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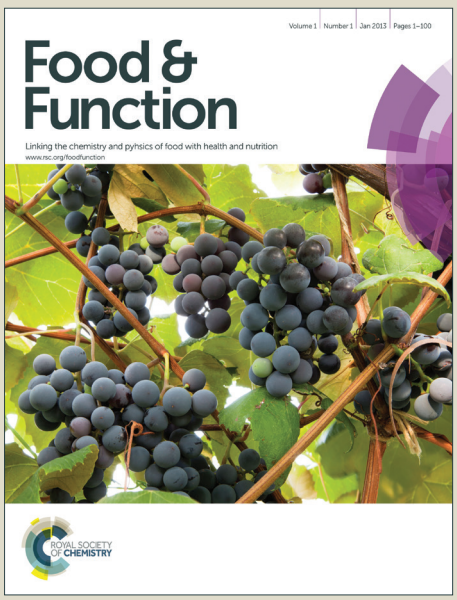

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Anti-inflammatory Effect and Prostate Gene Expression Profiling of Steryl Ferulate on Experimental Rats with Non-bacterial Prostatitis

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ABSTRACT: Steryl ferulate (SF) is a bioactive mixture extracted from rice bran and shows higher inhibitory activity against inflammation than the corresponding free sterols. In this study, it aims to investigate the anti-inflammatory effect and prostate gene expression profiling of SF using a Xiaozhiling-induced non-bacterial prostatitis (NBP) rat model. The anti-inflammatory effect was evaluated by prostate weight, prostate index, acid phosphatase, density of lecithin corpuscles (DLC), white blood cell count (WBC), and prostatic histologic section. The prostate gene expression profiling was assessed by cDNA microarray and validated by quantitative real-time PCR of five selected genes. Pathway analysis and Gene ontology (GO) analysis were applied to determine the roles of these differentially expressed genes played in these biological pathways or GO terms. SF treatment could significantly inhibit prostate weight, prostate index, total acid phosphatase, prostatic acid phosphatase and WBC, suppress the severity of histological lesion and increase the DLC. Compared with the control group, the SF treatment group contained **238** up-regulated genes and **111** down-regulated genes. GO analysis demonstrated that the most significant expression genes were closely related to the terms of fibrinolysis, inflammatory response, high-density lipoprotein particle, protein-lipid complex, enzyme inhibitor activity, peptidase inhibitor activity and others. Canonical pathway analysis indicated **five** pathways were significantly regulated, which were associated with inflammation and tumorgenesis. In conclusion, SF may be used as a health supplement to prevent NBP, in that it could inhibit prostate inflammation in NBP patients by affecting the expression of genes in the related GO terms and pathways.

KEY WORDS: Steryl ferulate; Non-bacterial prostatitis; Anti-inflammatory; Phytosterol; Gene expression profiling

1 Introduction

Prostatitis is one of the most prevalent conditions in urology[1, 2] and accounts for nearly two million ambulatory care cases annually in the United States[3]. Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), the most common types of prostatitis, cause approximately 90% of prostatitis cases [4]. CP inflammation is usually non-bacterial. It is also an important factor of symptom progression in patients with benign prostatic hyperplasia and associated with an increased risk of prostate cancer [5]. The CP/CPPS patients with manifest severe pain as the main physical complaint experience lower quality of life than normal individuals[6]. However, few studies have provided information about the use of non-pharmacological modalities to treat this symptom[7, 8].

Steryl ferulate (SF, commonly known as γ-oryzanol) is a bioactive mixture extracted from rice bran[9, 10]; SF contains ferulic acid esterified to the hydroxyl group of phytosterols[11]. The ester bond between ferulic acid and phytosterols can be cleaved during mammalian digestion, thereby liberating ferulic acid and phytosterol moieties[12]. Ferulic acid is a ubiquitous plant constituent that arises from the metabolism of phenylalanine and tyrosine[13]. It has been shown to have significant antioxidant effects in many studies[14]. Phytosterols are natural constituents of plants and have many essential functions in plant cells, similar to those of cholesterol in animal cells [15, 16]. A few studies have reported that various plant extracts with phytosterols as the main active compound can elicit protective effects on prostatitis. For instance, pollen extract[17, 18], bamboo shoot oil[19]， and *Pygeum africanum* extract[20] affect the smooth muscles of the bladder and the urethra; these extracts also elicit strong anti-inflammatory and anti-proliferative effects.

SF provides health-promoting effects of these two active components and shows a higher inhibitory activity against inflammation in mice than corresponding free sterols in previous studies[21]. With the evident contribution of phytosterols to inhibit inflammation and prevent prostate diseases, it is reasonable to suppose that SF is possibly more beneficial for the treatment of non-bacterial prostatitis (NBP). However, as far as we know, there have been no attempts to evaluate inhibitory activity of SF

against inflammation in non-bacterial prostatitis.

In this study, the protective effects of SF against NBP were evaluated in experimental rats. Microarray gene expression profile analysis was used to determine the mechanism of inflammatory prevention of SF. To the best of our knowledge, this is the first investigation to use microarray technology for studying the role of SF in the prevention of prostate inflammatory. This study also aimed to elucidate the use of SF as a health supplement and provide ideas and methods to prevent and treat prostatitis.

2 Results

2.1 Effects of SF on weight, prostate weight, prostate volume, prostate density, and prostate index

During the four-week test period, no clinical signs or effects could be attributed to SF administered in rats. No significant difference was observed between the body weights of the control group and the Sham-ope group. After Xiaozhiling solution was administered, the control group showed severe edema and inflammation. These rats also revealed an increase in prostate weight, particularly in the ventral and lateral lobes. In SIT and SF groups, the increase in the total prostate weight was inhibited significantly ($p < 0.05$). SF 2000 and SF 1000 groups showed that the increase in total prostate weight was inhibited significantly ($p < 0.01$). Prostate index was significantly increased in the control group compared with the Sham-ope group ($p < 0.05$), and such parameter could be significantly decrease by 1000 and 2000 mg/kg SF in SF 1000 and SF 2000 groups, respectively ($p < 0.05$, Fig.1).

