Original Article

Histopathological Investigation of the Anti-inflammatory Effects of Metformin in an Experimental Model of Chronic Non-bacterial Prostatitis in Rats

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ABSTRACT

Background: Chronic prostatitis is one of the most common diseases of the prostate gland, significantly impacting men's quality of life. Today, metformin is recognized as a medicine with multiple therapeutic potentials, including enhanced anti-inflammatory and antioxidant activity, antitumor effects, benign prostatic hyperplasia, etc.

Objectives: The present study aimed to investigate the histopathological effects of metformin on chronic non-bacterial prostatitis (CNP) induced by carrageenan in rats.

Methods: Twenty-four male rats were divided into four groups (n=6): Group I (Sham: pseudo CNP-untreated), group II (control: CNP-untreated), group III (cernilton: CNP-standard treatment), and group IV (MET: CNP-under treatment). To induce CNP, 0.1 mm carrageenan (1%) was injected into the prostate of groups II, III, and IV. In contrast, an equal amount of normal saline was injected into group I. Subsequently, group IV was treated with MET (100 mg/kg), group III with cernilton (100 mg/kg), and groups I and II with 0.5 mL normal saline, orally daily for up to 3 weeks (21 days). After 21 days, the rats were euthanized, and prostate tissue sections were prepared for histological staining with hematoxylin-eosin.

Results: Group IV had a significant difference in inflammation compared to group II ($P<0.05$). Additionally, from a histopathological perspective, group IV exhibited a significant reduction in inflammation but in hyperplasia of acinar epithelial cells and hyperemia. Although there were no statistically significant differences among all groups, the reduction of hyperplasia and the increase in hyperemia were notable in group IV, with the tissue appearance closely resembling that of normal prostate tissue.

Conclusion: In the present study, it was demonstrated for the first time that metformin reduces tissue inflammation and its associated complications in the prostate.

Keywords: Chronic Prostatitis, Histopathology, Metformin

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Introduction

The prostate is the target of a number of common diseases that can affect male fertility at different ages. In both young and aged men, prostatic diseases or an unhealthy prostate can affect spermatozoa functioning and, therefore, male fertility (Verze et al., 2016). Prostatitis has been recognized as a significant medical condition in recent decades; however, a comprehensive and appropriate treatment for it remains elusive. The prevalence of this disease has been reported to range from 2.2% to 8.9%. Nickel et al. employed questions similar to the NIH chronic prostatitis symptom index (NIH-CPSI) pain domain to determine the prevalence of prostatitis-like symptoms in 20–74-year-old men from Eastern Canada. Of 868 participants, 84(9.7%) had chronic prostatitis-like symptoms. Prostatitis-like symptoms occurred in 11.5% of men younger than 50 years old and in 8.5% of men ≥50 years old (Krieger et al., 2007). Prostatitis is the most common issue in young men and one of the most prevalent problems in men over the age of 50. Genital infections, including prostatitis, epididymitis, and testicular swelling, account for 12% of infertility causes in men (Lu et al., 2011). According to NIH reports, prostatitis is classified into four clinical types: acute bacterial prostatitis, chronic bacterial prostatitis, chronic prostatitis or chronic pelvic pain syndrome, and asymptomatic inflammatory prostatitis. Chronic prostatitis or chronic pelvic pain syndrome can present in inflammatory or non-inflammatory forms (Yang et al., 2014). Inflammatory chronic prostatitis has the highest prevalence (40% to 65% of cases) and is eight times more common than chronic bacterial prostatitis (5% to 10% of cases).

Symptoms of non-bacterial inflammatory chronic prostatitis include urinary problems, blood in semen, chronic pelvic pain, and erectile dysfunction. The pain associated with this condition is often intermittent and can be felt in various areas, including the anus, testicles, penis, and abdomen (Alshahrani et al., 2013). The factors contributing to this disease include immunological issues, urine backflow into the prostate, neurological influences, recurrent smooth muscle contractions in the prostate and bladder neck, psychological factors, increased pressure in the prostate, and hormonal imbalances. Recent studies show that oxidative stress and inflammatory mediators worsen complications associated with chronic prostatitis. The imbalance between reactive oxygen species (ROS) and antioxidants, along with the activation of inflammation and cytokine feedback, leads to tissue damage and complications from the disease (Zhao et al., 2019). Chronic inflammatory prostatitis, or chronic pelvic pain syndrome, is associated with elevated levels of pro-inflammatory cytokines, like interleukin-17 and interferon-gamma, which support the use of anti-inflammatory medications (Yoon et al., 2013). Common treatments include antibiotics, non-steroidal anti-inflammatory drugs, muscle relaxants, and alpha receptor blockers, though no definitive treatment exists (Chen et al., 2014). Research indicates that oxidative stress contributes to prostatitis, with the detection of radicals, such as nitric oxide and ROS in prostate tissues confirming inflammation. Therefore, compounds that eliminate free radicals and boost antioxidant activity may help in treatment (Yang et al., 2014).

