Role of Prostatic Exosome miRNA-146a/TLR-4/NF- B Pathway in Chronic Inflammation in EAP Rats and Dahuo Cao Intervention: Post-print

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Abstract

Background Current treatments for chronic prostatitis (CP) have not achieved the expected efficacy, and there is an urgent need to search for new therapeutic agents. Our research group' s previous clinical observations have found that Dahuocao exhibits good therapeutic effects on CP, but its specific mechanism of action remains to be further investigated.

Objective To investigate the role of prostatic exosome-derived miRNA-146a in regulating the TLR-4/NF- B pathway in chronic inflammation in EAP rats and the possible mechanism of Dahuocao intervention.

Methods Forty-two Wistar rats were divided into 7 groups by random number table, namely the normal group, model group, agonist group, inhibitor group, low-dose Dahuocao group, medium-dose Dahuocao group, and high-dose Dahuocao group, with 6 rats per group. After drug intervention, RT-PCR was used to detect prostatic exosome miRNA-146a-5p mRNA, Western blot (WB) to detect TLR-4, I B, p-I B, TRAF6 proteins, and ELISA to detect inflammatory cytokines.

Results Compared with the normal group, prostatic exosome miRNA-146a-5p mRNA expression was downregulated in all groups ($P<0.01$). Compared with the model group, prostatic exosome miRNA-146a-5p mRNA expression was upregulated in the medium- and high-dose Dahuocao groups and the miRNA-146a mimics group ($P<0.01$); the miRNA-146a inhibitor group showed downregulated prostatic exosome miRNA-146a-5p mRNA expression ($P<0.01$). Dahuocao and miRNA-146a mimics significantly decreased prostate tissue expression of TLR-4, p-I Bα, TRAF6 proteins ($P<0.01$); promoted upregulation of I Bα protein expression in prostate tissue ($P<0.01$); and significantly reduced serum

levels of TNF-α, IL-6, IL-8, INF-γ in rats ($P<0.01$). Dahuocao and miRNA-146a mimics alleviated inflammatory cell infiltration in prostate tissue and improved pathological damage.

Conclusion Prostatic exosome-derived miRNA-146a can regulate TLR-4/NF- B pathway activation and participates in local pathological changes in the prostate of EAP rats. The mechanism of Dahuocao against chronic inflammation in EAP rats may be related to its regulation of the prostatic exosome-derived miRNA-146a/TLR-4/NF- B pathway.

Full Text

Preamble

Title: Role of the Prostasome miRNA-146a/TLR-4/NF- B Pathway in Chronic Inflammation in EAP Rats and the Therapeutic Effect of Anemone Tomentosa

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Abstract

Background: Current treatments for chronic prostatitis (CP) have not achieved satisfactory efficacy, necessitating the search for novel therapeutic agents. Our preliminary clinical observations have demonstrated that Anemone Tomentosa exhibits promising effects against CP, though its specific mechanism of action requires further investigation.

Objective: To investigate the role of prostasome-derived miRNA-146a in regulating the TLR-4/NF- B pathway in chronic inflammation in experimental autoimmune prostatitis (EAP) rats and to explore the potential therapeutic mechanism of Anemone Tomentosa.

Methods: Forty-two Wistar rats were randomly divided into seven groups (n=6 per group): normal control, model, miRNA-146a mimics, miRNA-146a inhibitor, low-dose Anemone Tomentosa, medium-dose Anemone Tomentosa, and high-dose Anemone Tomentosa. Following drug interventions, prostasomal miRNA-146a-5p mRNA was detected by RT-PCR; TLR-4, I B, p-I B, and TRAF6 protein levels were measured by Western blot; and inflammatory cytokines were quantified by ELISA.