2.2 Effects of SF on various physiological indexes of prostatitis and prostate cancer

Compared with the Sham-ope group, the white blood cell count (WBC) was significantly increased and the density of lecithin corpuscles (DLC) was significantly decreased in the prostate fluid of the control group (Fig. 1). SF 2000, SF 1000, and SIT groups could significantly alleviate symptoms ($p < 0.01$). SF 500 group showed a significant decrease in WBC ($p < 0.05$).

The total acid phosphatase (ACP) and prostatic acid phosphatase (PAP) activities increased significantly in the control group compared with the Sham-ope group ($p < 0.001$). Tartrate resistant acid phosphatase (TrACP) activity remained unchanged. By contrast, ACP and PAP activities decreased significantly in SF 2000, SF 1000, and SIT groups ($p < 0.05$) compared with the control group. In SF 500 group, ACP and PAP remained unaffected by SF (Fig. 2).

2.3 Effects of SF on prostate histopathology

In HE staining (haematoxylin and eosin staining) of the prostate lateral lobe (Fig. 3), severe diffuse chronic inflammation symptoms characterized by acinar atrophy, leukocyte infiltration, and interstitial fibrosis were found in the control group. By contrast, SF and SIT treatments could significantly suppress these changes. In most glands, leukocyte infiltration and interstitial fibrosis were decreased as SF concentration was increased. No significant difference was observed in the epidermis area ratio of the rats in the control group and the Sham-ope group. Glandular number, glandular area, glandular cavity area, and their corresponding ratios were significantly lower ($p < 0.01$ or $p < 0.001$) in the control group than in the Sham-ope group. Glandular number, glandular area, and glandular cavity area were significantly higher in SF and SIT groups than in the control group, but no significant difference was observed in the corresponding ratios. Epidermis height, epidermis area, interstitial area, and interstitial area ratio in the control group were significantly higher ($p < 0.01$) than those in the Sham-ope group. Epidermis height and area were higher ($p < 0.01$) in the SF and SIT groups than in the control group, but no significant difference was observed in the interstitial area and the interstitial area ratio among the groups.

2.4 Effects of SF on prostate gene expression response

The control group (three samples) and the SF 2000 group (three samples) were subjected to microarray gene expression profile analysis to determine the mechanism of inflammatory prevention of SF. A total of 349 differentially expressed genes (DE genes), including 238 up-regulated genes and 111 down-regulated genes (fold change ≥ 1.5, $p < 0.05$), were found in the SF 2000 group compared with the control group. 20 most differentially expressed genes are shown in Table 1; most of these genes are

closely related to inflammatory response (*Ahsg*, *Serpina1*, *Gal*, *Apoa2*, *Apoc3*) and immune response (*Hp*, *Rbp4*, *F2*, *C3*). These genes may be related to NBP protection and thus require further studies.

Pro-inflammatory cytokines, interleukin-8, interleukin-1β, tumor necrosis factor-α and C-reactive protein are common biomarkers of inflammation in prostatitis Fourteen genes concerned with these biomarkers in the microarray were investigated and the results were shown in Table 2. However, only C-reactive protein was significantly down-regulating expressed between the two groups, and the rest showed no significant difference.

2.5 Effects of SF on related Gene ontology (GO) terms

GO analysis is a functional analysis, in which DE genes are associated with GO categories derived from GO (www.geneontology.org), a community bioinformatics resource that represents a gene product function by using structured and controlled vocabularies[22]. The result demonstrated that the significantly DE genes ($p < 0.05$, FDR < 0.05) comprised a total of 44 GO terms, including those involved in biological processes (28 GO terms), cell components (11 GO terms) and molecular function (5 GO terms). In these terms, the most differentially expressed genes appears mainly related to the response to the stimulus (91 DE genes), response to stress (44 DE genes), response to external stimulus (38 DE genes), response to organic substance (32 DE genes), response to wounding (26 DE genes), regulation of the response to the stimulus (22 DE genes), defense response (21 DE genes), the immune response (21 DE genes)， inflammatory response (16 DE genes), enzyme regulator activity (29 DE genes), extracellular region (60 DE genes), extracellular region part (40 DE genes) and extracellular space (32 DE genes), etc.

The highest enrichment GO terms are shown in Table 3. These GO terms were related to fibrinolysis, regulation of coagulation, activation of plasma proteins involved in acute inflammatory response, inflammatory response, lipid metabolic process, sterol metabolic process, hemostasis, regeneration, response to wounding, lipoprotein particle, platelet alpha granule lumen, cytoplasmic membrane-bounded vesicle lumen, vesicle lumen, enzyme inhibitor activity and so on.

159

160 **2.6 Effects of SF on related pathways**

161 Kyoto Encyclopedia of Genes and Genomes（KEGG） is a database resource
162 that integrates genomic, chemical, and systemic functional information[23]. Using the
163 latest KEGG database, we conducted a pathway analysis of DE genes. Five significant
164 pathways ($p < 0.05$; FDR < 0.05) were obtained in Table 4, including Complement
165 and coagulation cascades (rno04610), Metabolism of xenobiotics by cytochrome P450
166 (rno00980), PPAR signaling pathway (rno03320), Retinol metabolism (rno00830),
167 Drug metabolism –cytochrome P450 (rno00982). Two pathways were selected to
168 explain this mechanism further (Fig. 4). We mapped the DE genes onto the KEGG
169 pathways to attain a linkage between key molecules and biochemical pathways
170 associated with SF. Yellow marked nodes are associated with up-regulated genes,
171 orange marked nodes are associated with down-regulated or only whole dataset genes,
172 and green nodes have no significance.

