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Original Article

Wogonoside ameliorates carrageenan-induced chronic nonbacterial prostatitis in rats

Qiong Zhou[1*], Lu Zhang[1*], Cuihua Feng[2]

Departments of [1]Urology, [2]Gastrointestinal Surgery, Wuhan No.6 Hospital, Wuhan, Hubei, China. [*]Equal contributors.

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Abstract: Chronic nonbacterial prostatitis (CNP) is the most common type of prostatitis, the etiology and pathogenesis of CNP still remain unknown and the new therapeutic strategies for CNP are urgent needed to be developed. In the present study, we investigated the effect of wogonoside on carrageenan-induced nonbacterial prostatitis in rats. After the experimental period, we found wogonoside treatment played anti-inflammatory roles against experimental model of CNP. Compared with the model group, wogonoside treatment significantly decreased the level of prostate index (PI), prostatic acid phosphatase (PAP), total acid phosphatase (ACP) and suppressed the production of inflammatory cytokines, as well as the increased activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx). In addition, we found treatment with wogonoside obviously inhibited the activation of PI3K/Akt/NF-κB signaling pathway in carrageenan-injected rats. In conclusion, our study demonstrated that wogonoside exert anti-inflammatory effect against carrageenan-induced chronic nonbacterial prostatitis in rats via modulation of PI3K/Akt/NF-κB signaling, suggesting that wogonoside might be a potential effective drug for CNP.

Keywords: Wogonoside, carrageenan, prostatitis, PI3K/Akt/NF-κB signaling pathway

Introduction

Prostatitis is the most prevalent problems in andriatry and urinary surgery, nearly 2 to 16% of men worldwide suffer from this disease [1, 2]. According to the National Institutes Health (NIH)/National Institute of Diabetes Digestive and Kidney Disease (NIDDK) classification of prostatitis in 1999, prostatitis mainly includes four types: the acute bacterial prostatitis (ABP), the chronic bacterial prostatitis (CBP), the chronic nonbacterial prostatitis (CNP) and the noninflammatory chronic pelvic pain syndrome (ICPS) [3]. In all categories, CNP is the most common and debilitating prostatitis syndrome types of prostatitis which causes approximately 90% of prostatitis cases [4]. Despite the rapid progress of modern medicine, there is still a lack of effective treatment for CNP. Therefore, it is of great significance to explore the precise etiology and underlying mechanisms of CNP and strengthen the screening and development of potent drugs for CNP.

Wogonoside is the main flavonoid component derived from the root of *Scutellaria baicalensis* Georgi, it has been shown to have anti-tumor, antithrombotic, antifibrotic and antioxidant effects [5-9]. In addition, recently, studies reported that wogonoside could inhibit inflammatory cytokines production in LPS-stimulated RAW-264.7 cells, and wogonoside played anti-inflammatory roles in experimental colitis by inhibiting NF-κB and NLRP3 inflammasome activation [10, 11]. But the roles of wogonoside in prostatitis, especially in CNP and the underlying mechanisms still remain unknown.

In this report, we investigated the role of wogonoside in CNP. We found wogonoside ameliorated carrageenan-induced CNP in rats. The level of prostate index (PI), and the concentration of the bio-markers for the diagnosis of prostate diseases such as prostatic acid phosphatase (PAP) and total acid phosphatase (ACP), as well as the production of inflammatory cytokines were all decreased in wogonoside-treated group compared with the model group. And wogonoside treatment increased the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx). Furthermore, we found treatment with wogonoside significantly inhibited the acti-

vation of PI3K/Akt/NF-κB signaling pathway. In conclusion, in the present study, we illustrated the anti-inflammatory role of wogonoside in CNP, and suggested wogonoside as a potential drug candidate for the treatment of CNP.

Materials and methods

Animals and treatments

Eight-week-old male Sprague-Dawley (SD) rats weighed 200-220 g, were obtained from Huazhong University of Science and Technology (Wuhan, China). Rats were housed in an animal room maintained at constant temperature and humidity with a 12-hour light/dark cycle, food and water were supplied ad libitum. The experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals approved by the Animal Ethics Committee of the Scientific Investigation Board of Wuhan No.6 Hospital. Thirty SD rats were randomly divided into sham-operated group (group A), carrageenan-induced CNP model group (group B), and carrageenan-induced CNP with wogonoside treatment group (group C) with each animal number of 10. Group B and group C were injected 20 μl of 1% carrageenan into their prostatic lateral lobes and group A was injected with 20 μl physiological saline instead. Seven days after the operation, rats in group C were kept for oral administration of wogonoside (50 mg/kg, Langze Pharmaceutical Co, Ltd., Nanjing, China) and those of group B and group A were orally treated with the same volume of physiological saline for 4 weeks.