Results: Compared with the normal group, prostasomal miRNA-146a-5p expression was downregulated in all other groups (P<0.01). Relative to the model group, miRNA-146a-5p expression was significantly upregulated in the medium- and high-dose Anemone Tomentosa groups and the miRNA-146a mimics group (P<0.01), while it was further downregulated in the miRNA-146a inhibitor group (P<0.01). Both Anemone Tomentosa and miRNA-146a mimics significantly reduced protein expression of TLR-4, p-I Bα, and TRAF6 in prostate tissue (P<0.01), promoted I Bα expression (P<0.01), and markedly decreased serum levels of TNF-α, IL-6, IL-8, and IFN-γ (P<0.01). Histopathological examination revealed that both Anemone Tomentosa and miRNA-146a mimics attenuated inflammatory cell infiltration and ameliorated pathological damage in prostate tissue.

Conclusion: Prostasome-derived miRNA-146a participates in local pathological changes in EAP rat prostate by regulating TLR-4/NF- B pathway activation. The anti-inflammatory mechanism of Anemone Tomentosa in EAP rats may involve modulation of the prostasome-derived miRNA-146a/TLR-4/NF- B pathway.

Keywords: Chronic prostatitis; Prostasome; miRNA-146a; TLR-4; NF- B

Introduction

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a common condition in urology and andrology, accounting for 90-95% of prostatitis cases. It is characterized by chronic pelvic pain and urinary dysfunction without clear association with bacterial infection [1]. The pathophysiology of CP/CPPS involves complex mechanisms including occult infection, immune dysregulation, inflammation, neurological factors, endocrine disturbances, intraprostatic urinary reflux, oxidative stress, and psychological factors, with frequent cross-interactions among these elements [2]. Due to its unclear and multifactorial etiology, single-modality therapy has proven largely unsuccessful, prompting a shift toward multimodal management strategies encompassing pharmacological and non-pharmacological approaches. Pharmacological options primarily include antibiotics, α-blockers, anti-inflammatory agents, and phytotherapy, while non-pharmacological interventions comprise pelvic floor physical therapy, myofascial trigger point release, acupuncture, psychological support, extracorporeal shockwave therapy, and local hyperthermia [3]. However, a systematic review and

meta-analysis concluded that contemporary medicine has yet to elucidate the specific pathogenesis of CP/CPPS, and current management approaches fail to achieve expected therapeutic outcomes [4].

In recent years, traditional Chinese medicine (TCM) using herbal extracts has gained attention as an alternative therapy for CP/CPPS due to its beneficial effects and distinctive advantages [5]. Our previous studies demonstrated that Anemone Tomentosa significantly improves clinical symptoms in CP patients [6] and modulates inflammatory cytokine levels in CP rat models [7], thereby exerting anti-prostatitis effects. Prostasomes, the first identified membranous vesicles in the male reproductive tract secreted by human prostatic epithelial cells, belong to the exosome family and play important pathophysiological roles in prostate diseases. Building upon this foundation, the present study employed an experimental autoimmune prostatitis (EAP) rat model to investigate the role of the prostasome-derived microRNA-146a (miRNA-146a)/Toll-like receptor 4 (TLR-4)/nuclear factor- B (NF- B) pathway in local chronic inflammation in EAP rats and to explore the potential therapeutic mechanism of Anemone Tomentosa, a single herb with "heat-clearing and dampness-removing, diuretic and strangury-relieving, qi-supplementing and kidney-tonifying" properties, in treating CP/CPPS.

Materials and Methods

1.1 Experimental Animals

Forty-two clean-grade male Wistar rats aged 8 weeks, weighing 200$±$15 g, were purchased from Changsha Tianqin Biotechnology Co., Ltd. (License No. SCXK2019-0013). Animals were housed in the Guangxi Key Laboratory of Molecular Biology of Preventive Medicine of Traditional Chinese Medicine at 18-25°C with ad libitum access to standard chow and water.