Metformin (1,1-dimethyl biguanide hydrochloride), a first-line treatment for type 2 diabetes since the 1950s, has shown a variety of additional therapeutic benefits, including applications in weight loss, cardiovascular diseases, cancer, kidney and liver diseases, and polycystic ovary syndrome. Its protective effects on neurons also suggest potential in managing Alzheimer's disease and dementia, highlighting its broad role in healthcare. The therapeutic benefits of metformin are not limited to those listed above, and it is also worth noting its role in controlling oxidative stress and reducing the production of ROS (Naseri et al., 2023). Administering metformin significantly enhances steroidogenesis and spermatogenesis in men with metabolic disorders, suggesting that metformin therapy is a promising method for improving reproductive function and fertility in men (Shpakov et al., 2021). Metformin affects the AMPK/mTOR (AMP-activated protein kinase/ mechanistic target of rapamycin) signaling pathway, which regulates various pro-inflammatory cytokines, including interleukin-1 (IL-1), IL-2, IL-6, IL-12, and tumor necrosis factor-alpha (TNF-α) (Chung et al., 2017). Metformin is an immunomodulatory drug with high tolerability that is used as an add-on treatment for certain malignant, autoimmune, and aging-related conditions. It exhibits antiproliferative and antioxidant effects and is beneficial for type 2 diabetes, obesity-associated inflammation, autoimmune diseases, and cardiovascular diseases, and neuroprotection (Yin et al., 2015; Lee et al., 2017; Jing et al., 2018; Kim et al., 2018; Schuiveling et al., 2018; Jang et al., 2020; Sciannimanico et al., 2020). In patients with chronic inflammation, metformin can be used as a protective or therapeutic option (Saisho, 2015; Ismaiel et al., 2016), including conditions, such as colitis-associated colon cancer (Koh et al., 2014), otitis media (Cho et al., 2016), and airway inflammation (Park et al., 2012). Metformin has been shown to help treat sepsis-related brain injury by reducing neuroinflammation, oxidative stress, and apoptosis

(Tang et al., 2017). By inhibiting insulin-like growth factor-1 (IGF-1) and its receptors, metformin can reduce the risk of benign prostatic hyperplasia (Mosli et al., 2015; Wang et al., 2017; Tseng et al., 2022).

Carrageenan is a sulfate-containing polysaccharide derived from marine algae (Lopes et al., 2020). Carrageenan possesses antiviral, antibacterial, antioxidant, anticancer, and immune-regulating properties (Das & Bal, 2024). One characteristic of carrageenan is its ability to induce inflammation in tissues, and it is commonly used in research to study inflammatory processes and anti-inflammatory agents (Thomson & Fowler, 1981). In the inflammatory responses induced by carrageenan, two phases are observed. The initial phase occurs 2 to 3 hours after induction, and the second phase manifests 3 hours after the first. In the acute phase, neutrophils are the predominant infiltrating cells, with histamine and serotonin as the mediators of this phase. In the second phase, the dominant populations comprise macrophages, lymphocytes, and eosinophils. Moreover, according to reports, nitric oxide is a significant mediator in carrageenan-induced inflammation (Barua et al., 2011). From a pathological perspective, following inflammation of the prostate, tissue exhibits proliferating immune cells, acinar atrophy, recruitment of inflammatory cells to the site, and accumulation of lymphocytes around the acini (De Marzo et al., 1999). Depending on the type of immune cells present at the site of inflammation, prostatitis is classified into four subgroups: acute, chronic, eosinophilic, and granulomatous. Although lymphocytes are the predominant cell population in chronic prostatitis, neutrophils dominate acute prostatitis. In eosinophilic prostatitis, there is extensive presence of eosinophils in the inflamed prostate tissue, while in the granulomatous type, multiple granuloma masses can be observed in the tissue (True et al., 1999). The onset of non-bacterial prostatitis (CNP) and the inflammation it induces results in changes in the histological appearance of the prostate tissue. Therefore, this study was conducted for the first time to investigate the therapeutic effects of metformin on the histopathological changes of CNP induced by carrageenan in rats.