173

174 **2.7 Quantitative real-time PCR (QRT-PCR) validation of the microarray**
175 **analysis**

176 QRT-PCR is one of the most common techniques used to detect and quantify
177 gene expression. This technique is also used to verify microarray data. A QRT-PCR
178 was performed on all of the five genes (*Plg*, *F2*, *Apoa2*, *Aqp7*, and *C3*) in order to
179 confirm the results obtained from the microarray. The results obtained from the
180 microarray analysis were compared with the data obtained by QRT-PCR for these
181 selected genes with differential expression and showed similar trends (Fig. 5). *Plg*,
182 *F2*, *Apoa2*, and *C3* expression levels were significantly higher in the control group
183 than in the sham-ope group ($p < 0.05$), while *Aqp7* expression was just the reverse. By
184 contrast, SF treatments could significantly suppress these changes.

185 **3 Discussion**

186 After Xiaozhiling solution was administered, severe edema, inflammation and
187 fibrosis in the prostatic lateral lobes and a remarkable increase in prostate mass were

found. Histological analysis showed severe diffuse chronic inflammation characterized by leukocyte infiltration and interstitial fibrosis in the lateral lobe of the prostate in the control group, similar to previous reports of other investigators[19,24] . By contrast, SF and SIT treatments could significantly suppress these changes. The inflammatory symptoms, for instance, in most glands, leukocyte infiltration and interstitial fibrosis were decreased as SF concentration was increased. This result indicated that SF could significantly alleviate the inflammatory symptoms of Xiaozhiling-induced NBP in rats.

As traditional markers of the severity of inflammation, the increase of WBC in prostate fluid is likely to be prostatitis and DLC is the opposite[24, 25]. PAP is a major source of serum acid phosphatase activity, mainly found in the prostate gland epithelium and lamina secretions. PAP is very low in normal serum levels, while it increases in patients with prostate cancer metastasis, prostatitis and prostatic hyperplasia. The prostate tumor size was positively correlated with the serum PAP activity. Therefore, PAP is used clinically as a serum biomarker of prostate cancer[26]. Similarly, ACP content has a certain significance in the clinical diagnosis of prostate disease. The experimental results show that WBC, PAP and ACP activities significantly increased and the DLC was just the reverse in the control group, but no effect on TrACP was found, while SF1000, SF2000 and SIT treatments could significantly restrain these parameters. It indicated that SF could effectively reduce inflammation and possibly decreased the risk of prostate cancer in the prostate tissues of rats with NBP.

Previous studies have shown that pro-inflammatory cytokines interleukin-8, interleukin-1β (*IL1b*) and tumor necrosis factor-α (*Tnfa*) are the reliable biomarkers of inflammation in prostatitis[27]. The expression of genes concerned with these biomarkers and the most differently expressed genes in part 2.4 showed that the addition of SF may not affect the pro-inflammatory transcription profile typical of these prostate tissues in the case of classical activation, but there was a decreased expression of genes involved in the GO terms related to inflammation and immune response.

Among the differently expressed GO terms, the terms related to the response to stimulus, stress, and defense were caused by SF absorption into the body. The GO terms of response to wounding, defense response, immune response, fibrinolysis, regulation of coagulation, activation of plasma proteins involved in acute inflammatory response, acute inflammatory response, platelet alpha granule lumen, cytoplasmic membrane-bounded vesicle lumen, vesicle lumen showed that SF could adjust and repair the tissue damage and inflammatory response caused by NBP. The emergence of these GO terms likely elucidated various molecular events of SF to prevent and treat NBP.

The significant pathways obtained by pathway analysis in part 2.6 are closely related to inflammatory and tumor-associated biological processes. For instance, peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors involved in regulating glucose and lipid homeostasis, inflammation, proliferation, and differentiation[28]. Complement and coagulation systems have fundamental clinical implications in the context of life-threatening tissue injury and inflammation[29, 30]. According to the mapping on the PPAR signaling pathway (Fig. 4a), all of these three subtypes, particularly PPARα and PPARγ, were affected by SF. On the regulation of complement and coagulation cascades (Fig. 4b), the three pathways of complement activation (classical, lectin, and alternative pathways)[31] were triggered and mediate complement activation by SF. Therefore, SF could alleviate inflammation and tissue fibrosis by down-regulating complement *C3* gene, the central component of the complement system associated with fibrin clot[32]. SF could also decrease the coagulation cascade reaction by down-regulating the genes of coagulation factors 2 and 12 (*F2* and *F12*) as well as other associated genes. It suggested that SF may significantly alleviate the inflammatory symptoms of Xiaozhiling-induced NBP by affecting the regulation of glucose and lipid homeostasis, inflammation, proliferation, and differentiation.

In addition, we also did a comparative gene expression analysis between SIT group and SF group. The result showed that there were no significant differences in both regulated GO terms and KEGG pathways between the two groups. It suggested

that SF had a similar anti-inflammatory mechanism as β-sitosterol did and the main active part of SF in the prevention and treatment of prostatitis process may be the steryl groups. However, the regulation mechanism of SF in these events remains to be studied further.

From a methodological perspective, this study applied a high-throughput whole gene expression microarray to investigate the behavior of anti-inflammatory effect of SF on Xiaozhiling-induced NBP rat model. The gene expression profiling analysis of the anti-inflammatory effect is completed by pathway analysis and GO analysis. The gene expression levels provided by microarray were validated by comparing with key mRNA transcripts assayed by QRT-PCR. This approach provides an overview of cellular processes in the anti-inflammatory effect, enabling the quantitative estimation of the change in expression of GO terms and pathways of the DE genes. It is, therefore, a powerful instrument to understand how the whole transcriptome is represented and annotated in prostate inflammation tissue in the presence of oral administration SF. However, the microarray presents an inherent limitation. The technique we used to investigate gene expression profiles, which does not automatically translate into proteomic profiles, and it has to be taken into account when evaluating the results of our experiments[33]. If a pathway is wanted to be studied completely, the corresponding proteomics experiments such as western blotting experiments and enzyme-linked immunosorbent assay (ELISA) must be performed. This is what we plan to implement in the further.