Samples

At the end of the experiment period, the samples of prostatic lateral lobes were rapidly separated and weighed, prostate index (PI) was calculated from the absolute prostatic weight divided by the body weight. The prostatic tissues were cut into three sections. The tissues were homogenized in physiological saline and centrifuged, the supernatants were collected for the biochemical analysis. Other parts were washed and stored at -80°C for the qRT-PCR analysis and western blot analysis.

Biochemical analysis

The commercial kits for the analysis of the prostatic acid phosphatase (PAP), total acid phosphatase (ACP), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China), the experiments were performed according to the manufacturer's instructions. Protein levels of IL-10, IL-6 and TNF-α were measured using IL-10, IL-6, TNF-α ELISA kit (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions.

qRT-PCR analysis

Prostatic tissues were collected and total RNA was extracted with TRIzol reagent according to the manufacturer's instructions (Invitrogen). SYBR RT-PCR kit (Takara Biotechnology) was used for qRT-PCR analysis. GAPDH was used as internal control. Primer sequences: TNF-α (sense): 5'-AGG GCC ATT CCT ACT CCC AT-3', TNF-α (anti-sense): 5'-TGT AGC CCC GGA TAC ACA GA-3'; IL-6 (sense): 5'-CAC TTC ACA AGT CGG AGG CT-3', IL-6 (anti-sense): 5'-TCT GAC AGT GCA TCA TCG CT-3'; IL-10 (sense): 5'-AAG GGT TAC TTG GGT TGC CA-3', IL-10 (anti-sense): 5'-TGC CTG GGG CAT CAC TTC TA-3'; GAPDH (sense): 5'-TGA TTC TAC CCA CGG CAA GTT-3', GAPDH (anti-sense): 5'-TGA TGG GTT TCC CAT TGA TGA-3'.

Western blot analysis

Whole cell lysates were prepared and subjected to Western blot analysis as described [12]. The antibodies for specific for p65, phospho-p65, IκBα, phospho-IκBα, PI3K, phospho-PI3K, Akt, phospho-Akt (Cell Signaling Technology Inc, Beverly, USA) and β-Actin (Santa Cruz Biotechnology, Santa Cruz, USA) were used in the present study. The process was in strict accordance with the instructions of manufactures.

Statistical analysis

One-way ANOVA was performed to compare three or more groups. If the ANOVA analysis result was significant, Tukey post hoc test was used to determine the specific pairs of groups showing statistically significant differences. $P < 0.05$ considered statistically significant.

Results

Wogonoside treatment attenuated the level of PI, PAP and ACP in rats with CNP induced by carrageenan

After the experimental period, the effect of wogonoside on the level of PI was detected. As

