Original Article

Protective effect of astaxanthin on chronic prostatitis/chronic pelvic pain syndrome in rat through modulating NF-κB signaling pathway

Jiahao Jiang[1#], Fei Xiao[1#], Lanxin Yang[2], Yan Zeng[1], Jing Chen[1], Hengcheng Zhu[1], Lingqi Liu[1]

[1]Department of Urology, Renmin Hospital of Wuhan University, Wuhan, China; [2]School of Pharmacy, Wuhan University, Wuhan, China

Contributions: (I) Conception and design: L Liu, H Zhu; (II) Administrative support: L Liu; (III) Provision of study materials or patients: J Jiang; (IV) Collection and assembly of data: F Xiao; (V) Data analysis and interpretation: L Yang, Y Zeng, J Chen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Dr. Hengcheng Zhu, MD, PhD; Dr. Lingqi Liu, MD, PhD. Department of Urology, Renmin Hospital of Wuhan University, 99 Zhangzhidong Road, Wuhan 430060, China. Email: zhcheng2018@outlook.com; llqfly@whu.edu.cn.

Background: Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a common male urological disease characterized by chronic pelvic pain and various discomforts. Astaxanthin (AST) has multiple functions, including anti-inflammatory property, but it is unclear whether AST plays a key role in CP/CPPS and how it works. This study aimed to investigate the protective effect of AST on CP/CPPS in rats and the underlying mechanism.

Methods: A CP/CPPS rat model was induced by intraprostatic injection of carrageenan and the blood specimens and prostates were harvested for further research after oral administration of AST for 4 weeks.

Results: Tactile allodynia test showed that AST ameliorated chronic pelvic pain in a dose-depended manner. In addition, histological evaluation indicated that AST alleviated CP/CPPS rat prostate histological inflammation. Meanwhile, AST suppressed the expression of proinflammatory cytokines, including interleukin-1β (IL-1β), IL-6, IL-8, and tumor necrosis factor-α (TNF-α). Besides, AST inhibited the activities of prostaglandin E2 (PGE2) and cyclooxygenase 2 (COX2). Furthermore, AST decreased the activation of the nuclear factor-κB (NF-κB) signaling pathway.

Conclusions: Our study has shown that AST exerts an anti-inflammatory and protective effect against CP/CPPS and the function is mediated at least through the suppression of NF-κB signaling pathway. These results provide evidence of AST as the potential agents for the treatment of CP/CPPS.

Keywords: Astaxanthin (AST); anti-inflammation; chronic prostatitis (CP); chronic pelvic pain syndrome (CPPS); NF-κB signaling pathway

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Introduction

Prostatitis is one of the most common urinary tract diseases among male outpatients <50 years of age in urology clinics (1). Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), also known as National Institutes of Health (NIH) Category III Prostatitis is the most common form of the symptomatic prostatitis subtypes, comprising approximately 90% of all prostatitis and with a prevalence of 5–10% worldwide (2). CP/CPPS is a chronic disease characterized by a variety of somatic and psychosocial symptoms including urinary irritation symptoms, pelvic or perineal pain, erectile dysfunction and painful ejaculation (3,4). In addition, the most common painful symptoms may be persistent or occur in numerous locations. Current

theories suggest that CP/CPPS may be induced by various complex factors, such as psychosocial factors, urinary pathologies, infection, muscular contractures and central sensitization of the nervous system. Many medicines and therapies have been utilized in the treatment of CP/CPPS such as antibiotics, alpha-receptor blockers, analgesics, phytotherapy, and other physical and behavior therapies (3-5). However, the definite etiology of CP/CPPS remains largely unknown and none of the present therapies obtain satisfactory and long-lasting efficacy. Therefore, it is still meaningful and necessary to develop new effective drugs for the treatment of CP/CPPS and investigate the underlying mechanisms.

Astaxanthin (AST) is a red pigment carotenoid primarily present in shrimp, salmon, lobster, crab, and asteroidean (6,7). Owing to the structure of a xanthophyll carotenoid with hydroxyl and keto moieties on both ends, AST could quench free radicals or other oxidants and protect the lipid bilayer from peroxidation. Several studies have corroborated the anti-oxidant efficacy of AST and it is extensively utilized in the nutrition and pharmaceutical industries (8-11). Moreover, a growing number of studies has shown that AST has anti-proliferative, anti-apoptotic and anti-inflammatory properties (8). Because of its strong activities, AST has been widely investigated by researchers as a multi-target pharmacological drug to treat numerous diseases (12). In addition, the anti-inflammatory mechanisms of AST have been reported to be associated with regulating inflammatory cytokines and multiple signaling pathways, such as PI3K/AKT, NF-κB, and MAPK signaling pathways (13,14).

Herein, the anti-inflammation effect of AST was systematically investigated on CP/CPPS rat model, which provides evidence that AST could be a potential effective choice in CP/CPPS treatment. We present this article in accordance with the ARRIVE reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-24-190/rc).

Highlight box

Key findings

- Astaxanthin (AST) exerts an anti-inflammatory and protective effect against chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) and the function is mediated at least through the suppression of nuclear factor-κB (NF-κB) signaling pathway.

What is known and what is new?

- AST ameliorated chronic pelvic pain in a dose-depended manner and alleviated CP/CPPS rat prostate histological inflammation. In addition, AST suppressed the expression of proinflammatory cytokines and inhibited the activities of prostaglandin E2 and cyclooxygenase 2.
- AST decreased the activation of the NF-κB signaling pathway in rat CP/CPPS model.

What is the implication, and what should change now?

- AST has specific protective effect on CP/CPPS. So, AST is a potential agent for the treatment of CP/CPPS.