Materials and Methods

Animal model

For the present study, 24 adult male Sprague-Dawley rats (7 weeks old, weighing 200-230 grams) were obtained from the laboratory animal breeding and maintenance section and housed in specialized cages. To avoid stress and allow the animals to acclimate to their environment, no experiments were conducted on the rats for one week. All animals were kept under uniform environmental and dietary conditions (temperature, humidity, light, type of diet, and frequency of feeding) and maintained on a 12-hour light/dark cycle. The rats were fed a specific laboratory animal pellet diet, and water (changed daily) was provided freely. All protocols of this study were approved by the Ethics Committee of Semnan University of Veterinary Medicine.

Study design

Rats were randomly divided into four groups (n=6).

Group I (sham: pseudo CNP-untreated): This group included rats that underwent a simulated prostatitis procedure (the rats were anesthetized, and a small incision was made in the midline of the abdomen). To both the right and left lobes of the prostate, an equivalent volume of normal saline was administered, and the animals received 0.5 mL of normal saline orally every 24 hours for up to 3 weeks.

Group II (control: CNP-untreated): This group included rats in which prostatitis was induced. The rats were anesthetized, and a small incision was made in the midline of the abdomen. To both the right and left lobes of the prostate, an equivalent volume of sterile 1% carrageenan solution was injected, and the rats received 0.5 mL of normal saline orally every 24 hours for up to 3 weeks.

Group III (cernilton: CNP-standard treatment): This group included rats in which prostatitis was induced. The rats received 100 mg/kg cernilton (the standard CNP treatment) orally daily for up to three weeks (Karimi et al., 2021).

Group IV (MET: CNP-under treatment): This group included rats with prostatitis induction that received 200 mg/kg metformin orally daily for up to 3 weeks.

Prostatitis induction

To induce prostatitis, rats were anesthetized with 10% ketamine (25 mg/kg intra peritoneally [i.p.]) and 2% xylazine (25 mg/kg i.p.). The hair at the surgical site was shaved, and after sterilizing the area, it was prepared for surgery. A small incision was made in the midline of the abdomen in the sterilized area, following which the bladder and prostate were carefully exteriorized. To the right and left lobes of the prostate in groups II, III, and IV, a sterile solution of 1% carrageenan was injected at a volume of 0.1 mL (Hajighorbani et al., 2017), whereas the prostate of group I rats received an equivalent volume of normal saline. After the injection, the excised organs were gently placed back into the pelvic cavity, and the wound was sutured. For three consecutive days post-surgery, antibiotic therapy was administered for all groups (ceftriaxone 1 g dissolved in 10 cc of distilled water, with 20 units of insulin injected intramuscularly for each rat).

After seven days (day 8), the rats in group III received 100 mg/kg cernilton, and group IV received 100 mg/kg metformin orally for 21 days. Rats in groups I and II received an equivalent volume of normal saline orally for 21 days. After the final administration (day 29), the rats were fasted for 12 hours, weighed, and euthanized, and their prostates were removed. To ensure that CNP was successfully induced in group II rats using carrageenan, the prostatic index (PI) was calculated by dividing the weight of the prostate in milligrams by the weight of each rat in grams (the normal level of this index in healthy rats aged 8 weeks is less than 1 mg/g). On day 8, one rat was randomly euthanized, and the histopathology of the prostate was checked and confirmed by a pathologist. CNP was confirmed through histopathological examination by identifying the presence of inflammation, hyperplasia, and hyperemia in the prostate tissue of a representative rat randomly selected from the group. The evaluation was conducted by a pathologist to ensure accurate confirmation of CNP induction.

Histopathological assessment

Prostate glands extracted from the body were fixed in 10% formalin for 24 hours, after which the fixative was replaced with fresh 10% formalin. A tissue processor was used for tissue processing, including dehydration, clearing, and infiltration. After processing, the tissues were embedded in melted paraffin and cut into five-micron sections using a microtome. For histological examination, hematoxylin and eosin (H&E) staining was performed, with hematoxylin staining nuclei blue and eosin staining cytoplasm pink. Subsequently, histopathological assessments under light microscopy reported the prostate inflammation status across groups I to IV both quantitatively and qualitatively. In this study, prostate gland tissue was analyzed for three lesions—inflammation, hyperplasia, and hyperemia—in five serial sections from each sample. Explanations regarding the scoring of each of the three aforementioned lesions are provided in this section. Five random fields of view were selected for each slide, and the inflammatory lesions, hyperplasia, and hyperemia were evaluated as described.