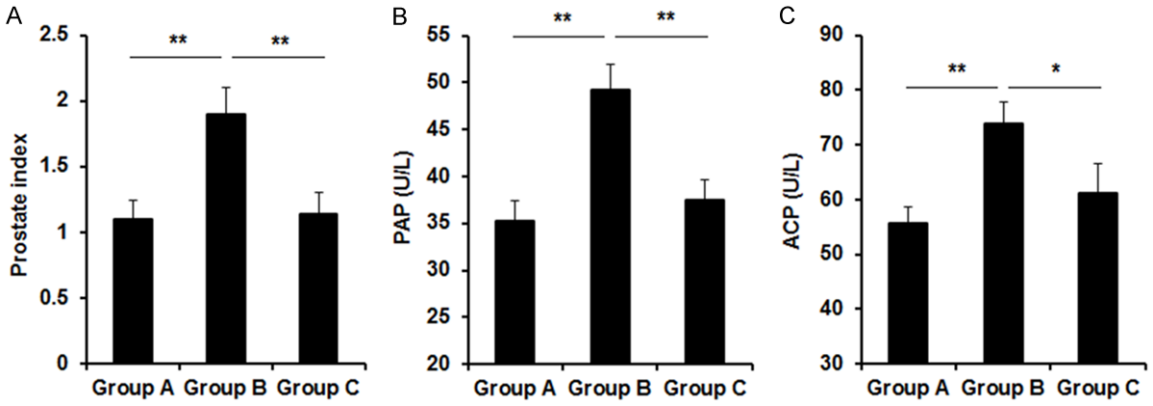

Figure 1. Wogonoside treatment attenuated the level of PI, PAP and ACP in rats with CNP induced by carrageenan. (A) Prostate index (PI) of group A, group B and group C, PI = absolute prostatic weight/body weight. (B, C) Concentration of PAP (B) and CAP (C) in prostate tissues of group A, group B and group C. Group A: sham-operated group (group A); group B: carrageenan-induced CNP model group; group C: carrageenan-induced CNP with wogonoside treatment group. The results were shown as mean ± SD from three representative independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

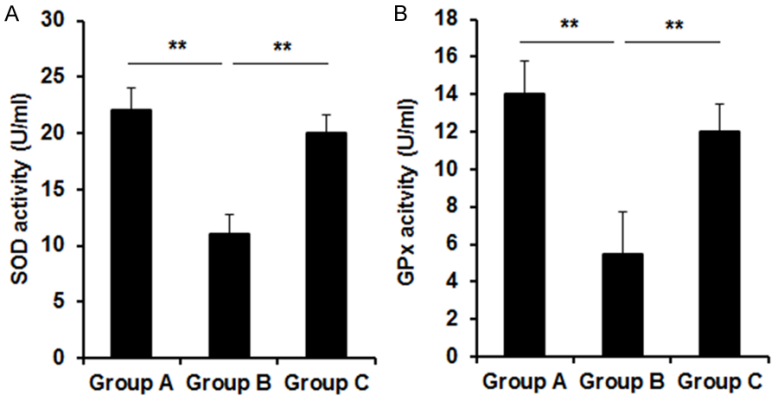

Figure 2. Wogonoside treatment increased the activity of SOD and GPx in rats with CNP induced by carrageenan. (A, B) Activity of SOD (A) and GPx (B) in prostate tissues of group A, group B and group C. Group A: sham-operated group (group A); group B: carrageenan-induced CNP model group; group C: carrageenan-induced CNP with wogonoside treatment group. The results were shown as mean ± SD from three representative independent experiments. **P < 0.01.

shown in **Figure 1A**, the level of PI was significantly increased in group B compared with group A, but in group C, the level was obviously decreased because of the treatment of wogonoside. In **Figure 1B** and **1C**, we examined the concentration of the bio-markers for the diagnosis of prostate diseases such as prostatic acid phosphatase (PAP) and total acid phosphatase (ACP) in prostatic tissues, and we found these concentrations were also attenuated by wogonoside treatment. These results indicated that intraprostatic carrageenan injection indeed induced the chronic nonbacterial prostatitis in rats, and the wogonoside treatment significantly ameliorated the prostatitis.

Wogonoside treatment increased the activity of SOD and GPx in rats with CNP induced by carrageenan

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) have been reported to play protective roles in the oxidation process in chronic prostatitis [13], thus we also examined the activities of SOD and GPx in prostatic tissues. As shown in **Figure 2A** and **2B**, the activities of SOD and GPx were decreased in group B compared with group A, but the activities were obviously restored after wogonoside treatment.

Wogonoside treatment inhibited the production of inflammatory cytokines in rats with CNP induced by carrageenan

Inflammatory cytokines have crucial function in the pathogenesis and diagnosis of chronic prostatitis [14, 15]. In **Figure 3A** and **3B**, we detected the mRNA levels and protein levels of inflammatory cytokines such as TNF-α, IL-6 and IL-10 in prostatic tissues by qRT-PCR and ELISA. Consistently, we observed that both mRNA and