Methods

Materials

AST was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China, #B25542). Carrageenan (#C121014) and olive oil (#O108686) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Anti-NF-κB p65 antibody (#PAB45935), and anti-I-κBα antibody (#PAB43967) were purchased from Wuhan Bio-swamp Biotechnology Co., Ltd. (Wuhan, China). Anti-phospho-NF-κB p65 antibody (#3033T) and anti-phospho-I-κBα antibody (#2859T) were obtained from Cell Signaling Technology (Beverly, MA, USA).

Animals

Animal studies were approved by the Wuhan University Biomedical Ethics Committee for animal use and protection (No. 20183060001). All experimental procedures were complied with Chinese or institutional guidelines for the Care and Use of Laboratory Animals. A protocol was prepared before the study without registration. A total of 48 six-week-old male Sprague-Dawley rats (weighting 220–250 g) were housed in groups of four in an environmentally controlled animal facility (temperature 24±2 ℃, relative humidity 50%±5%, 12 h light-dark cycle). All rats had free access to food and water and were allowed 1 week to acclimate to new environments.

Study design and treatment

The rats were randomly divided into four groups (n=12/group) (*Figure 1*): normal control group, CP/CPPS model group, AST group treated with 40 mg/kg/d, or 80 mg/kg/d AST (AST of 40 mg/kg or 80 mg/kg dissolved in olive oil by oral gavage daily) dissolved in olive oil orally. The CP/CPPS rat model was established by intra-prostatic injection of λ-carrageenan (*Figure 2*). Briefly, the rats in the model and treatment groups were anesthetized with 3% pentobarbital

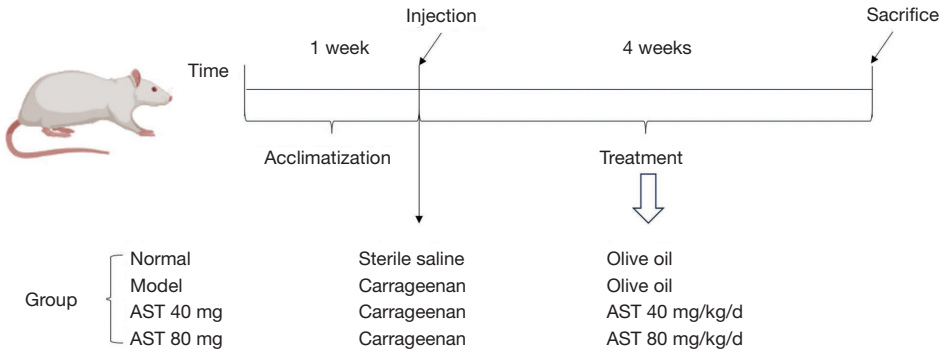

Figure 1 Schematic illustration of animal experimental design. Forty-eight SD rats were randomly divided into four groups (normal: normal group; model: chronic prostatitis model group; AST 40 mg: experimental group treated with 40 mg/kg/d AST; AST 80 mg: experimental group treated with 80 mg/kg/d AST). All rats were acclimatized for 1 week before inducing chronic prostatitis with injection of carrageenan. From the secondary day, rats received different treatments for another four weeks. AST, astaxanthin; SD, Sprague-Dawley.

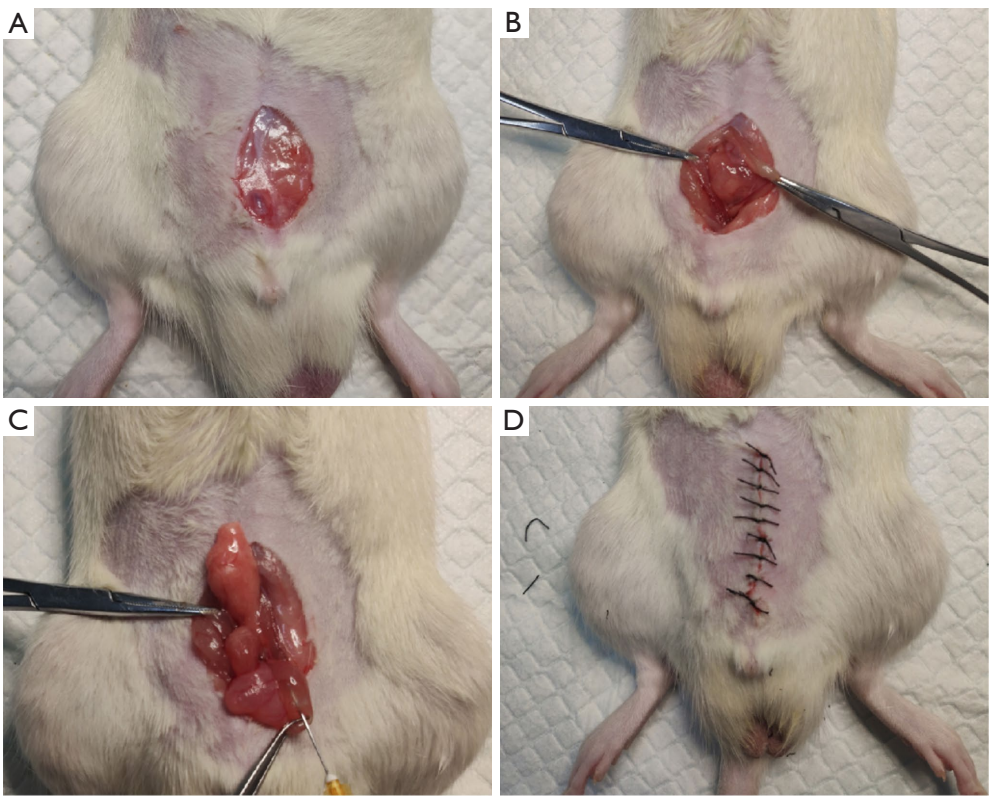

Figure 2 The surgical procedures of chronic prostatitis/chronic pelvic pain syndrome rat model. (A) Anesthetized rat was sterilized in the lower abdomen and cut through a longitudinal median incision with a length of approximately 1 cm. (B) Both prostate ventral lobes under the bladder were exposed. (C) 100 μL 1% carrageenan was injected into prostate gently using a 1-mL syringe. (D) The abdominal cavity was sutured layer by layer with absorbable suture.