1.2 Major Instruments and Reagents

Instruments: Transmission electron microscope (HITACHI HT7800/HT7700), nucleic acid/protein quantifier (DeNovix DS-11), electrophoresis apparatus (Beijing Liuyi DYCZ-24DN), semi-dry transfer system (ATTO WSE-4040, AE6675L), real-time PCR system (BIO-RAD CFX Connect™), biophotometer (Eppendorf BioPhotometer), optical microscope (OLYMPUS BX43), ultracentrifuge (Hitachi CP100MX), microplate reader (BIO-TEK Elx800).

Reagents: TLR-4 antibody (Affinity AF7017), tumor necrosis factor receptor-associated factor 6 (TRAF6) antibody (Affinity AF5376), p-I Bα antibody (Affinity AF5002), I Bα antibody (Affinity AF2002), miRcute miRNA isolation kit (Tiangen Biotech Cat# DP501), miRcute miRNA Detection Kit (SYBR Green) (Tiangen Biotech Cat# FP411), miRcute miRNA First-Strand cDNA Synthesis Kit (Tiangen Biotech Cat# KR211), complete Freund' s adjuvant

(Sigma-Aldrich), miRNA-146a mimics (GenePharma), miRNA-146a inhibitor (GenePharma), IL-6 (MultiSciences EK306), IL-8 (MultiSciences SEKR-0014), IFN-γ (MultiSciences EK380), TNF-α (MultiSciences EK382).

1.3 Preparation of Anemone Tomentosa Decoction

Appropriate amounts of Anemone Tomentosa were placed in a decoction container, soaked in 5-8 volumes of cold water for 1 hour, then boiled for 30 minutes and filtered. The residue was re-extracted with 3-6 volumes of water for another 30 minutes. The two filtrates were combined, centrifuged at 5000 r/min for 10 minutes, and the supernatant was concentrated using a rotary evaporator to a final concentration of 5.84 g crude drug per milliliter. Before administration, the concentrate was diluted with distilled water to 1.46 g/mL, equivalent to the human dose converted based on body surface area.

1.4 Establishment of EAP Rat Model

Preparation of rat prostatic protein extract: Five SPF-grade male Wistar rats (4 months old, 400$±$20 g) were euthanized and sterilized. The prostate was excised via abdominal incision, washed with cold saline, minced, and homogenized in an equal volume of 0.5% Triton X-100 in physiological saline (pre-sterilized). The homogenate was centrifuged at 15,000 r/min for 30 minutes at 4°C. The supernatant was collected, and protein concentration was determined by microplate reader after serial dilution. The remaining supernatant was diluted with 0.1 M PBS (pH 7.2) to 60 g/L, aliquoted, and stored at -80°C.

Immunization: Forty-two male Wistar rats (8 weeks old, 200$±$15 g) were randomly divided into seven groups (n=6): normal control (Group A), model (Group B), low-dose Anemone Tomentosa (Group C), medium-dose (Group D), high-dose (Group E), miRNA-146a inhibitor (Group F), and miRNA-146a mimics (Group G). Except for Group A, all rats received subcutaneous injections of 1 mL emulsion containing equal volumes of rat prostatic protein extract and complete Freund' s adjuvant at multiple sites on days 0 and 30, plus intraperitoneal injection of 0.5 mL pertussis-diphtheria-tetanus vaccine.

1.5 Drug Administration

After successful modeling, Groups A and B received normal saline (4 mL/kg · d) by gavage. Groups C, D, and E received Anemone Tomentosa decoction at concentrations of 1.46 g/mL, 2.92 g/mL, and 5.84 g/mL respectively (4 mL/kg · d). All treatments were administered once daily for 4 weeks. Group G received normal saline (4 mL/kg · d) for 4 weeks plus tail vein injection of miRNA-146a mimics (0.2 L/g) 72 hours before sacrifice. Group F received normal saline (4 mL/kg · d) for 4 weeks plus tail vein injection of miRNA-146a inhibitor (0.2 L/g) 72 hours before sacrifice.