4 Experimental

4.1 Preparation of SF

SF was purified from Chinese rice as described previously[34]. In brief, the SF was prepared from commercial rice bran oil by conventional silica gel column chromatography. Reverse-phase high-performance liquid chromatography revealed that the SF fraction was mainly composed of four major ferulates: cycloartenyl ferulae (31.4%), 24-methylene cycloartanyl ferulate (51.5%), β-sitosteryl ferulate (12.3%) and campesteryl ferulate (4.8%). The average molecular weight of SF was calculated

from the molar ratio of these ferulates and the value was 607.15.

4.2 Animals and treatments

A total of 84 adult male Sprague-Dawley rats were purchased from the Experiment Animal Center of Zhejiang Academy of Medical Sciences (Hangzhou, P. R. China) and fed with a common diet prepared according to state standard GB14924-1994 (Laboratory Animals Wholesome Feeds). Food and water were supplied ad libitum. The rats were housed under the following conditions: 12 h/12 h light/dark photocycle and 40% to 70% humidity. NBP was induced by injecting 0.1 mL of 25% Xiaozhiling solution (inflammatory inducer; Shuanghe Co., Ltd., Beijing, P. R. China) diluted in 0.5% sodium carboxymethyl cellulose (CMC-Na; Zhejiang Shapuaisi Pharma Co., Ltd., Zhejiang, P. R. China) in the prostatic lateral lobes.

SF was suspended at 500, 1000, and 2000 mg/kg in 0.5% CMC-Na. β-sitosterol (SIT; 95%, Sigma) used as the positive control treatment was also suspended at 2000 mg/kg in 0.5% CMC-Na. The rats were randomly divided into six treatment groups with 14 rats in each group: sham-operated (Sham-ope) group; control group; SF 500 group; SF 1000 group; SF 2000 group; and SIT group. In the Sham-ope group, the rats receive an oral treatment with 0.5% CMC-Na (15 mL/kg). In the control group, the rats were injected with 0.1 mL of 25% Xiaozhiling solution in the prostatic lateral lobes under ether anesthesia on the first day and then orally treated with 0.5% CMC-Na (15 mL/kg). In SF 500, SF 1000, SF 2000, and SIT groups, 500, 1000, and 2000 mg/kg SF as well as 2000 mg/kg β-sitosterol were orally administered for four weeks, respectively. These procedures were conducted in accordance with the institutional guidelines for animal care and with Chinese government animal protection and management laws.

The general physical condition of each rat was monitored throughout the test period. The amounts of ACP, PAP and TrACP were analyzed using acid phosphatase assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, P. R. China). The prostate was extirpated and weighed; prostate index was calculated from the absolute prostatic weight divided by the body weight. The parts of the right prostatic lateral lobes were crushed. Afterward, DLC and WBC in crushed prostate samples were

observed under a microscope. Other parts of the prostatic lateral lobes were stored in liquid nitrogen for use in functionally focused cDNA microarray techniques.

4.3 Prostate histology

The left prostatic lateral lobes were used to confirm the presence of prostate inflammation. The ventral and dorsolateral prostate lobes were fixed at 10% neutral buffered formalin, embedded in paraffin, and stained with haematoxylin and eosin (HE staining). The images of the prostate lateral lobe were initially obtained at random fields of view. Prostatic histomorphological parameters (including glandular number, epithelial height, epithelium area, glandular cavity area, glandular area, and matrix area) were then analyzed quantitatively[35]. Relative epithelium area, glandular area, glandular cavity area, and matrix area were calculated using VNT QuantLab-MD biomedical image analysis system (Visual New Technology Developing Co., Ltd., Beijing, P. R. China).

4.4 cDNA microarray

The total RNAs of SF 2000 and control groups (three examples from each group) were extracted according to methods described previously[19], Total RNA from each sample was quantified by the NanoDrop 1000 and RNA integrity was assessed by standard denaturing agarose gel electrophoresis. The total RNAs of SF 2000 and control groups were used for labeling and array (NimbleGen 12x135K microarrays) hybridization, following the manufacturer's instructions.

Array scanning using the Axon GenePix 4000B microarray scanner (Molecular Devices Corporation).Scanned images were then imported into NimbleScan software (version 2.5) for grid alignment and expression data analysis. Expression data were normalized through quantile normalization and the Robust Multichip Average (RMA) algorithm included in the NimbleScan software. The 6 gene level files were imported into Agilent GeneSpring GX software (version 11.0) for further analysis. Differentially expressed genes were identified through Volcano Plot filtering. Pathway analysis and GO analysis were applied to determine the roles of these differentially expressed genes played in these biological pathways or GO terms.

4.5 Validation of microarray data by quantitative real-time PCR

QRT-PCR was performed to determine and confirm gene expression. The primers of *Plg* (Assay Identification Number: NM_053491), *F2* (NM_022924), *Apoa2* (NM_013112), *Aqp7* (NM_019157), and *C3* (NM_016994) were designed and synthesized by Superarray Bioscience Corp. The rat's reduced glyceraldehyde phosphate dehydrogenase (*GAPDH*, NM_017008) was used as an internal control. The thermal cycler conditions were listed as follows: 95 °C for 5 min (for initial denaturation); denaturation at 95 °C for 10 s; 59 °C for 15 s; and 72 °C for 20 s (35 cycles). QRT-PCR was conducted according to the manufacturer's protocol. The standard curve was drawn using the threshold cycle of the templates in known numbers of copies. The threshold cycle of each sample was plotted on the standard curve, and mRNA copy number was calculated using Rotor-gene 6.0 (Corbett Research). The results were expressed relative to *GAPDH*. All of the assays were performed in triplicate.

4.6 Statistical analysis

Each value was presented as mean ± SD. Data were analyzed by ANOVA. Significant difference was determined at $p < 0.05$, and the differences between treatments were determined by one-way ANOVA in SAS (SAS Institute Inc., 2002).

5 Conclusion

In conclusion, SF may be useful for the treatment of NBP, in that it could inhibit prostate inflammation in nonbacterial prostatitis patients by affecting the expression of genes in the related GO terms and pathways. This study elucidated SF may be used as a health supplement to prevent and treat NBP. However, the regulation mechanism of SF in these events remains to be studied further.