sodium solution. Anesthetized rats were sterilized with iodophor in lower abdomen and cut through a longitudinal median incision with a length of approximately 1 cm. Subsequently, the prostate was exposed and 0.1 mL of 1% λ-carrageenan saline solution was injected into the prostate of both lobes to establish a CP/CPPS rat model. Animals in normal group were injected with the same volume of sterile saline at the same location. The abdominal cavity was sutured layer by layer and the incision was sterilized. After CP/CPPS modeling, AST was utilized from the second day after previous procedure and continued for 4 weeks. Finally, the total experimental rats were anesthetized and weighed, and the blood specimens were gathered from the heart. The prostate was dissected rapidly and weighed and the majority part of prostate tissues were preserved in the freezer at −80 ℃ for subsequent detection. A small part of prostate was fixed in 4% formaldehyde for histological assessment.

Evaluation of chronic pelvic pain

Rats were measured for hyperalgesia before CP/CPPS (0 weeks) and on days 14 (2 weeks) and 28 (4 weeks) after modeling. One day before the measurement, experimental rats were placed in a quiet room to prevent any animals from being disturbed by external events. The procedures were performed in a separate plastic chamber on a stainless-steel mesh floor. Referred hyperalgesia and tactile allodynia were assessed and quantified by the electronic von Frey with force of 6 g applied to the pubic region or the scrotal base (15). The operation was repeated a total of 10 times, and each time for 1 to 2 seconds with an interval of 2 minutes. The positive response was recognized as immediate licking or scratching of the stimulated area, rapid contraction of the abdomen, and jumping. All measurements were conducted by independent research.

Enzyme-linked immunosorbent assay (ELISA) for pro-inflammatory cytokine levels in prostate tissue

The level of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6 and IL-8 in prostate tissue were assessed by commercial ELISA kits. The prostate tissues were weighed and collected in 0.9% sodium chloride solution with the ratio of tissue weight/0.9% sodium chloride solution (100 mg: 1 mL). The concentration of TNF-α, IL-1β, IL-6 and IL-8 were measured by TNF-α ELISA kit (Bioswamp, Wuhan, China), IL-1β (Bioswamp), IL-6 (Bioswamp) and IL-8 (Merck Millipore, Darmstadt, Germany) following the manufacturer's protocol.

Paraffin section and histopathological staining

After fixation, prostate tissue specimens were treated by dehydration, clearing, and paraffin wax immersion. The paraffin-embedded specimens were cut into 5 μm sections and then stained with hematoxylin and eosin (H&E). Images were captured and observed under an optical microscope (Nikon E100, Nikon Corp., Tokyo, Japan). According to the literature (16), the severity of inflammation was evaluated by the inflammation score of 0–4, briefly, 0 indicated normal; 1 suggested mild inflammation; 2 indicated mild infiltration of perivascular monocytes; 3 suggested significant infiltration of perivascular monocytes; 4 indicated significant infiltration of perivascular monocytes and hemorrhages. The inflammation score was graded using a double-blind method.

Analysis of PGE2 activity in prostate tissue and serum

The PGE2 level in the prostate supernatants or serum samples was assessed by the PGE2 ELISA kit (#RA20013, Bioswamp) following the manufacturer's protocol.

Immunohistochemical staining

The prostate tissue sections were incubated overnight at 4 ℃ with primary antibodies against cyclooxygenase 2 (COX2; 1:200 dilution; PAB31107, Bioswamp). Then, the sections were incubated with goat anti-rabbit IgG-labeled secondary antibodies for 20 minutes at 37 ℃. Staining was observed under an optical microscope (Nikon E100). The optical density (OD) was measured by ImageJ software. The average optical density (AOD) [AOD = integrated optical density (IOD) SUM/area SUM] was measured by ImageJ software (15).

Western blot analysis

The prostate samples were lysed in lysis buffer and then subjected to Western blot as previously described (17,18). Briefly, total protein was extracted from prostate tissue specimens. An equal amount of prostate was loaded onto a 12% sodium dodecyl sulfate-polyacrylamide gel, and subsequently transferred to nitrocellulose membranes for electrophoresis. The primary antibodies were as follows: anti-NF-κB/P65 (1:1,000, Bioswamp), anti-I-

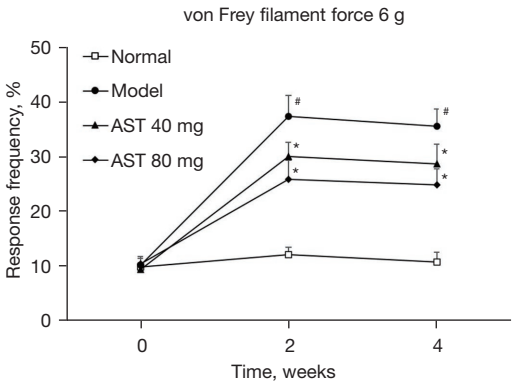

Figure 3 AST ameliorated chronic pelvic pain. The positive responses frequencies of pelvic pain of rats in different groups were measured by the von Frey test. The force of the von Frey filaments was 6 g. Data were shown as mean ± SD (n=12). #, P<0.05 *vs.* normal and *, P<0.05 *vs.* model. AST, astaxanthin; SD, standard deviation.