Prostatic inflammation examination

The assessment of inflammation severity in prostate tissue is based on the method by Nickel et al. (2001). Accordingly, the severity of inflammation in CNP is classified into grade 1: mild, 2: moderate, and 3: severe. The histological description of each grade is presented in Table 1.

Prostatic hyperplasia examination

Prostatic hyperplasia is assessed based on the increase in acini epithelial cells and is reported as either present or absent.

Prostatic hyperemia examination

Due to increased blood supply to the tissue, prostatic hyperemia resulting from inflammation presents microscopically as a hyperemia of red blood cells filling the vascular space. Like hyperplasia, hyperemia is reported as either present or absent.

Statistical analysis

The data were analyzed using SPSS software, version 23 (SPSS Inc., Chicago, IL). The non-normally distributed data were compared using the Kruskal–Wallis test with a Mann–Whitney U post hoc test. $P<0.05$ were considered statistically significant.

Results

Histopathological findings

The microscopic appearance of the complications of CNP (inflammation, hyperplasia, and hyperemia) in prostate tissue is shown in Figures 1A, 1B and 1C. The comparison of histopathology of CNP complications in prostate tissue across the four studied groups is shown in Figure 2.

Effect of metformin on the inflammation of prostate tissue

Prostate tissue inflammation in the cernilton and MET groups showed significant improvement compared to the control group and was approaching the sham group. According to the mean rank index, treatments in groups IV and III reduced the severity of tissue inflammation by approximately half compared with group II, bringing it closer to group I. Group II was significantly different from group IV, and group I was significantly different from group II ($P<0.05$). However, group III did not differ significantly from any other group (Table 2).

Effect of metformin on prostatic tissue hyperplasia

The statistical analysis results indicated a significant reduction in the severity of hyperplasia in the Met group compared to the control and sham groups. The therapeutic effects of metformin were effective in reducing hyperplasia, and the severity after treatment was lower than that in the sham group. No significant difference was found among the studied groups (Table 2).

Effect of metformin on prostate tissue hyperemia

Post-treatment hyperemia after metformin administration showed little reduction and was similar to the control group. Hyperemia in the cernilton group increased compared to the control group. Tissue hyperemia in the MET group showed a slight decrease compared to the control group, while an increase was observed in the cernilton group. No significant differences were found among the studied groups (Table 2).

Discussion

In the present study, CNP was induced by intraprostatic injection of carrageenan. One advantage of this method is that the protocol is relatively simple to carry out, Sprague-Dawley rats are readily available, and the induction of pain and inflammation closely resembles the clinical manifestation of chronic prostatitis in humans. The current study aimed to investigate the protective effects of metformin on a Sprague-Dawley rat model of CNP. To date, no studies have examined the effects of metformin on the reduction of inflammation caused by CNP in animal models or clinical trials in humans, with prior research primarily focusing on the impact of metformin on prostate cancer (Hou et al., 2019). Therefore, the results of this research may provide valuable insights regarding the therapeutic potential of metformin against CNP. Given the anti-inflammatory, antimicrobial, and blood glucose-lowering effects of metformin, it is plausible to hypothesize that its administration could reduce the incidence of prostatitis. Although sufficient information to demonstrate a direct effect of metformin on prostatitis is lacking at this time, this drug may have the potential to reduce infection and inflammation in the prostate.

