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Protective effects of grape seed-derived procyanidin extract against carrageenan-induced abacterial prostatitis in rats

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ABSTRACT

The potential of grape seed-derived procyanidin extract (GSP) to protect against carrageenan-induced abacterial prostatitis in rat was investigated. After the experimental period, the effects of GSP on the levels of prostatic interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), interleukin-6 (IL)-6, IL-10 and basic fibroblast growth factor (bFGF) were measured by enzyme linked immunosorbent assay. Prostatic oxidative stress was evaluated by detecting the activities of antioxidant enzymes. The prostatic levels of extracellular signal-regulated kinases (ERK) and its phosphorylation state were determined using Western blot analysis. It was found that GSP could ameliorate the carrageenan-induced ERK over-phosphorylation state as well as the disordered levels of proinflammatory cytokines, lipid peroxidation and antioxidant enzymes. These results suggest that the GSP exerts prostate protective effects *via* suppressing elevated levels of ERK phosphorylation state, proinflammatory cytokines and lipid peroxidation, and normalizing antioxidant enzyme activities.

1. Introduction

More and more people suffer with prostatitis which is one of the most prevalent problems in andriatry and urinary surgery (Wilson, Woodson, Wiehr, Reddy, & Shinha, 2004). Prostatitis mainly includes four types: the acute bacterial prostatitis (ABP), the chronic bacterial prostatitis (CBP), the chronic abacterial prostatitis (CAP) and the noninflammatory chronic pelvic pain syndrome (ICPS) (Nickel, Nyberg, & Hennenfent, 1999). Among them, ABP and CBP are better understood, but they have low incidence in all the prostatitis subtypes. CAP is the most frequent prostatitis that occurs in much more frequents than the others. However, the cause of CAP still remains elusive (Motrich et al., 2005). Chronic prostatitis, especially the CAP, is a condition of long-term prostatic inflammation state that may contribute to the generation of benign prostatic hyperplasia (BPH) and prostatic carcinoma (Pca) (Steenkamp, Gouws, Gulumian, Elgorashi, & van Staden, 2006). In addition, patients with chronic prostatitis experience an increased risk of depressive disorder (Chung, Huang, & Lin, 2011). Although it is not a life-threatening disease in the near future, CAP seriously obstructs the life and work of patients.

Herbal remedies with proven anti-inflammatory and antioxidant activities have been used in the treatment of

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prostatitis for many years (Steenkamp et al., 2006). Grape (*Vitis vinifera*) is a member of Vitaceae and worldwide. It is used in Chinese folk medicine for improving blood circulation, relieving thirst and restlessness, diuretics and nourishing the kidneys. According to the records, grape is traditionally used for the treatment of edema, painful urination, prostatitis and BPH for patients suffering from kidney/prostate problems due to its satisfactory therapeutic effectiveness (The editorial board of China Herb of State Administration of Traditional Chinese Medicine., 1999).

More recently, grape seed has attracted much attention owing to a number of its diverse biological activities. The main composition of grape seed includes natural polyphenolic substances like (+)-catechin, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin-3-gallate, dimeric procyanidins B1, B2, B3, B4 and trimeric procyanidin C1, among others (Quesada et al., 2011). As a nutritional supplement, grape seed-derived procyanidin extract (GSP) contains many benefits such as anti-inflammatory, anti-diabetic, cardioprotective, improving lipid metabolism and limiting adipogenesis (EI-Alfy, Ahmed, Fatani, & Kader, 2008; Montagut et al., 2009; Yamakoshi, Saito, Kataoka, & Kikuchi, 2002), and so on. Furthermore, procyanidin oligomers (OPC) are successfully detected in the plasma after GSP oral administration, and the absorption rate of dimers is higher than trimers and tetramers (Ou & Gu, 2013). Additionally, GSP is reported to have the ability of inhibiting the growth of prostate cancer PC-3 cells and maybe used as a new drug for the treatment of prostate cancer (Huang et al., 2008).

Cernilton (Prostat Tablet), derived from Secale cereale, is one of the most commonly used clinical agents for the treatment of prostate diseases such as BPH and CAP. Each tablet of cernilton contains 60 mg of the aqueous extract of Secale cereale (P-5) and 3 mg of the acetone extract (EA-10). In this study, 200 mg/kg/d cernilton and 200 mg/kg/d GSP were used as positive control and low dose GSP, respectively (Lu et al., 2011). The potential of GSP to protect against carrageenan-induced abacterial prostatitis in a rat model was investigated for the evaluation of its possible mechanisms.