κBα (1:1,000, Bioswamp), anti-p- NF-κB/P65 (1:1,000, Cell Signaling Technology), anti- p-I-κBα (1:1,000, Cell Signaling Technology) and anti-β-actin. The horseradish peroxidase (HRP)-conjugated secondary antibodies were goat anti-rabbit IgG (1:5,000, Cell Signaling Technology). Autoradiograms were scanned and analyzed with Quantity One (Bio-Rad, Hercules, CA, USA) to quantify band densities.

Statistical analysis

Data obtained from this experiment were presented as mean ± standard deviation (SD) and analyzed with SPSS 19.0. The Student's *t*-test was used for comparison of two groups and one-way analysis of analysis of variance (ANOVA) was used for multiple comparison. A significant difference was defined as P<0.05.

Results

AST ameliorated chronic pelvic pain induced by carrageenan

In order to assess the ability of AST on chronic pelvic pain in rat model, we performed tactile allodynia test. Under certain force (6 g) of the von Frey filaments, we observed a significant increasement about the frequency of pelvic pain positive responses in the CP/CPPS rat model group compared with the normal control group (*Figure 3*, P<0.05), indicating that CP/CPPS model rat was successfully induced. Moreover, following four weeks of treatment, AST produced markedly inhibition of the frequency of pelvic pain positive responses in AST (40 mg, 80 mg) groups compared with the model group on day 14 (2 weeks) and 28 (4 weeks), indicating the pelvic pain was ameliorated after 4 weeks administration of AST in CP/CPPS model rats. Notably, AST showed the best therapeutic efficacy on pelvic pain in a dose-dependent manner.

AST alleviated prostate histological damage in CP/CPPS rat

To extend the above findings that AST exacted an effect in CP/CPPS, we next utilized H&E staining to analyze the histological structure of prostate in each group and evaluate the effect of AST on CP/CPPS. As shown in *Figure 4*, although few inflammatory cell infiltrations were observed in normal group, we found varied degrees of inflammatory cell infiltrations in the mesenchyme in the model group and this phenomenon is the most important histopathological feature in CP/CPPS model rats. Moreover, inflammatory vacuoles and edema were found in CP/CPPS model rat prostate tissue. However, AST markedly alleviated the inflammation degree in rat prostate induced by carrageenan. Furthermore, in order to assess the inflammation degree, we next analyzed the inflammation score of the prostatic histopathological alterations in each group. As expected, the inflammation score was significantly decreased in the AST groups compared with the model group (*Figure 4E*, P<0.05).

AST reduced the expression of proinflammatory cytokine levels in prostate tissue

Since proinflammatory cytokines are direct biomarkers of inflammation, some key factors were utilized to assess inflammatory degrees in each group. As shown in *Figure 5*, the expression of IL-1β, IL-6, IL-8 and TNF-α remarkably enhanced by 5.2-, 4.3-, 7.4- and 5.2-fold, respectively, in the model group compared with those in the normal control group. However, AST effectively inhibited the up-regulation of these proinflammatory cytokines (*Figure 5*, P<0.05).

AST inhibited the expression of prostaglandin E2 (PGE2) in prostate tissue and serum

PGE2 is a principal mediator of inflammation in diseases.

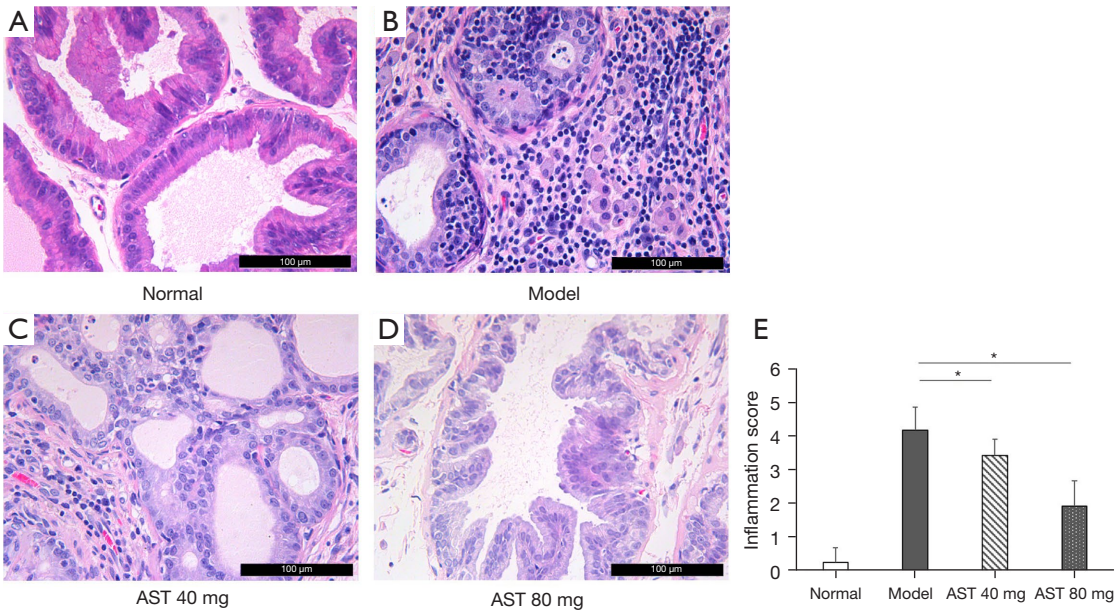

Figure 4 AST alleviated CP/CPPS rat prostate histological damage. After fixation, prostate tissue specimens were treated by dehydration, clearing, and paraffin wax immersion. The paraffin-embedded specimens were cut into 5 μm sections and then stained with H&E. (A) No abnormal histological changes were observed in the normal group. (B) The model group had different degree of inflammatory cell infiltration in the mesenchyme. (C,D) In AST treated groups, the infiltration of inflammatory cells and vacuoles were decreased in a dose-depended manner. (E) Inflammation scores for each prostate specimens were analyzed. Magnification is 400×. Data were shown as mean ± SD (n=6). *, P<0.05. AST, astaxanthin; CP/CPPS, chronic prostatitis/chronic pelvic pain syndrome; H&E, hematoxylin and eosin; SD, standard deviation.