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TABLE

Table 1. The most differentially expressed genes whose changes due to Xiaozhiling treatment could be reversed by SF

SEQ_ID	GeneSymbol	COMMENTS	*p*-value	FC[a]
NM_134326	*Alb*	albumin	0.000949	-17.78041
NM_001009626	*Apoh*	apolipoprotein H	0.002639	-8.134782
NM_012901	*Ambp*	alpha 1 microglobulin/bikunin	0.001694	-6.252965
NM_012898	*Ahsg*	alpha-2-HS-glycoprotein	0.002458	-6.047176
NM_022519	*Serpina1*	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	0.010285	-5.692349
NM_053491	*Plg*	plasminogen	0.001675	-5.606492
NM_033237	*Gal*	galanin	0.007393	-5.174481
NM_133526	*Tspan8*	tetraspanin 8	0.026491	-5.14902
NM_001003409	*LOC298116*	alpha-2u-globulin (L type)	0.006671	-4.585438
NM_012582	*Hp*	haptoglobin	0.016404	-4.555831
NM_173094	*Hmgcs2*	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	0.008361	-4.427111
NM_013112	*Apoa2*	apolipoprotein A-II	0.018252	-4.424937
XM_001080229	*Rbp4*	retinol binding protein 4, plasma	0.003067	-4.28228
NM_031598	*Pla2g2a*	phospholipase A2, group IIA (platelets, synovial fluid)	0.026909	+3.775508
XM_001079837	*Tm4sf5*	transmembrane 4 superfamily member 5 (predicted)	0.004134	+3.601309
NM_001008859	*Mrps10*	mitochondrial ribosomal protein S10	0.027233	+3.586477
NM_012824	*Apoc1*	apolipoprotein C-I	0.009531	-3.519669
NM_012501	*Apoc3*	apolipoprotein C-III	0.024263	-3.470708
NM_022924	*F2*	coagulation factor II	0.007944	-3.375911
NM_053021	*Clu*	clusterin	0.02292	-3.362452

[a] FC: Fold change, the ratio of normalized intensities between two groups. "-", stands for down-regulating expression; "+", stands for up-regulating expression.

Table 2. The expression level of genes concerned with proinflammatory cytokines

SEQ_ID	GeneSymbol	COMMENTS	FC[a]
NM_031512	*Il1b*	Rattus norvegicus interleukin 1 beta (Il1b), mRNA.	-1.18
NM_053953	*Il1r2*	Rattus norvegicus interleukin 1 receptor, type II (Il1r2), mRNA.	-1.43
NM_177935	*Il1rapl1*	Rattus norvegicus interleukin 1 receptor accessory protein-like 1 (Il1rapl1), mRNA.	1.18
NM_133575	*Il1rl2*	Rattus norvegicus interleukin 1 receptor-like 2 (Il1rl2), mRNA.	1.14
NM_022194	*Il1rn*	Rattus norvegicus interleukin 1 receptor antagonist (Il1rn), mRNA.	1.40
NM_019310	*Il8ra*	Rattus norvegicus interleukin 8 receptor, alpha (Il8ra), mRNA.	1.14
NM_001014039	*Tnfaip8l2*	Rattus norvegicus tumor necrosis factor, alpha-induced protein 8-like 2 (Tnfaip8l2), mRNA.	1.09
NM_182950	*Tnfaip1*	Rattus norvegicus tumor necrosis factor, alpha-induced protein 1 (endothelial) (Tnfaip1), mRNA.	-1.49
XM_001056720	*Tnfaip8*	PREDICTED: Rattus norvegicus tumor necrosis factor, alpha-induced protein 8 (predicted) (Tnfaip8_predicted), mRNA.	1.23
XM_001061429	*Tnfaip8l1*	PREDICTED: Rattus norvegicus similar to tumor necrosis factor, alpha-induced protein 8-like 1 (LOC301131), mRNA.	1.10
XM_001071736	*Tnfaip2*	PREDICTED: Rattus norvegicus similar to Tumor necrosis factor, alpha-induced protein 2 (Primary response gene B94 protein) (LOC299339), mRNA.	-1.03
XM_001072284	*Tnfaip8l3*	PREDICTED: Rattus norvegicus tumor necrosis factor, alpha-induced protein 8-like 3 (predicted) (Tnfaip8l3_predicted), mRNA.	1.25
XM_236246	*Tnfaip8l3*	PREDICTED: Rattus norvegicus tumor necrosis factor, alpha-induced protein 8-like 3 (predicted) (Tnfaip8l3_predicted), mRNA.	1.36
NM_017096	Crp	Rattus norvegicus C-reactive protein, pentraxin-related (Crp), mRNA.	-3.61

[a] FC: Fold change, the ratio of normalized intensities between two groups. "-", stands for down-regulating expression; "+", stands for up-regulating expression.

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Table 3. The most enrichment GO terms could be improved by SF