2. Materials and methods

2.1. Reagents

Carrageenan was purchased from Dingguo Changsheng Biotechnology, Co, Ltd. (Wuhan, Hubei, China). The commercial kits for the analysis of the prostatic acid phosphatase (PACP), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The total protein extraction kit was purchased from ProMab Biotechnologies, Inc. (Richmond, CA, USA). The bicinchoninic acid (BCA) kit used for the detection of protein content was purchased from Beyotime Institute of Biotechnology (Shanghai, China). The commercial enzyme linked immunosorbent assay (ELISA) kits used for the measurement of interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), interleukin -6 (IL)-6, IL-10 and basic fibroblast growth factor (bFGF) were purchased from R&D Systems, Inc. (Minneapolis, MN, USA). The antibodies of extracellular signal-regulated kinases (ERK) antibody (about 44 kD) and p-ERK antibody (about 44 kD) used for Western blot were purchased from Epitomics Biotechnology, Inc. (Burlingame, CA, USA). Cernilton (Prostat Tablet) was procured from Meirui Pharmacy, Co, Ltd. (Nanjing, Jiangsu, China). GAPDH was bought from Sigma–Aldrich (St. Louis, MO, USA). (+)-Catechin, (−)-epicatechin and procyanidin B2 (all 98% purity) were purchased from Chinese Standard Material Institute (Beijing, China). Vitexin (98% purity) used as the internal standard (IS) was obtained from Chengdu Biopurify Technology Development, Co, Ltd. (Chengdu, Sichuan, China). The acetonitrile (HPLC grade) was purchased from Tedia, Inc. (Fairfield, OH, USA). All other solvents and chemicals used in the study were of analytical grade and purchased from Sinopharm Chemical Reagent, Co, Ltd. (Shanghai, China).

2.2. Preparation of GSP and analysis for the content of procyanidin

Red grape was collected from Wuhan, Hubei, China. The air-dried grape seed was powdered using an oscillating high speed universal grinder. The powder (5.0 kg) was soaked in 70% ethanol (solid (g)–liquid (ml) ratio = 1:7) and extracted under hot reflux at 50 °C for 3 times, each for 1 h. The extract was filtered through Whatman No. l membrane filter, the filtrate was freed of solvent under vacuum by means of a rotatory evaporator. The concentrated extract was dried in lyophilizer (Virtis 2K-XL, Gardiner, NY, USA). The resulting crude extract was purified by macroporous resin column and washing with ethanol/water: the resin column was gradient eluted with 0%, 20%, 60% and 80% of ethanol, respectively. The 60% eluted fractions were collected and identified as GSP.

The content of total polyphenol in GSP was determined by Folin–Ciocalteau spectrophotometric method using gallic acid as a standard (Martín et al., 2010). The content of total procyanidin in GSP was detected *via* BuOH–HCl assay according to the method of Bozan, Tosun, and Özcan (2008). Total determination for OPC (⩽4) in GSP was performed based on the method of Fan, Wu, and Tao (2007). The compounds catechin, epicatechin and procyanidin B2 in GSP were determined by HPLC–MS/MS. The grape seed extract powder (100 mg) with 10 ml of 80% methanol was ultrasonic extraction for 30 min, then the extract was centrifuged for 10 min at 11,000*g*, the supernatant was appropriately diluted and filtered for HPLC–MS/MS analysis.

Shimadzu LC-20A series HPLC (Kyoto, Japan) was linked to an API 4000 Mass Spectrometer (AB SCIEX, Framingham, MA, USA). A 5 μl of sample was injected into a Sepax GP C_{18} column (150 × 2.1 mm, 3 μm) (Newark, DE, USA), held at 25 °C, with a constant flow rate of 200 μl/min. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile containing 0.1% formic acid) with an initial concentration of 10% B. The binary gradient was as follows: 10% B (0–2 min), 10–14% B (2–2.5 min), 14% B (2.5–10 min), 14–20% B (10–15 min), 20–50% B (15–18 min), 50–97% (18–20 min) and held until 22 min and then returned to the initial conditions over 1 min and equilibrated for a further 2 min until injection of the next analytical sample. The column effluent was introduced into the Mass Spectrometer detecting negative ions. The ion spay voltage was set at −4500 V, the nebulizer gas 50 psi (heated at 450 °C), the auxiliary gas