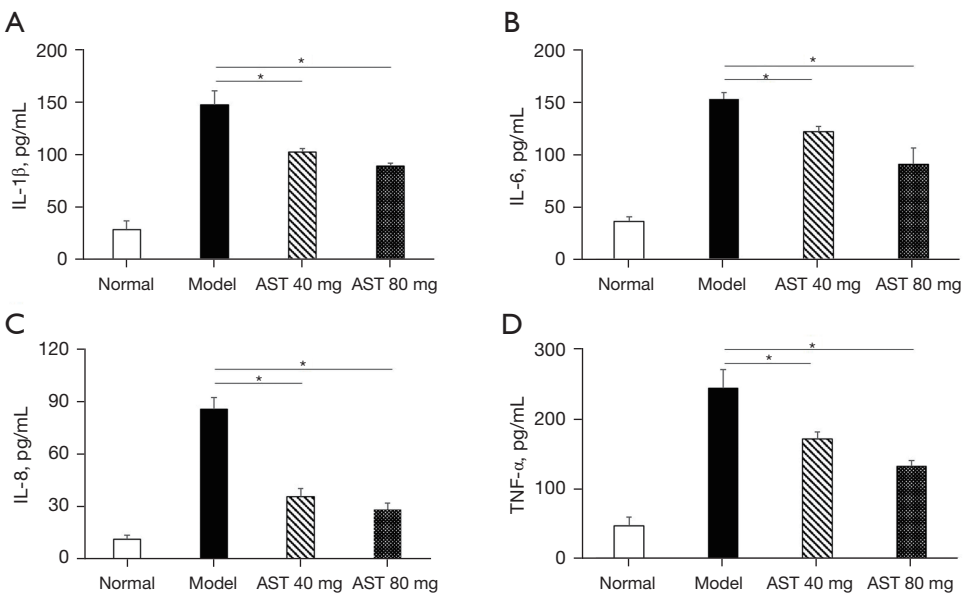

Figure 5 AST showed anti-inflammatory activity by inhibiting inflammatory cytokines expression in prostate tissue. (A-D) A significant enhancement in the levels of IL-1β, IL-6, IL-8 and TNF-α was found in model rats *vs.* normal rats. While in AST treated groups, AST downregulated inflammatory cytokines including IL-1β, IL-6, IL-8 and TNF-α. Data were shown as mean ± SD (n=6). *, P<0.05. AST, astaxanthin; IL, interleukin; TNF-α, tumor necrosis factor-α; SD, standard deviation.

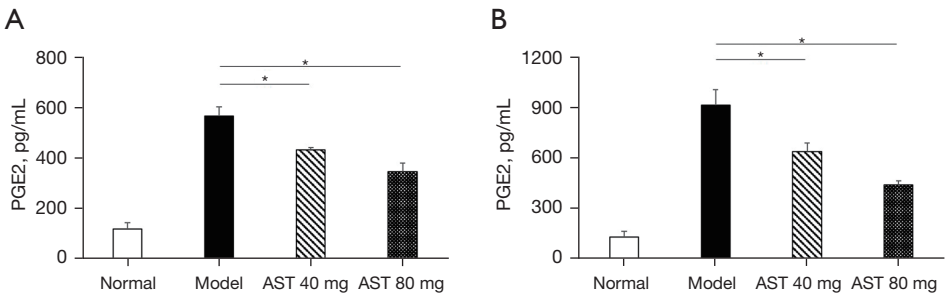

Figure 6 AST inhibited the expression of PGE2 in CP/CPPS rat prostate tissue and serum. (A) AST decreased the expression of PGE2 in CP/CPPS rat prostate tissue. (B) AST decreased the expression of PGE2 in CP/CPPS rat serum. Data were presented as mean ± SD (n=6). *, P<0.05. AST, astaxanthin; PGE2, prostaglandin E2; CP/CPPS, chronic prostatitis/chronic pelvic pain syndrome; SD, standard deviation.