1.6 Detection Methods

1.6.1 Histopathological Examination of Prostate Tissue Prostate tissues were fixed in formalin, paraffin-embedded, sectioned, stained with hematoxylin-eosin, and examined under an optical microscope.

1.6.2 Electron Microscopic Identification of Prostasomes in Prostatic Fluid Prostasomes were extracted from prostatic fluid by ultracentrifugation. Samples (10 L) were applied to copper grids for 1 minute, blotted, then stained with 10 L uranyl acetate for 1 minute, blotted again, air-dried, and examined by transmission electron microscopy.

1.6.3 Detection of Prostasomal miRNA-146a-5p Expression Prostasomes were extracted by ultracentrifugation. Total RNA was isolated from 200 L samples using the miRcute miRNA isolation kit according to the manufacturer' s protocol. Reverse transcription was performed using the miRcute miRNA First-Strand cDNA Synthesis Kit (37°C for 60 minutes). Real-time PCR was conducted using the miRcute miRNA Detection Kit with U6 as internal control. Relative quantification employed the 2^(-ΔΔCT) method. PCR conditions: initial denaturation at 94°C for 2 minutes, followed by 40 cycles of 94°C for 20 seconds and 60°C for 34 seconds.

1.6.4 Detection of TLR-4, I B, p-I B, and TRAF6 Protein Expression Prostate tissue (50 mg) was minced, ground in liquid nitrogen, lysed in 500 L RIPA buffer, and total protein was extracted. Western blot was performed according to standard protocols using primary antibodies: TLR-4 (1:1000), TRAF6 (1:1000), p-I Bα (1:1000), and I Bα (1:1200). Protein expression levels were normalized to internal control and quantified by densitometry.

1.6.5 ELISA Detection of Serum TNF-α, IL-6, IL-8, and IFN-γ Serum concentrations of TNF-α, IL-6, IL-8, and IFN-γ were measured strictly according to ELISA kit instructions.

1.7 Statistical Analysis

Data were analyzed using SPSS 22.0 software and expressed as mean ± standard deviation ($\bar{x}\pm s$). Normally distributed data with homogenous variance were compared between two groups using t-test and among multiple groups using one-way ANOVA followed by LSD post-hoc test. Non-parametric tests were used for non-normally distributed data.

Results

2.1 Histopathological Analysis of Prostate Tissues

Under optical microscopy, the model group exhibited severe prostatic architectural damage compared with the normal group, including highly irregular and distorted glandular lumens, nuclear pyknosis and necrosis of epithelial cells, severe interstitial hemorrhage, and mild inflammatory cell infiltration. In contrast, the miRNA-146a mimics group showed mild structural damage with slightly disorganized glands, focal epithelial necrosis and shedding, but no significant inflammatory infiltration. The miRNA-146a inhibitor group displayed severe damage with markedly disorganized glands, diminished luminal areas, epithelial sloughing, abundant interstitial erythrocytes, and minimal inflammatory cells.

Among Anemone Tomentosa-treated groups, the low-dose group showed noticeable damage with disorganized architecture, irregular lumens, epithelial necrosis, moderate interstitial hemorrhage, and moderate inflammatory infiltration. The medium-dose group exhibited less severe damage with mildly disorganized glands, irregular and smaller lumens, and minimal inflammatory infiltration. The high-dose group demonstrated only mild injury, with predominantly well-arranged epithelium, regular glandular lumens, mild hyperplasia, loose connective tissue with fibroblasts, and no inflammatory cells [Figure 1: see original paper].

2.2 Electron Microscopic Identification of Prostasomes

Prostasomes are membranous vesicles approximately 30-150 nm in diameter, exhibiting characteristic "cup-shaped" morphology under electron microscopy. Electron microscopic examination confirmed successful extraction of prostasomes from prostatic fluid in all groups [Figure 2: see original paper].