Research into the anti-inflammatory mechanisms of metformin has revealed that it plays a significant role in halting the inflammatory cascade and preventing cytokine storms through two primary pathways: AMPK-dependent and AMPK-independent. In the AMPK-dependent pathway, metformin enters the cytoplasm and inhibits complex I in the mitochondria, leading to an increased AMP:ATP ratio, and subsequent activation of AMPK. The active AMPK then exerts broad inhibitory effects on various inflammatory molecules, including TNF-α, NF-κB, and pathways related to mTOR signaling. In addition to the AMPK-dependent pathway, metformin can also enter the cytoplasm and reduce the expression of several pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β, and NF-κB, without involving AMPK (Kristófi & Eriksson, 2021). In male C57BL/6 rats, coronary artery ligation resulted in ischemia-reperfusion injury, leading to myocardial apoptosis, inflammation, and replacement with collagen fibers. Treatment with metformin significantly reduced the pathological consequences of ischemia-reperfusion injury and cellular apoptosis in the hearts of these rats. Metformin inhibits the production of superoxide species in the heart by increasing the levels of antioxidant enzymes, such as superoxide dismutase 2, peroxiredoxin-3 and -5, and thioredoxin reductase 2. Metformin administration in adult albino male rats exposed to gamma radiation resulted in a reduction of biomarkers for cardiac injury (lactate dehydrogenase and CK-MB). Additionally, cardiac catalase levels, superoxide production, and NF-κB, IL-6, and TNF-α levels were significantly decreased in the treated rat group (Bai & Chen, 2021). In a study investigating the anti-inflammatory properties of metformin on kidney tissue, metformin inhibited MCP-1 stimulation by TGF-β1, as well as the expression of collagen type IV and fibronectin in human proximal tubular cells. Metformin treatment in pregnant Sprague-Dawley rats subjected to a high-fat diet and suffering from gestational diabetes resulted in decreased serum levels of IL-6 and TNF-α, alongside a reduction in MAPK1/3, MAPK14, and MAPK8 proteins in the kidneys.

In diabetic nephropathy in GK rats, metformin reduced IL-1β and TGF-β1 levels in kidney tissues. In rats with renal ischemia, metformin reduced tubule cell necrosis and decreased caspase-3 and COX-2 levels by increasing AMPK activation (Bai & Chen, 2021). Cytokine storm is one of the leading causes of death in patients with COVID-19. In a study investigating the anti-inflammatory properties of metformin in rats suffering from COVID-19, metformin prevented the action of a wide range of pro-inflammatory cytokines, such as IL-6 and TNF-α, ultimately affecting the cytokine storm induced by COVID-19 (Taher et al., 2023). The anti-inflammatory effects of metformin on lipopolysaccharide (LPS)-induced inflammation in human middle ear epithelial cell lines were investigated. LPS increased the levels of TNF-α and cyclooxygenase-2. However, pretreatment with metformin reduced the production of these inflammatory factors (Cho et al., 2016). Metformin

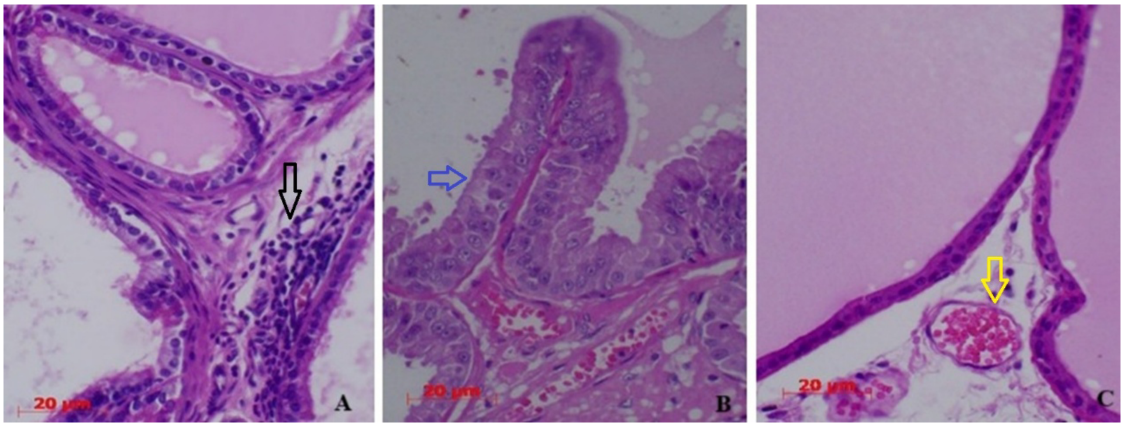

Figure 1. Microscopic appearance of chronic prostatitis complications in prostate tissue in the control group

A) Dense aggregation of inflammatory cells (black arrow), B) Hyperplasia of epithelial cells (blue arrow), C) Hyperemia in the arterioles (yellow arrow) (H&E staining, magnification: ×400)

also decreased the risk of brain injury caused by sepsis in inflammatory cytokines in rat, such as IL-6, IL-1β, and TNF-α (Tang et al., 2017). In the present study, a reduction in inflammation in the prostatitis group treated with metformin is clearly evident. A significant difference was found between the untreated prostatitis group and the prostatitis group treated with metformin. These results are consistent with previous research.