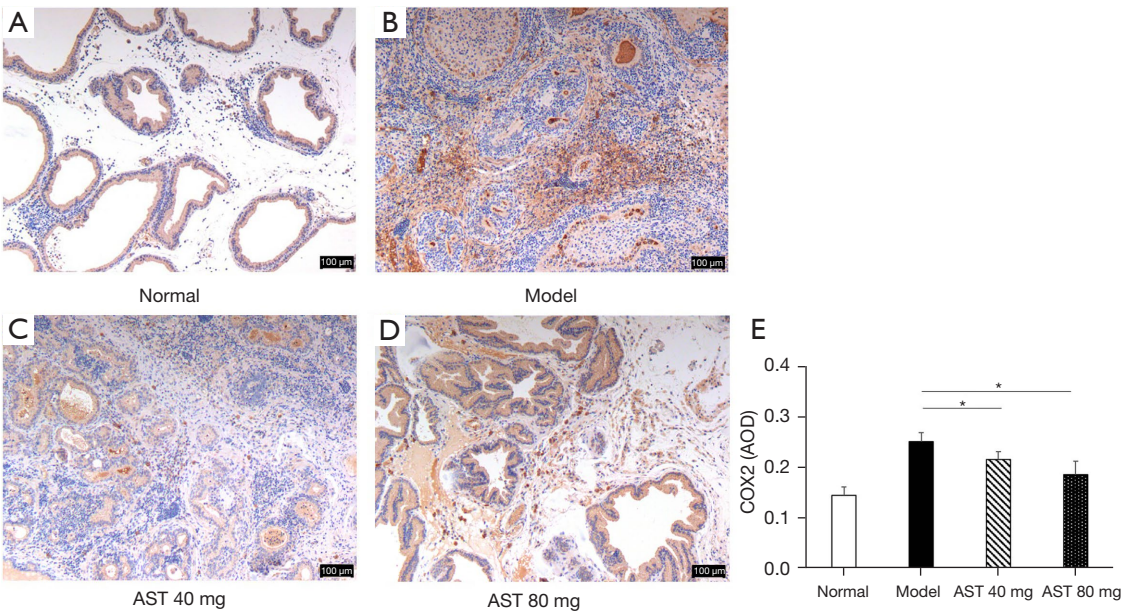

Figure 7 AST decreased the expression of COX2 in prostate tissue. Representative immunohistochemical staining of COX2 in the prostate tissue of different groups of rats. (A) Immunohistochemical staining of COX2 in the normal group. (B) Immunohistochemical staining of COX2 in the model group. (C,D) In AST treated groups, the levels of COX2 were decreased in a dose-depended manner after AST administration. (E) The AOD was measured by ImageJ software. Magnification is 100×. Data were shown as mean ± SD (n=6). *, P<0.05. AST, astaxanthin; COX2, cyclooxygenase 2; AOD, average optical density; SD, standard deviation.

The effect of AST on the PGE2 activity of prostate tissue and serum in the CP/CPPS model rats is shown in *Figure 6*. As compared with the normal control group, after carrageenan stimulation, the level of PGE2 remarkedly increased both in prostate tissue (*Figure 6A*, P<0.05) and serum (*Figure 6B*, P<0.05). In contrast, AST markedly reversed the increasement of PGE2 caused by carrageenan stimulation.

AST decreased the expression of COX2 in prostate tissue

Increased expression of COX2 is involved in the development of chronic pelvic pain. We thus performed immunohistochemistry to assess the COX2 activity in the CP/CPPS rat prostate tissue in each group (*Figure* 7). As expected, the activity of COX2 was significantly enhanced in the CP/CPPS model group compared with the normal

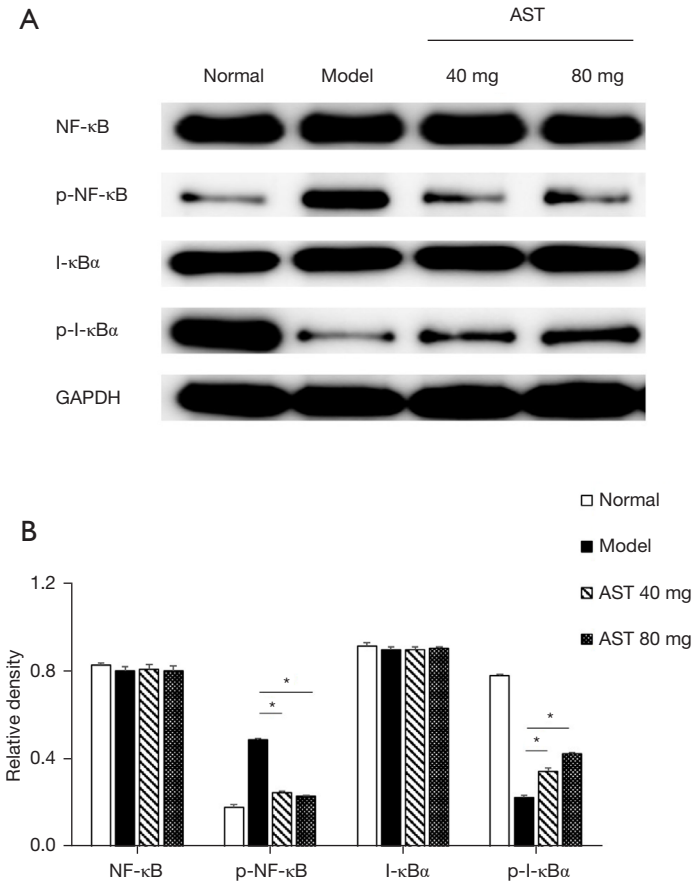

Figure 8 AST suppressed the activation of the NF-κB signaling pathway. (A) AST suppressed the phosphorylation of NF-κB and increased the phosphorylation of I-κBα as shown by Western blotting. (B) Relative density analysis of each band compared with corresponding GAPDH band. Data were shown as mean ± SD (n=3). *, P<0.05. AST, astaxanthin; SD, standard deviation; NF-κB, nuclear factor-κB.

control group. On the contrary, AST markedly reversed the elevation of COX2 activity (*Figure* 7, P<0.05).

AST inhibited the phosphorylation of nuclear factor-κB (NF-κB)

To further demonstrate the underlying molecular mechanism of AST on CP/CPPS, the NF-κB signaling pathway was explored. As shown in *Figure 8A*, compared with the normal control group, NF-κB was markedly phosphorylated in the model group, which indicates the activation of NF-κB signaling pathway. However, AST treatment in CP/CPPS rats markedly inhibited the phosphorylation level of NF-κB in a dose-dependent manner. Moreover, the phosphorylation of I-κBα was significantly suppressed in the model group compared with the normal group. As expected, AST treatment in CP/CPPS rats markedly up-regulated the phosphorylation level of I-κBα in a dose-dependent manner (*Figure 8A*,*8B*, P<0.05).