GO.ID	Term	Ontology[a]	Count[b]	Fold. Enrichment[c]
GO:0042730	Fibrinolysis	BP[d]	5	26.37
GO:0030195	Negative regulation of blood coagulation	BP	6	21.09
GO:0050819	Negative regulation of coagulation	BP	6	18.08
GO:0030193	Regulation of blood coagulation	BP	6	13.56
GO:0050818	Regulation of coagulation	BP	6	11.17
GO:0002541	Activation of plasma proteins involved in acute inflammatory response	BP	5	9.04
GO:0018904	Organic ether metabolic process	BP	7	7.03
GO:0006639	Acylglycerol metabolic process	BP	6	6.66
GO:0002526	Acute inflammatory response	BP	11	6.45
GO:0006638	Neutral lipid metabolic process	BP	6	6.44
GO:0051605	Protein maturation by peptide bond cleavage	BP	7	6.33
GO:0006662	Glycerol ether metabolic process	BP	6	6.33
GO:0008203	Cholesterol metabolic process	BP	7	5.99
GO:0016125	Sterol metabolic process	BP	7	5.61
GO:0007596	Blood coagulation	BP	7	5.15
GO:0007599	Hemostasis	BP	7	5.09
GO:0031099	Regeneration	BP	9	4.13
GO:0051241	Negative regulation of multicellular organismal process	BP	11	4.02
GO:0006954	Inflammatory response	BP	16	3.75
GO:0009611	Response to wounding	BP	26	3.21
GO:0034364	High-density lipoprotein particle	CC[e]	6	22.21
GO:0034361	Very-low-density lipoprotein particle	CC	4	16.78
GO:0034385	Triglyceride-rich lipoprotein particle	CC	4	16.78
GO:0032994	Protein-lipid complex	CC	6	16.42
GO:0034358	Plasma lipoprotein particle	CC	6	16.42
GO:0031093	Platelet alpha granule lumen	CC	5	7.67
GO:0060205	Cytoplasmic membrane-bounded vesicle lumen	CC	5	7.15
GO:0031983	Vesicle lumen	CC	5	6.42
GO:0005615	Extracellular space	CC	34	4.12
GO:0044421	Extracellular region part	CC	40	3.56
GO:0005576	Extracellular region	CC	60	3.01
GO:0030234	Endopeptidase inhibitor activity	MF[f]	17	7.51
GO:0004857	Serine-type endopeptidase inhibitor activity	MF	10	7.48
GO:0030414	Peptidase inhibitor activity	MF	18	7.39
GO:0004867	Enzyme inhibitor activity	MF	23	6.17

[a] Ontology: the ontology of listed term belongs to;

[b] Count: the number of DE genes associated with the listed GO term;

[c] Fold. Enrichment: the Fold Enrichment value of the GO term;

[d]BP: biological processes ;

[e]CC: cell components;

[f]MF: molecular function.

Table 4. The most enrichment pathways could be improved by SF

PathwayID	Definition	Fisher-Pvalue	E-Score[a]	Genes[b]
rno04610	Complement and coagulation cascades	1.62E-06	5.78	*C3//C4B//CFI//F12//F2//FGG //PLG//SERPINA1//SERPINF2*
rno00980	Metabolism of xenobiotics by cytochrome P450	1.08E-05	4.96	*AHRR//CYP2B3//CYP2C23//GSTA2 //GSTA2//GSTM7//MGST1//UGT1A1*
rno03320	PPAR signaling pathway	2.54E-05	4.59	*A2//APOC3//AQP7//CYP8B1// HMGCS2//PCK1//RXRG//SLC27A6*
rno00830	Retinol metabolism	9.28E-05	4.03	*AHRR//AWAT2//CYP2B3//CYP2C23// RDH12//RDH7//UGT1A1*
rno00982	Drug metabolism – cytochrome P450	0.000267	3.57	*CYP2B3//CYP2C23//GSTA2//GSTA2// GSTM7//MGST1//UGT1A1*

[a]E-Score: the Enrichment Score value of the PathwayID, it equals -log10(P-value)

[b]Genes: the DE genes associated with the PathwayID. The abbreviations of DE genes are case-insensitive.

FIGURES

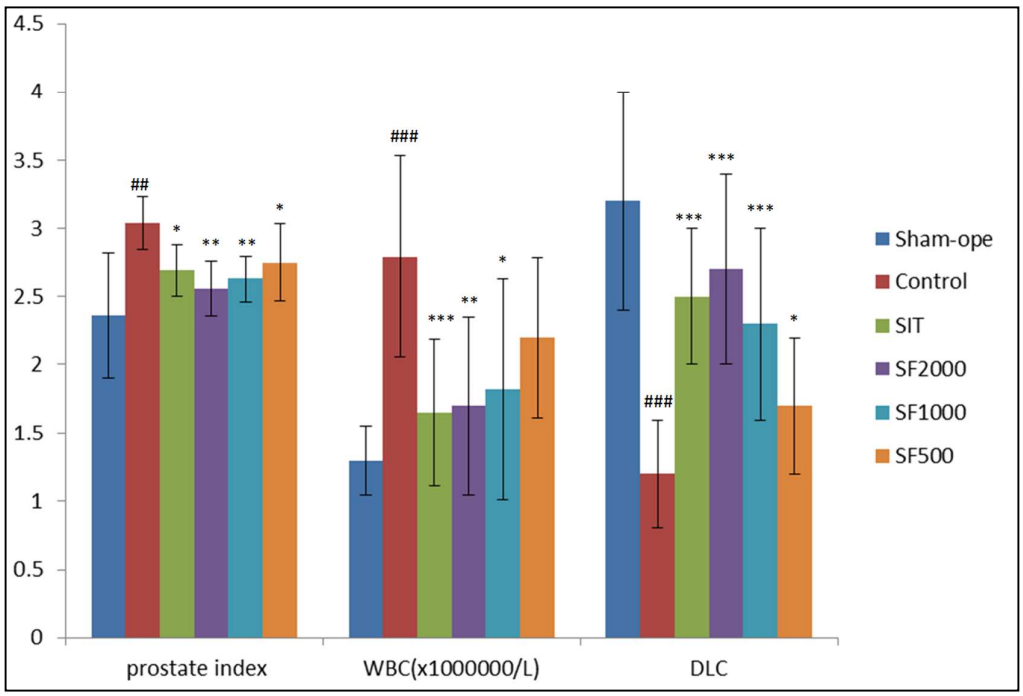


Figure 1. Effect of SF on prostate index, WBC and DLC in prostate fluid（n=10）

SF: Steryl ferulates; SIT: β-Sitosterol; DLC: density of lecithin corpuscle in crushing prostates; WBC: white blood cells counting in crushing prostates; Mean±SD , ###p<0.001, versus Sham-ope; * p<0.05, ** p<0.01, ***p<0.001, versus controls.