2.3 Expression of Prostasomal miRNA-146a-5p

Compared with the normal group, miRNA-146a-5p expression was significantly downregulated in all other groups ($P<0.01$). Relative to the model group, miRNA-146a-5p expression was upregulated in the medium- and high-dose Anemone Tomentosa groups and the miRNA-146a mimics group ($P<0.01$), while it was further downregulated in the miRNA-146a inhibitor group ($P<0.01$)
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Table 1 Primer sequences for RT-PCR detection - miRNA-146a-5p forward: 5' -TGAGAACTGAATTCCATGGGTT-3' - miRNA-146a-5p reverse: 5' -CGCAAGGATGACACGCAAAT-3'

Table 2 Comparative analysis of prostasomal miRNA-146a-5p expression among groups ($\bar{x}\pm S$, n=6) | Group | miRNA-146a-5p | |—-|———| | Normal | 1.123$±0.139||$Model$|0.439±0.116$^{\Delta}$Δ||$Low-dose$|0.535±0.118$^{\Delta}$Δ▲▲||$Medium-dose$|0.724±0.105$^{\Delta}$Δ▲▲||$High-dose$|0.747±0.116$^{\Delta}$Δ▲▲||$miRNA-$

146*ainhibitor*|0.212±0.028$^{\Delta}$ΔAA||*miRNA*−146*amimics*|0.848±$0.092^ΔΔ
|

Note: ^ΔP<0.05, ^ΔΔP<0.01 vs. Group A; ^ P<0.05, ^ P<0.01 vs. Group B.

2.4 Protein Expression of TLR-4, I Bα, p-I Bα, and TRAF6 in Prostate Tissue

TLR-4 expression was significantly upregulated in all groups compared with the normal group (P<0.01), except in the high-dose Anemone Tomentosa group which showed levels comparable to normal (P>0.05). Relative to the model group, TLR-4 expression was decreased in the miRNA-146a mimics and all Anemone Tomentosa groups (P<0.01), with the most pronounced reduction in the high-dose group, while the inhibitor group showed increased expression (P<0.05).

p-I Bα expression was upregulated in all groups versus the normal group (P<0.01). Compared with the model group, p-I Bα was downregulated in the miRNA-146a mimics and Anemone Tomentosa groups (P<0.01), again with the high-dose group showing the greatest effect, whereas the inhibitor group exhibited increased expression (P<0.01).

I Bα expression was downregulated in all groups compared with the normal group (P<0.01 or P<0.05), except in the miRNA-146a mimics and high-dose groups which were comparable to normal (P>0.05). Relative to the model group, I Bα was upregulated in the miRNA-146a mimics and high-dose groups (P<0.01).

TRAF6 expression was upregulated in all groups versus the normal group (P<0.01 or P<0.05). Compared with the model group, TRAF6 was downregulated in the miRNA-146a mimics and all Anemone Tomentosa groups (P<0.01), most significantly in the high-dose group, while the inhibitor group showed a non-significant increasing trend [TABLE:3, FIGURE:3].

Table 3 Comparative analysis of protein expression in prostate tissues among groups ($\bar{x}$±S, n=6) | Group | TLR4 | I Bα | p-I Ba | TRAF6 | |—-|—|—|—-|—-| | Normal | 0.225$±0.035|0.787±0.100|0.342±0.043|0.410±0.075||*Model*|0.552±0.060$^{\Delta}$Δ|0.383±0.035$^{\Delta}$Δ|1.
dose|0.439±0.081$^{\Delta}$ΔAA|0.576±0.033$^{\Delta}$ΔAA|0.777±0.051$^{\Delta}$ΔAA|0.893±0.119$^{\Delta}$ΔAA||*Medium*−
dose|0.394±0.081$^{\Delta}$ΔAA|0.725±0.166$^{\Delta}$ΔAA|0.576±0.033$^{\Delta}$ΔAA|0.725±0.166$^{\Delta}$ΔAA||*High*−
dose|0.288±0.026$^{\blacktriangle}$A|0.931±0.125$^{\Delta}$A|0.456±0.034$^{\Delta}$ΔAA|0.655±0.088$^{\Delta}$AA||*miRNA*−
146*ainhibitor*|0.555±0.044$^{\blacktriangle}$A|0.379±0.087$^{\Delta}$Δ|1.179±0.079$^{\Delta}$ΔAA|0.861±0.094$^{\Delta}$ΔA||*miRNA*−
146*amimics*|0.379±0.087$^{\Delta}$Δ|0.575±0.102$^{\blacktriangle}$A|0.599±0.013$^{\Delta}$ΔAA|0.494±$0.034^ΔΔ
|