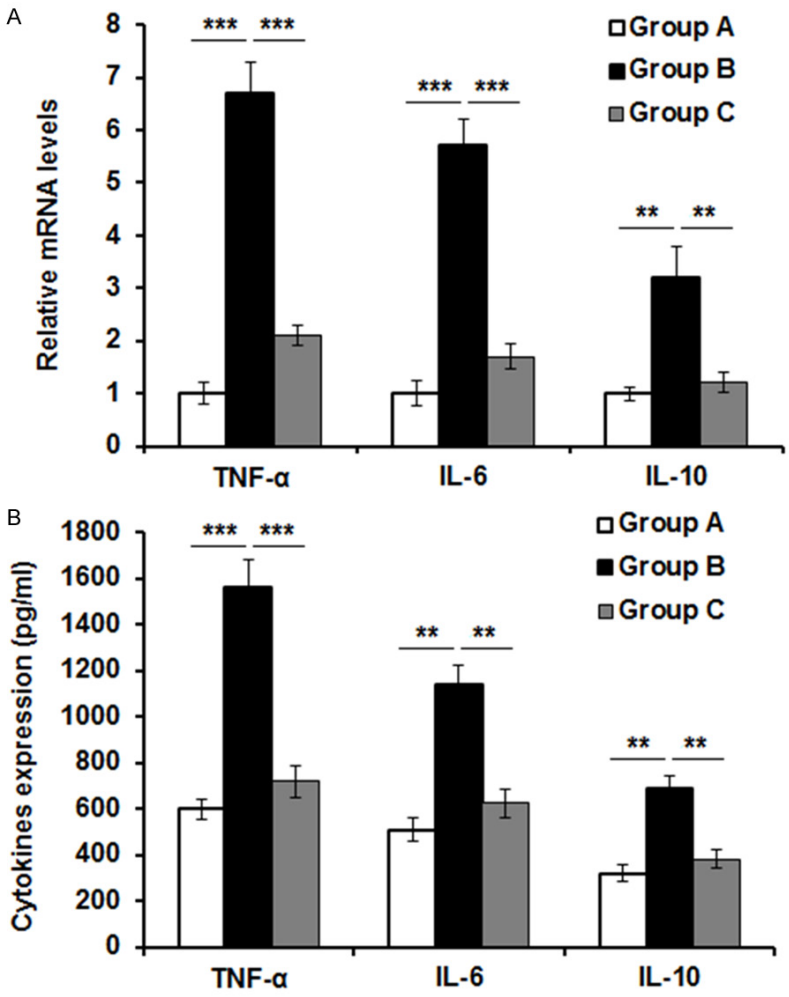

Figure 3. Wogonoside treatment inhibited the production of inflammatory cytokines in rats with CNP induced by carrageenan. (A, B) Relative mRNA level (A) and secreted protein level (B) of TNF-α, IL-6, IL-10 in prostate tissues of group A, group B and group C. Group A: sham-operated group (group A); group B: carrageenan-induced CNP model group; group C: carrageenan-induced CNP with wogonoside treatment group. The results were shown as mean ± SD from three representative independent experiments. **P < 0.01, ***P < 0.001.

protein levels of these inflammatory cytokines were decreased in group C compared with group B after carrageenan challenged.

Wogonoside treatment suppressed the activation of NF-κB signaling in rats with CNP induced by carrageenan

Production of inflammatory cytokines depends mainly on the NF-κB activation, so we hypothesized if wogonoside could affect NF-κB activation. We found activation of NF-κB was significantly increased in group B compared with group A, most importantly, we found treatment of wogonoside markedly inhibited the activation of NF-κB in group C (**Figure 4A**). The relative expression of p-P65 (**Figure 4B**) and p-IκBα (**Figure 4C**) was also identified.

Wogonoside treatment inhibited the activation of NF-κB through PI3K/Akt pathway in rats with CNP induced by carrageenan

It has been documented that NF-κB is the downstream of PI3K/Akt pathway [16, 17]. Previous studies showed that wogonoside could inhibit NF-κB activation through PI3K/Akt pathway in human colon cancer cells [18], therefore we examined if wogonoside treatment inhibited the activation of NF-κB through PI3K/Akt pathway in CNP rats. We found that in group C, wogonoside treatment significantly reduced the phosphorylation level of PI3K and Akt, which indicated the inhibitory effect of wogonoside on PI3K/Akt pathway in rats with CNP induced by carrageenan (**Figure 5A**). The relative expression of p-PI3K (**Figure 5B**) and p-Akt (**Figure 5C**) was also examined.

Discussion

In the present study, we investigated the effect of wogonoside on carrageenan-induced chronic nonbacterial prostatitis in rats. We found that wogonoside treatment had anti-inflammatory effects against experimental model of CNP. Compared with the model group, wogonoside treatment significantly decreased the level of PI, PAP, and ACP and suppressed the production of inflammatory cytokines such as TNF-α, IL-6 and IL-10, as well as increased the activities of SOD and GPx. In addition, we found treatment with wogonoside obviously inhibited the activation of NF-κB signaling through suppressing phosphorylation of PI3K and Akt in carrageenan-treated rats. To the best of our known, this is the first study the role of wogonoside in prostatitis.