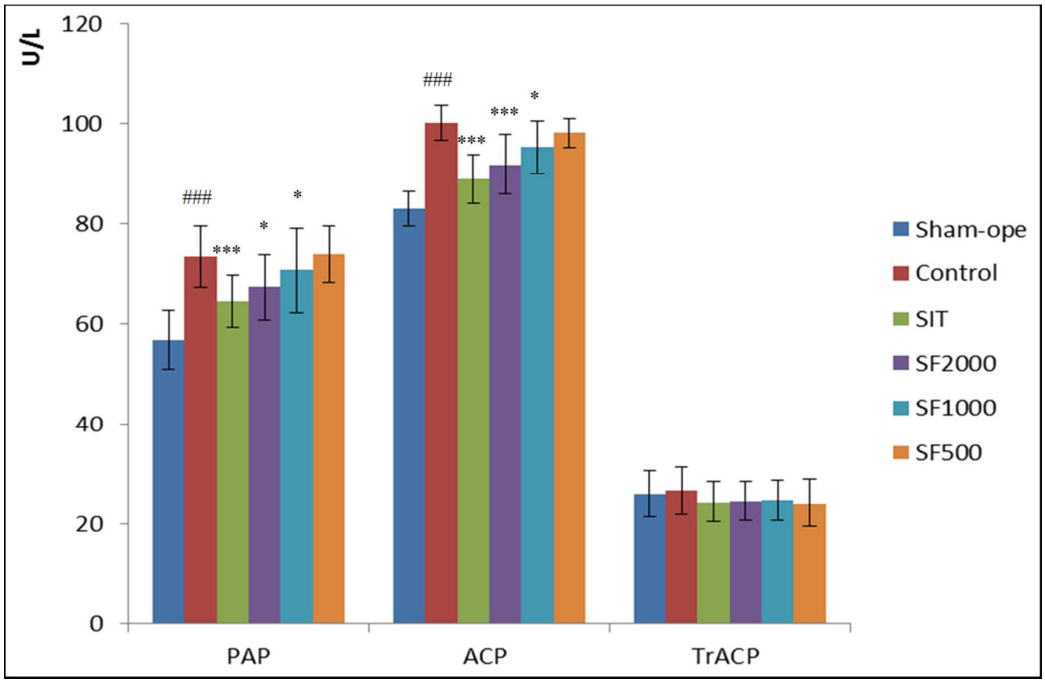


Figure 2. Effect of SF on ACP, PAP and TrACP in serum（n=10）

SF: Steryl ferulates; SIT: β-Sitosterol; PAP: prostatic acid phosphatase; ACP: total acid phosphatase; TrACP: tartrate resistant acid phosphatase; Mean±SD, ###p<0.001, versus Sham-ope; * p<0.05, ** p<0.01, ***p<0.01 versus controls.

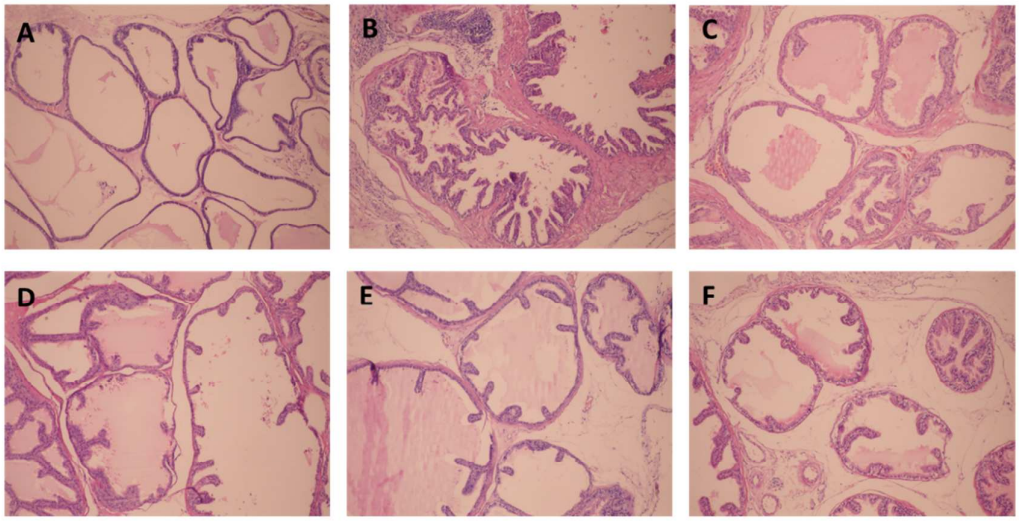
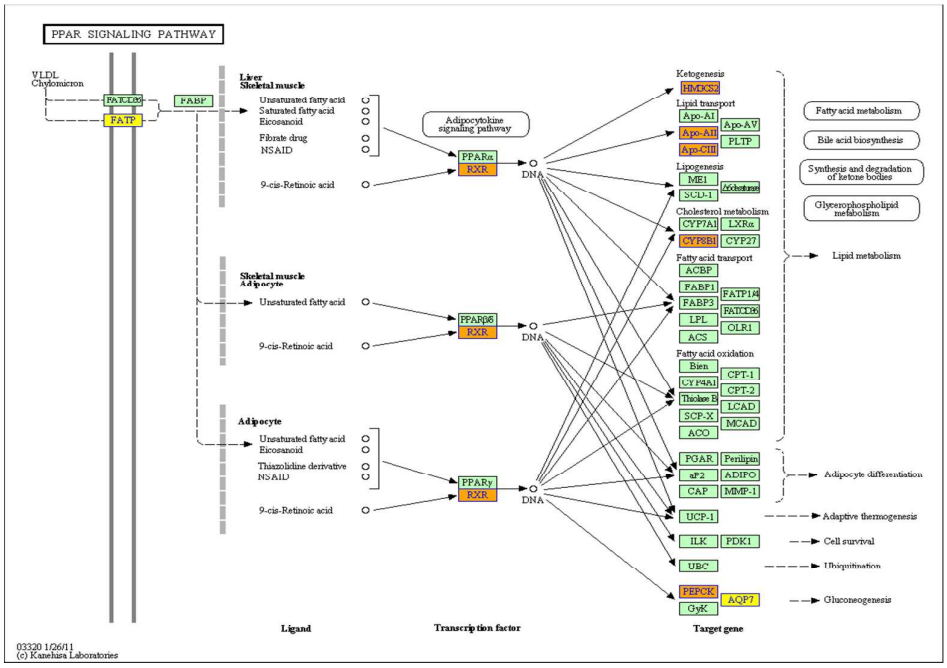


Figure 3. HE staining of the prostate in experimental nonbacterial prostatitis rat

(A) Sham-ope group; (B) Control group; (C) SIT group; (D) SF 2000 group; (E) SF 1000 group; (F) SF 500 group. ×100.