In a study conducted on Sprague-Dawley rats that developed benign prostatic hyperplasia due to testosterone or metabolic syndrome, metformin was able to reduce the risk of developing benign prostatic hyperplasia by inhibiting IGF-1 and its receptors (Mosli et al., 2015; Wang et al., 2017; Tseng et al., 2022). Metformin restores the balance of androgen hormones in rats and prevents cell proliferation in vitro and in vivo through the activation of AMPK (Yang et al., 2023). The combined treatment of metformin and GSK126 (an enhancer of zeste homolog 2 (EZH2) enzyme inhibitor) has shown a synergistic effect on the inhibition of cancer cell growth both in vitro and in vivo. Additionally, metformin alone contributes to the reduction of EZH2 by increasing miR-26a-5p (a tumor-suppressing microRNA). The increased expression of the EZH2 enzyme is associated with the exacerbation of prostate cancer and leads to a decline in the prognosis of this disease (Kong et al., 2020). Metformin can effectively treat endometrial hyperplasia, and the expres-

Table 1. Grading the severity of prostate tissue inflammation (Nickel et al., 2001)

Grade	Morphological Description (Typical Inflammatory Cell Density, Cells/mm²)
I	Individual inflammatory cells, most of which are separated by distinct intervening spaces (less than 100).
II	Moderate confluent sheets of inflammatory cells with no tissue destruction or lymphoid nodule/follicle formation (between 100 and 500).
III	Severe confluent sheets of inflammatory cells with tissue destruction or nodule/follicle formation (more than 500).

Table 2. Comparison of tissue changes in the prostate among the study groups based on the mean rank index

Prostate Tissue Changes	Shame (n=6)	Control (n=6)	Cernilton (n=6)	Metformin (n=6)
Inflammation	3*	7.5*	3.67	3.5*
Hyperplasia	3.8	6.5	4.58	3.75
Hyperemia	4.9	5.13	6	5.5

*Statistically significant difference compared to the sham and control groups (P<0.05)

Note: There was a significant difference in inflammation between the MET group and the shame and control groups. There was no significant differences between the other groups with respect to hyperplasia and hyperemia.

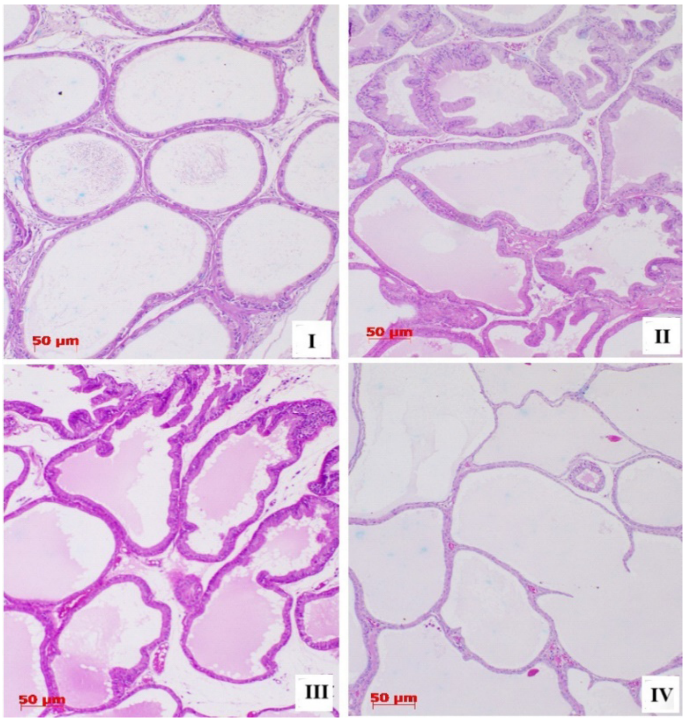

Figure 2. Comparison of the histopathology of CNP complications in prostate tissue among the four studied groups

Note: I: Shame, II: Control, III: Cernilton, IV: MET; (H&E staining, magnification: ×250).