Discussion

In the present study, we successfully induced a reliable CP/CPPS rat model by intraprostatic injection of 1% carrageenan, which is consistent with the previous report (19,20). Based on this specific CP/CPPS rat model, our observation suggested that AST could ameliorate chronic pelvic pain induced by carrageenan. Moreover, the histological findings indicated that AST at different concentrations could alleviate the tissue inflammatory damage of rat prostate. With the help of this typical model, our research revealed that AST could reduce the expression of proinflammatory mediator levels of IL-1β, IL-6, IL-

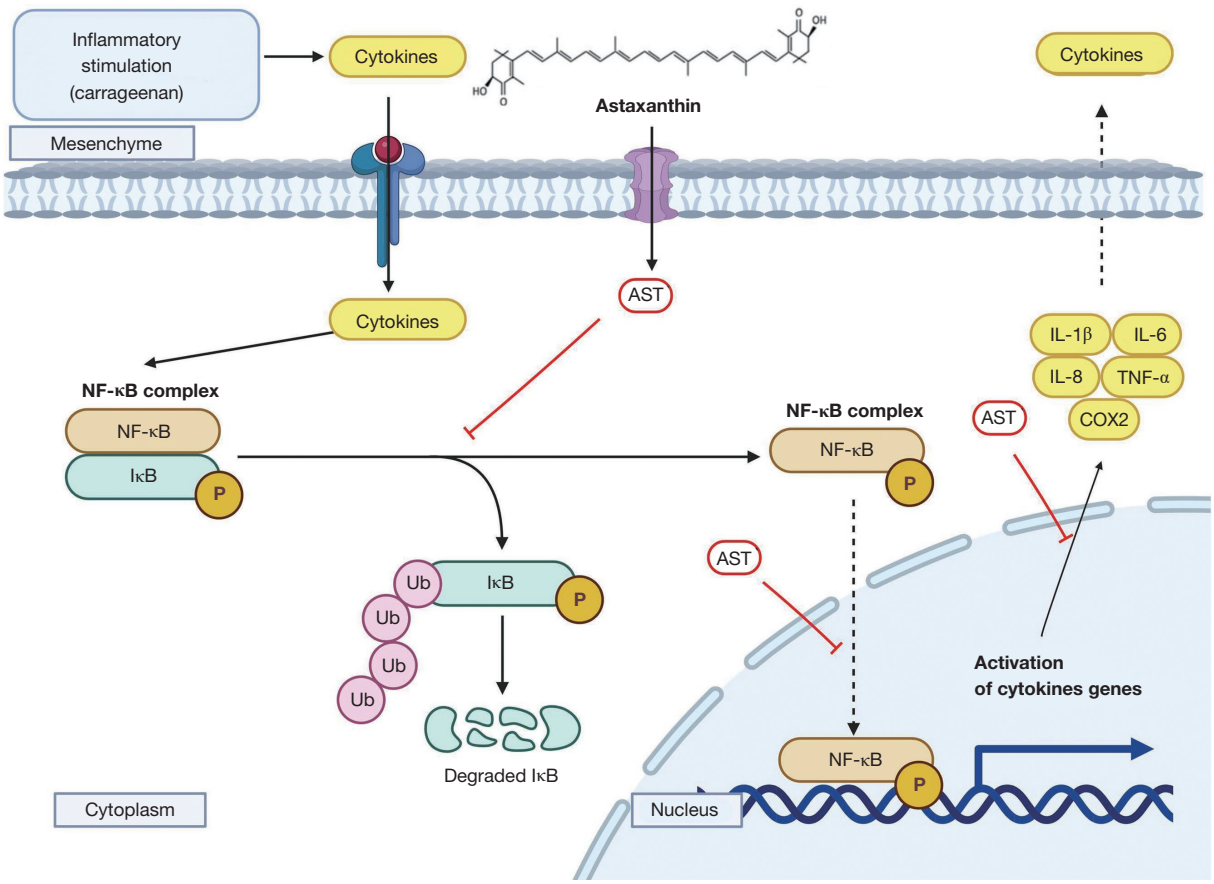

Figure 9 Schematic of proposed mechanisms of anti-inflammatory effect for AST in CP/CPPS rat model. Created with BioRender.com. As depicted, AST might be an efficacious treatment to alleviate pain and inflammation in CP/CPPS, possibly due to decreased activation of NF-κB signaling pathway. AST, astaxanthin; IL, interleukin; TNF-α, tumor necrosis factor-α; COX2, cyclooxygenase 2; NF-κB, nuclear factor-κB; CP/CPPS, chronic prostatitis/chronic pelvic pain syndrome.

8, and TNF-α, inhibit PGE2 activity, and decrease COX2 level. All these findings proved that AST protected against prostatitis through anti-inflammatory effects. Furthermore, we investigated the role of AST on CP/CPPS in the classic inflammatory signaling pathway and it indicated that AST inhibited the phosphorylation of NF-κB and increased the phosphorylation of I-κBα (*Figure 9*).