Note: ^ΔP<0.05, ^ΔΔP<0.01 vs. Group A; ^ P<0.05, ^ P<0.01 vs. Group B.

2.5 Serum Levels of TNF-α, IL-6, IL-8, and IFN-γ

Serum TNF-α levels were significantly elevated in all groups compared with the normal group (P<0.01), except in the high-dose group. Relative to the model group, TNF-α was significantly reduced in all groups except the miRNA-146a inhibitor group (P<0.01).

IL-6 levels were markedly increased in all groups versus the normal group (P<0.01), except in the high-dose group. Compared with the model group, IL-6 was significantly decreased in the low-, medium-, and high-dose Anemone Tomentosa groups and the miRNA-146a mimics group (P<0.01).

IL-8 levels were elevated in all groups compared with the normal group (P<0.01), except in the high-dose group. Relative to the model group, IL-8 was significantly reduced in all groups except the miRNA-146a inhibitor group (P<0.01).

IFN-γ levels were significantly increased in all groups versus the normal group (P<0.01 or P<0.05). Compared with the model group, IFN-γ was markedly decreased in all groups except the miRNA-146a inhibitor group (P<0.01) .

Table 4 Comparative analysis of serum cytokine levels among groups ($\bar{x}\pm S$, n=6) | Group | TNF-α | IL-6 | IL-8 | IFN-γ | |—-|—-|—|—|—-| | Normal | 20.473$±1.891|25.491±1.886|34.566±6.379|77.897±5.967||$Model$|58.851±6.991$^{\Delta}$Δ|55.744±6.726$^{\Delta}$Δ|84.312±9. $dose$|47.247±5.914$^{\Delta}$Δ▲▲|44.726±6.803$^{\Delta}$Δ▲▲|71.388±7.438$^{\Delta}$Δ▲▲|140.326±17.663$^{\Delta}$Δ▲▲||$Medium-$ $dose$|37.793±5.017$^{\Delta}$Δ▲▲|38.329±3.305$^{\Delta}$Δ▲▲|60.698±6.885$^{\Delta}$Δ▲▲|123.284±14.446$^{\Delta}$Δ▲▲||$High-$ $dose$|24.994±3.490$^{\blacktriangle}$▲|30.482±3.813$^{\blacktriangle}$▲|44.589±4.616$^{\Delta}$▲▲|85.925±7.733$^{\blacktriangle}$▲||$miRNA-$ $146ainhibitor$|64.084±7.110$^{\Delta}$Δ|65.151±6.715$^{\Delta}$Δ▲|93.779±9.689$^{\Delta}$Δ▲|176.452±22.310$^{\Delta}$Δ||$miRNA-$ $146amimics$|31.618±3.749$^{\Delta}$Δ▲▲|35.114±7.191$^{\Delta}$Δ▲▲|56.866±8.626$^{\Delta}$Δ▲▲|105.578±$16.281^ΔΔ |

Note: ^ΔP<0.05, ^ΔΔP<0.01 vs. Group A; ^ P<0.05, ^ P<0.01 vs. Group B.