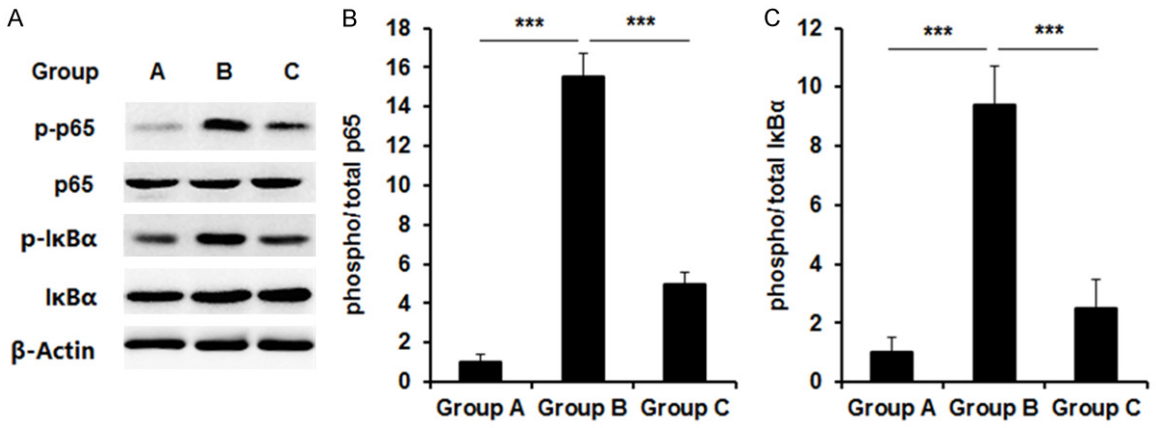

Figure 4. Wogonoside treatment suppressed the activation of NF-κB signaling in rats with CNP induced by carrageenan. (A) Expression of p-p65, p65, p-IκBα, IκBα and β-Actin in prostate tissues of group A, group B and group C. Group A: sham-operated group (group A); group B: carrageenan-induced CNP model group; group C: carrageenan-induced CNP with wogonoside treatment group. (B, C) Quantification of protein levels of p-p65 (B) and p-IκBα (C) in (A). The results were shown as mean ± SD from three representative independent experiments. ***P < 0.001.

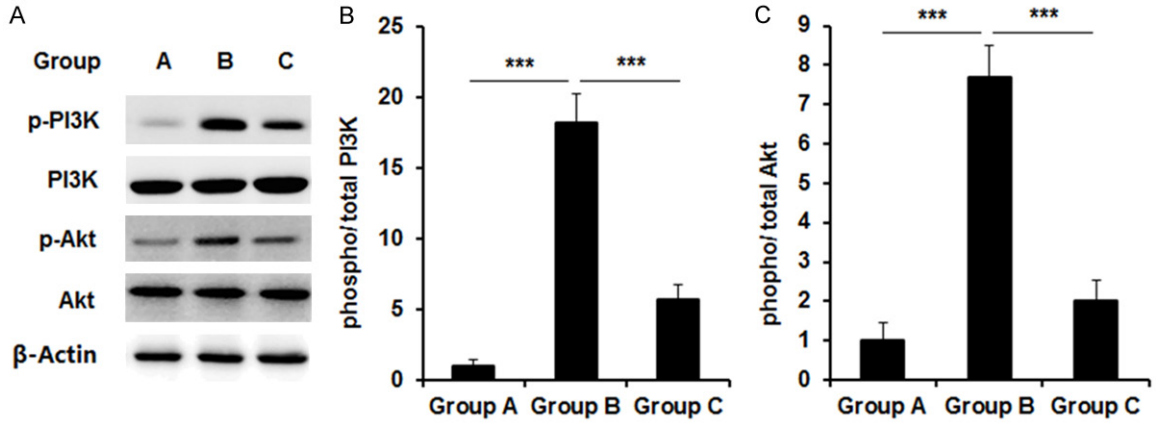

Figure 5. Wogonoside treatment inhibited the activation of NF-κB through PI3K/Akt pathway in rats with CNP induced by carrageenan. (A) Expression of p-PI3K, PI3K, p-Akt, Akt and β-Actin in prostate tissues of group A, group B and group C. Group A: sham-operated group (group A); group B: carrageenan-induced CNP model group; group C: carrageenan-induced CNP with wogonoside treatment group. (B, C) Quantification of protein levels of p-PI3K (B) and p-Akt (C) in (A). The results were shown as mean ± SD from three representative independent experiments. ***P < 0.001.