Clinically, CP has now been concerned as an indispensable part of the symptom progression in the disease of benign prostatic hyperplasia (BPH) and lower urinary tract symptoms, meanwhile, most CP/CPPS patients are suffering from chronic genitourinary pain (21-24). Although the etiology of CPPS is heterogenous and the underlying mechanism is still largely unclear, CP has been recognized as a key component (25,26). The current results confirmed that the establishment of CP/CPPS rat model by intraprostatic injection with 1% carrageenan was an effective method. Electric von Frey experiments indicated increased response frequency in CP/CPPS rats, which could be concerned as the chronic prostatic inflammation having a sensitizing effect on pain. Besides, histopathological changes in the prostate were found in the chronic prostatic inflammation rat model, which showed as markedly prostate tissue damage with significant inflammatory infiltration. These observations are consistent with the previous studies (15,27,28). In addition, administration of AST could alleviate the chronic pelvic pain. Furthermore, qualitative observation and analysis of the prostatic histopathology indicated that AST remarkedly inhibited the inflammatory infiltration in the CP/CPPS rats.

Inflammatory cytokines are pivotal proteins that play a prominent role in modulating physiological beneficial

inflammatory response. Increased levels of inflammatory cytokines including IL-1β, IL-6, IL-8, and TNF-α have been described in clinical samples from CP/CPPS patients (29,30). IL-1β is a key pro-inflammatory cytokine that participates in the host's response to pathogens and tissue damage, and IL-1β is mainly derived from monocytes, macrophages, and dendritic cells and its release is in response to stimuli such as pathogen-related or risk-related molecular patterns (31,32). Ashok *et al.* reported that the expression of IL-1β in prostate secretions from CP/CPPS patients was increased (33). Yang *et al.* showed that the expression of IL-1β was increased in the prostate tissue in the CP mice (34). A previous study has found that IL-8 plays a key role in inducing pain hypersensitivity by acting as a chemokine to attract monocytes and neutrophils into the inflammatory location (35). IL-6, is one of the most important immune factors involved in inflammatory response, which modulates the production of IL-1β and TNF-α (36). Zhang *et al.* reported that the expression of IL-6 was significantly related to the inflammatory level in prostatic tissue (37). TNF-α is synthesized by macrophages and monocytes, which exerting an important role in diseases associated with inflammation (30). In agreement with previous studies, our data suggested that inflammatory cytokines increased significantly in the prostate tissue of CP/CPPS rats. At the same time, the anti-inflammatory activity of AST in CP/CPPS rat model was confirmed by inhibiting inflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α levels.

Apart from the proinflammatory cytokines, other cytokines, including PGE2 and COX2, also play a pivotal role in modulating the inflammatory response. PGE2 plays a critical role in regulating various aspects of the inflammatory response. The role of PGE2 in driving acute inflammation is well known. However, PGE2 also elicits powerful immunosuppressive properties that contribute to the resolution phase of acute inflammation, facilitating tissue regeneration and the return to homeostasis (38). Activation of PGE2 leads to unphosphorylated β-catenin accumulation in the cytoplasm and then reaching the nucleus to suppress the transcription factor NF-κB. The suppression of NF-κB by β-catenin is a common mechanism that inhibits inflammatory level (39). In addition, COX2 has been found to be increased in CP/CPPS rats (40). Consistent with previous studies, our results indicated that both PGE2 and COX2 were upregulated in CP/CPPS rat model, interestingly, AST significantly down-regulated the activities of PGE2 and COX2, suggesting that the alleviation of pelvic pain might be beneficial to the inhibitory effect of AST on inflammation in CP/CPPS.

NF-κB signaling pathway has been found to have a pivotal role in the occurrence and development of inflammation such as CP/CPPS (41-43). In unstimulated cells, NF-κB is combined with the inhibitor I-κBα, and the latter chelates NF-κB in the cytoplasm. The activation of NF-κB is triggered by the degradation of I-κB proteins which are induced by certain signals (13). Once stimulated, I-κBα is phosphorylated and separated from the complexes, and subsequent ubiquitination and degradation leads to liberation of the heterodimeric NF-κB, and it could move into the nucleus and modulate transcription of downstream genes like proinflammatory factors IL-1β, IL-6, IL-8, and TNF-α (44). Alaimo *et al.* reported that prostate cells secret transient receptor potential cation channel subfamily M member 8 gene RNA into extracellular vesicles (EVs), which primes TLR3 (toll-like receptor)/NF-κB-mediated inflammatory signaling after EV endocytosis by epithelial cancer cells (45). Recently, AST has been widely studied because of its plenty of interesting bioactivities, such as anti-microbial and anti-inflammatory activities (46,47). For instance, Lee *et al.* reported that AST exhibited protective effects against gastrointestinal diseases through multiple mechanisms including suppression of pro-inflammatory cytokines (48). Yin *et al.* indicated that AST markedly suppressed the expression of IL-1β, IL-17, and TGF-β proteins induced by lipopolysaccharides and showed intense antioxidant activities in dendritic cells associated with inflammatory responses, which was expected to be a potential treatment for sepsis (49). In the present study, we observed that AST with various concentration could markedly inhibit phosphorylation of NF-κB, which would further modulate the expression of various pro-inflammatory cytokines. Based on the above results, we demonstrated that NF-κB participates in carrageenan-induced rat CP/CPPS model, and AST can alleviate the injure degree of prostate tissue at least partly through inhibiting of NF-κB signaling pathway.

Conclusions

The results of our study indicated for the first time that AST might be an effective treatment to alleviate pain and inflammation in CP/CPPS, possibly due to the inhibition of NF-κB signaling pathway. However, the detailed potential

mechanisms of the impact of AST on the processes are still unclear, so further research is needed to investigate the interference of AST on various signaling pathways.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-24-190/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal studies were approved by the Wuhan University Biomedical Ethics Committee for animal use and protection (No. 20183060001). All experimental procedures were complied with Chinese or institutional guidelines for the Care and Use of Laboratory Animals.

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