Discussion

CP/CPPS symptoms, including varying degrees of chronic pelvic pain (prostatic, perineal, urethral) and urinary dysfunction, significantly impair quality of life. The estimated prevalence among Chinese men is approximately 8.4-25% [10], with recurrence rates reaching 50% with advancing age [11]. An evidence-based systematic review identified phytotherapy, α-blockers, anti-inflammatory agents, and antibiotics as potentially beneficial therapies, though the quality of evidence was moderate to low, indicating unsatisfactory efficacy of current treatments [12]. The variable etiology and complex mechanisms of CP/CPPS contribute to its high incidence, refractory nature, and recurrence. Traditional Chinese medicine has emerged as an effective alternative therapy for CP/CPPS management, attracting researcher and clinician attention due to its minimal side effects and multimodal actions [5]. While TCM lacks a specific disease classification for CP/CPPS, practitioners generally regard it as a condition of

"deficiency in origin and excess in superficiality" associated with "kidney deficiency, damp-heat, and blood stasis" [13].

Anemone Tomentosa, a perennial herb belonging to the Ranunculaceae family, is primarily distributed in Guangxi and Yunnan provinces and represents an authentic Zhuang medicine. With a pungent, slightly bitter flavor and neutral property, it possesses "heat-clearing and dampness-removing, diuretic and strangury-relieving, qi-supplementing and kidney-tonifying" effects [14], aligning closely with the fundamental pathogenesis of "kidney deficiency and damp-heat" in CP/CPPS.

MicroRNAs are multifunctional non-coding RNAs that regulate gene expression at the post-transcriptional level. Toll-like receptors constitute the first line of immune defense through selective recognition of pathogen-associated molecular patterns, and excessive activation of TLR signaling pathways can lead to inflammation and immune disorders. MicroRNAs can finely modulate the initiation, termination, and intensity of TLR signaling, thereby participating in the development of immune-inflammatory responses [15]. TRAF6 enhances transcription of pro-inflammatory cytokine genes to initiate inflammatory cascades and serves as a crucial adaptor protein for the TNF superfamily and TLR superfamily [16]. TRAF6 activates NF- B signaling by stimulating I B kinase to promote I B phosphorylation and degradation, representing a key positive regulator of NF- B signal transduction [17].

Previous reports have confirmed that miR-146a exerts anti-inflammatory effects by targeting TRAF6. Studies show that miRNA-146a inhibits NF- B transcriptional activity and reduces downstream inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α) by suppressing TRAF6 expression in the TLR-4/NF- B pathway [18]. Yang et al. [19] reported that exosome-derived miR-146a was upregulated in N9 microglial cells and negatively regulated inflammatory responses by modulating TRAF6 expression in the TLR4 signaling pathway, significantly reducing IFN-β, TNF-α, and IL-1β levels.

The present study investigated the regulatory role of prostasome-derived miRNA-146a on the TLR-4/NF- B pathway in EAP rats. Our findings demonstrate that miRNA-146a suppresses TLR-4/NF- B transcriptional activity, inhibits excessive NF- B phosphorylation, and reduces downstream inflammatory cytokines (IL-6, IL-8, IFN-γ, TNF-α) by inhibiting TRAF6 expression, thereby alleviating local prostatic inflammation. Anemone Tomentosa intervention enhanced prostasomal miRNA-146a-5p expression, suppressed TRAF6 expression, inhibited TLR-4/NF- B pathway activation, reduced downstream inflammatory cytokine release, and mitigated local prostatic inflammation. The regulatory effect on the prostasome-derived miRNA-146a/TLR-4/NF- B pathway became more pronounced with increasing Anemone Tomentosa dosage. Although limited by the small sample size, this study provides preliminary evidence supporting the potential research value of prostasomes in CP pathogenesis and development as a novel direction for future investigation.

Conflict of Interest: The authors declare no conflict of interest.

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Note: Figure translations are in progress. See original paper for figures.