Chronic nonbacterial prostatitis is the most common urological diagnosis in men younger than 50 years and the third most common urologic diagnosis in men older than 50 years [19]. However, the specific pathogenesis of CNP is still unclear, and no standard therapies are confirmed to be available and effective for the treatment of CNP. Carrageenan-induced nonbacterial prostatitis in rat model is the most common model used in the research of CNP, and the model has greatly promoted the research on the treatment of CNP. By using this disease model, previous studies showed that grape seed-derived procyanidin extract could protect the rats from carrageenan-induced nonbacterial prostatitis [20], and N-acetylcysteine has been reported to ameliorate CNP via miR-141 regulating Keap1/Nrf2 signaling [12]. In this study, we used carrageenan-induced chronic nonbacterial prostatitis model in rats to research the effect of wogonoside in CNP, and we found wogonoside could obviously decreased the level of PI, PAP, and ACP and suppressed the production of inflammatory cytokines, therefore the chronic nonbacterial prostatitis in rats could be ameliorated.

Flavonoids possess diverse biological and pharmaceutical properties and have been widely studied in the physiological and pathological

processes of diverse diseases. In the research of prostatitis model in rats, many kinds of flavonoids extracted from Chinese herbal medicine have been studied. Flavonoid-rich fraction from Cyclosorus acuminatus was found to have prostatic protective nature [21]. Protective potential of the methanol extract of Macrothelypteris oligophlebia rhizomes for CNP has also been shown [22]. Anthocyanin extracted from black soybean was recently reported has anti-inflammatory and antimicrobial effects on chronic bacterial prostatitis rat model [23]. Consistent with their findings, we found that wogonoside, which is a flavonoid extract from *Scutellaria baicalensis*, also exist anti-inflammatory effect on CNP in rats model. Most importantly, we found wogonoside could inhibit the activation of NF-κB signaling mainly through the suppression of the phosphorylation of PI3K and Akt in CNP rat model, which may also indicate the importance of these signaling pathways in the treatment of CNP.

In conclusion, in the current study, we revealed the anti-inflammatory effect and the potent potential of wogonoside in the treatment of chronic nonbacterial prostatitis.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Cuihua Feng, Department of Gastrointestinal Surgery, Wuhan No.6 Hospital, Wuhan 430015, Hubei, China. E-mail: cuihua_feng01@163.com

References

[1] Collins MM, Stafford RS, O'Leary MP and Barry MJ. How common is prostatitis? A national survey of physician visits. J Urol 1998; 159: 1224-1228.

[2] Cheah PY, Liong ML, Yuen KH, Teh CL, Khor T, Yang JR, Yap HW and Krieger JN. Chronic prostatitis: symptom survey with follow-up clinical evaluation. Urology 2003; 61: 60-64.

[3] Litwin MS, McNaughton-Collins M, Fowler FJ Jr, Nickel JC, Calhoun EA, Pontari MA, Alexander RB, Farrar JT and O'Leary MP. The national institutes of health chronic prostatitis symptom index: development and validation of a new outcome measure. Chronic prostatitis collaborative research network. J Urol 1999; 162: 369-375.

[4] Clemens JQ, Calhoun EA, Litwin MS, McNaughton-Collins M, Kusek JW, Crowley EM, Landis JR; Urologic Pelvic Pain Collaborative Research N. Validation of a modified national institutes of health chronic prostatitis symptom index to assess genitourinary pain in both men and women. Urology 2009; 74: 983-987, quiz 987, e981-983.

[5] Yu C, Zhang Z, Zhang H, Zhen Z, Calway T, Wang Y, Yuan CS and Wang CZ. Pretreatment of baicalin and wogonoside with glycoside hydrolase: a promising approach to enhance anticancer potential. Oncol Rep 2013; 30: 2411-2418.

[6] Gao Z, Huang K, Yang X and Xu H. Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of scutellaria baicalensis Georgi. Biochim Biophys Acta 1999; 1472: 643-650.