sion of glucose transporter type 4 insulin-responsive is closely associated with the development of endometrial hyperplasia (Liu et al., 2022). Quercetin is a type of plant flavonoid that is beneficial in fighting prostate hyperplasia cells. Meanwhile, *Nigella sativa* seed oil has shown promise in relieving benign prostatic hyperplasia symptoms (Jafari et al., 2024). In this study, the histopathological findings from the rats in the MET group indicated a reduction in the severity of epithelial cell hyperplasia in the prostatic acini. Although no significant differences were observed among the groups studied, the level of hyperplasia in the treated groups decreased and became comparable to that of the control group. Therefore, the results of this study regarding the antiproliferative properties of metformin align with previous research. It is important to note that the manipulations performed to induce a pseudo-prostatitis process (specifically, needle penetration into the prostatic tissue and the injection of saline) caused stress in the prostatic tissue, which plays a significant role in the development of hyperplasia.

Regarding the effects of metformin on arthritogenic erectile dysfunction, three mechanisms have been reported: endothelial-related vasodilation, sympathetic nerve hyperactivity, and atherosclerosis, which are associated with insulin resistance occurring in obesity, diabetes, hypertension, and dyslipidemia. Insulin resistance leads to a reduction in the expression of nitric oxide synthase. Consequently, it decreases nitric oxide production. Metformin reduces insulin resistance, restoring conditions for nitric oxide production and contributing to vasodilation. Additionally, metformin plays a role in vasodilation by reducing sympathetic nerve hyperactivity, as evidenced by heart rate and blood pressure assessments in individuals treated with metformin. Therefore, metformin induces vasodilation and increases tissue blood flow through the two mechanisms described (Patel et al., 2017). The beneficial effect of metformin in reducing metabolic resistance to insulin, independent of changes in glucose metabolism, indicates that metformin has a direct vascular effect. Metformin improved endothelium-dependent forearm blood flow in individuals with metabolic syndrome (de Aguiar et al., 2006). A 36-week treatment with metformin led to an improvement in microvascular blood flow in the skin of patients with type 2 diabetes. In contrast, 3 months of metformin treatment improved microvascular reactivity in the skin of healthy individuals with metabolic syndrome (Pistrosch et al., 2013; de Aguiar et al., 2006). Women with type 2 diabetes and obesity showed improved functional capillary density during hyperemia following occlusion after receiving metformin for 30 days (Schiapaccassa et al., 2019; Schiapaccassa et al 2020). In the present study, prostate tissue hyperemia continued after the treatment period. Considering the effects of metformin on vascular dilation, the persistence of hyperemia in prostatic tissue is understandable; moreover, the reduction in inflammation in the prostatitis group treated with metformin is clearly evident. A significant difference was found between the untreated prostatitis group and the prostatitis group treated with metformin, although metformin was effective in reducing hyperplasia. No significant difference has been found among the studied groups.

Conclusion

The present study is the first to investigate the effect of metformin on improving complications arising from chronic CNP in male rats. Based on the results obtained from this study, oral administration of metformin led to a reduction in the inflammatory effects associated with CNP. Therefore, leveraging the pleiotropic properties of metformin, the potential of this drug as an alternative in the treatment protocol for CNP is posited. However, further research with similar themes in other animal models, with a larger sample size, and from the perspective of other histopathological and clinical pathology indicators is necessary to confirm the stated hypothesis.

Limitation

This study presents a comprehensive evaluation of the histopathological effects of metformin on experimentally induced chronic prostatitis in mice; however, it did not include gene expression analysis, which could have provided deeper insights into the molecular mechanisms. Nevertheless, our research team has concurrently conducted a study on inflammatory cytokines, such as IL-17 and IFN-γ, in the same model, and the results are currently under review for publication. Due to the review process and ethical considerations, it was not possible to present these findings in the current article. Future publications are expected to complement the data from this research and provide a more comprehensive understanding of the role of metformin in reducing chronic prostate inflammation.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Institutional Animal Care and Ethics Committee of the Faculty of Veterinary Medicine, Semnan University, Semnan, Iran (Code: IR.SU.REC.1402). All experimental procedures were conducted under the strict supervision of the committee, ensuring compliance with the guidelines for the care and use of laboratory animals.

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Authors' contributions

Conceptualization and study design: All authors; Material preparation, data collection, and analysis: Sahar Ghaffari Khaligh and Mahmood Ahmadi-Hamedani; Initial draft preparation: Sahar Ghaffari Khaligh; Review and editing: Sahar Ghaffari Khaligh and Mahmood Ahmadi-Hamedani; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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