A



B

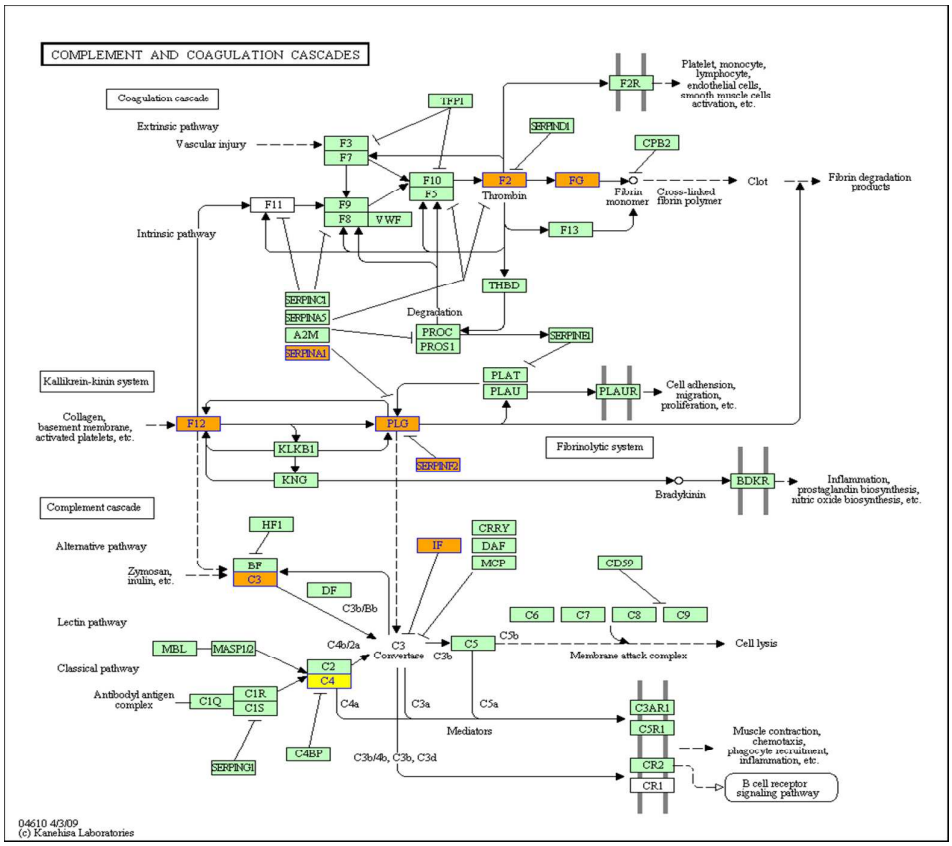


Figure 4. The reference KEGG pathway representing the significantly different expression signal pathways

(A) The regulation of PPAR signaling transduction; (B) The regulation of complement and coagulation cascades; DE genes were mapped onto the KEGG pathways to attain a linkage between key molecules and biochemical pathways associated with SF. Yellow marked nodes are associated with up-regulated genes, orange marked nodes are associated with down-regulated or only whole dataset genes, and green nodes have no significance.

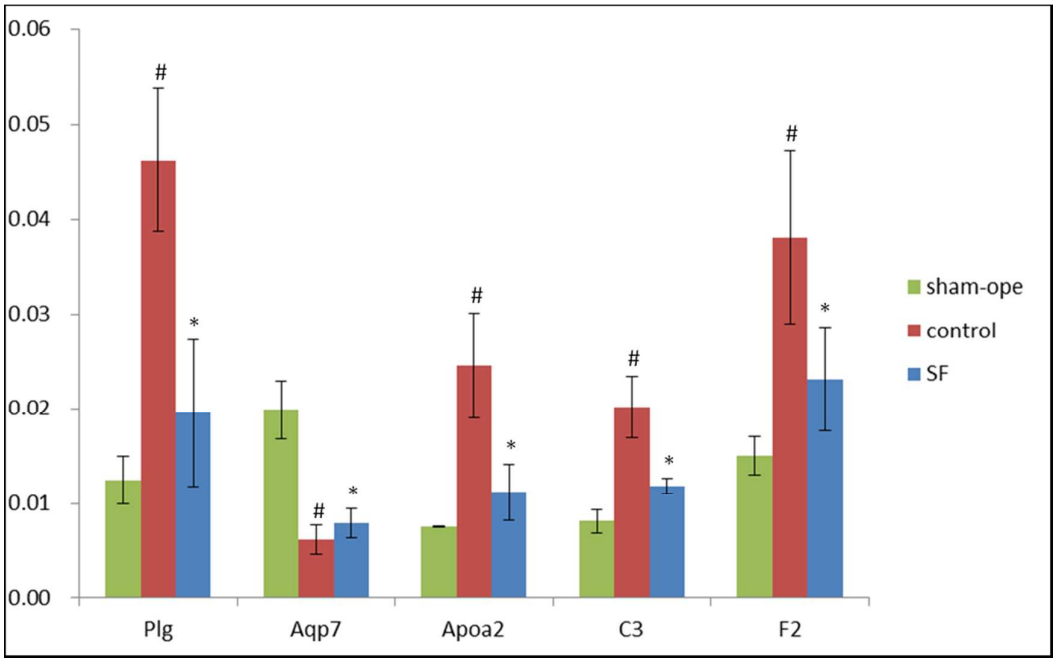


Figure 5. QRT-PCR validation of microarray results for *Plg, Aqp7, Apoa2, C3,* and *F2* (n=3)

The results are expressed relative to the *GAPDH* internal control. Mean±SD, #$p<0.05$, versus sham-ope; * $p<0.05$, versus control.

Abstract graphics