Apoptotic activity was also suggested as a key cofactor in BPH development and progression. Bcl-2 is a relative protein involved in the regulation of prostate apoptosis and regarded as a potent apoptosis suppressor (Kyprianou et al., 1996). Previous studies have shown that activation of both Akt and NF-κB may cause Bcl-2 to generate, as well as further promote cell growth (Mortenson et al., 2003). Namely, the activated Akt and NF-κB enhance the expression of Bcl-2. The results indicated that AHT and TFA could suppress the enhancement of the Bcl-2 expression compared with model group. And at 200 mg/kg/d, the decreased level of Bcl-2 was observed significantly. This may contribute to the pro-apoptotic effects of AHT and TFA by inhibition of Bcl-2 expression through PI3K/Akt pathway and NF-κB pathway in BPH.

5. Conclusion

In conclusion, oral treatment of AHT and TFA for 4 weeks showed dose dependent inhibition of BPH induced by testosterone in rats. The preventive effect is likely due to regulating inflammatory responses, reducing oxidative stress and anti-proliferative. In addition, AHT and TFA can guard against the p-Akt and NF-κB expressions induced in BPH. However, the deep molecular mechanisms need to further describe. Additionally, many efforts on analysis of bioactive compounds from AP are being taken in progress.

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