[7] Kimura Y, Okuda H and Ogita Z. Effects of flavonoids isolated from scutellariae radix on fibrinolytic system induced by trypsin in human umbilical vein endothelial cells. J Nat Prod 1997; 60: 598-601.

[8] Wang Y, Yin RF and Teng JS. Wogonoside induces cell cycle arrest and mitochondrial mediated apoptosis by modulation of Bcl-2 and Bax in osteosarcoma cancer cells. Int J Clin Exp Pathol 2015; 8: 63-72.

[9] Wang Q, Wen R, Lin Q, Wang N, Lu P and Zhu X. Wogonoside shows antifibrotic effects in an experimental regression model of hepatic fibrosis. Dig Dis Sci 2015; 60: 3329-3339.

[10] Yang YZ, Tang YZ and Liu YH. Wogonoside displays anti-inflammatory effects through modulating inflammatory mediator expression using RAW264.7 cells. J Ethnopharmacol 2013; 148: 271-276.

[11] Sun Y, Zhao Y, Yao J, Zhao L, Wu Z, Wang Y, Pan D, Miao H, Guo Q and Lu N. Wogonoside protects against dextran sulfate sodium-induced experimental colitis in mice by inhibiting NF-kappaB and NLRP3 inflammasome activation. Biochem Pharmacol 2015; 94: 142-154.

[12] Wang LL, Huang YH, Yan CY, Wei XD, Hou JQ, Pu JX and Lv JX. N-acetylcysteine ameliorates prostatitis via miR-141 regulating Keap1/Nrf2 signaling. Inflammation 2016; 39: 938-947.

[13] Kim DS, Lee EJ, Cho KS, Yoon SJ, Lee YH and Hong SJ. Preventive effects of oligomerized polyphenol on estradiol-induced prostatitis in rats. Yonsei Med J 2009; 50: 391-398.

[14] Hu C, Yang H, Zhao Y, Chen X, Dong Y, Li L, Dong Y, Cui J, Zhu T, Zheng P, Lin CS and Dai J. The role of inflammatory cytokines and

ERK1/2 signaling in chronic prostatitis/chronic pelvic pain syndrome with related mental health disorders. Sci Rep 2016; 6: 28608.
[15] Jang TL and Schaeffer AJ. The role of cytokines in prostatitis. World J Urol 2003; 21: 95-99.
[16] Zha L, Chen J, Sun S, Mao L, Chu X, Deng H, Cai J, Li X, Liu Z and Cao W. Soyasaponins can blunt inflammation by inhibiting the reactive oxygen species-mediated activation of PI3K/Akt/NF-kB pathway. PLoS One 2014; 9: e107655.
[17] Arlt A, Gehrz A, Muerkoster S, Vorndamm J, Kruse ML, Folsch UR and Schafer H. Role of NF-kappaB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. Oncogene 2003; 22: 3243-3251.
[18] Sun Y, Zhao Y, Wang X, Zhao L, Li W, Ding Y, Kong L, Guo Q and Lu N. Wogonoside prevents colitis-associated colorectal carcinogenesis and colon cancer progression in inflammation-related microenvironment via inhibiting NF-kappaB activation through PI3K/Akt pathway. Oncotarget 2016; 7: 34300-34315.
[19] Coulthard MG. Adult and pediatric urology. Mosby Year Book 1996.
[20] Lei Y, Ren X, Chen J, Liu D and Ruan J. Protective effects of grape seed-derived procyanidin extract against carrageenan-induced abacterial prostatitis in rats. Journal of Functional Foods 2014; 7: 416-424.
[21] Chen J, Song H, Ruan J and Lei Y. Prostatic protective nature of the flavonoid-rich fraction from cyclosorus acuminatus on carrageenan-induced non-bacterial prostatitis in rat. Pharm Biol 2013; 491-497.
[22] Han P, Lai YJ, Chen J, Zhang XN, Chen JL, Yang X, Xue PP and Ruan JL. Protective potential of the methanol extract of macrothelypteris oligophlebia rhizomes for chronic non-bacterial prostatitis in rats. Pak J Pharm Sci 2016; 29: 1217-1221.
[23] Yoon BI, Bae WJ, Choi YS, Kim SJ, Ha US, Hong SH, Sohn DW and Kim SW. The anti-inflammatory and antimicrobial effects of anthocyanin extracted from black soybean on chronic bacterial prostatitis rat model. Chin J Integr Med 2013; 1-6.