Trauma Monthly 2026; 31(2): 1737-1749

10.30491/tm.2026.533587.1853

Trauma Monthly
Research Article

Nano Zeolite Mitigates Inflammation and Oxidative Stress in Carrageenan Induced Inflammatory Prostatitis in Rats

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Received 2025-07-10 ; **Accepted** 2026-02-01; **Online Published** 2026-04-29

Abstract

Introduction: Inflammatory prostatitis (IP) is a common clinical condition associated with persistent pelvic pain, inflammation, and increased oxidative stress, all of which negatively affect male quality of life and reproductive function. Given the limited effectiveness of existing therapeutic interventions, this study presents the first methodological analysis of the anti-inflammatory and antioxidant properties of zeolite (ZEO) in a carrageenan-induced IP rat model.

Method: Thirty male Sprague-Dawley rats were randomly assigned to five experimental groups: Sham, IP, Cernilton, ZEO-IP, and ZEO-Oral. Prostatitis was induced by carrageenan injection, and the corresponding treatments were administered over three weeks. Serum levels of prostate-specific antigen (PSA), interleukin-6 (IL-6), and interleukin-17 (IL-17) were measured using enzyme-linked immunosorbent assay (ELISA). Histopathological analysis was performed to assess inflammatory infiltration, epithelial hyperplasia, and local hyperemia in prostate tissue.

Result: The highest levels of PSA, IL-6, and IL-17 were observed in the IP group, whereas the ZEO-treated groups showed reductions in these markers. However, these reductions were not statistically significant ($p > 0.05$). Histopathological examination revealed that the severe inflammation observed in the IP group was reduced in the ZEO-treated groups, particularly in the ZEO-Oral group.

Conclusion： ZEO demonstrated a promising therapeutic effect in IP, improving prostatic architecture by reducing inflammation and oxidative stress. Notably, histopathological analysis showed greater resolution of inflammatory pathology, hyperplasia, and hyperemia in ZEO-treated cohorts compared to changes in quantitative biomarkers such as PSA, IL-6, and IL-17. Although these biomarker reductions were not statistically significant, they suggest that ZEO may serve as a complementary treatment for IP. Further research is warranted to confirm its clinical efficacy.

Keywords: Zeolite, Prostatitis, Carrageenan, Rat.

Introduction

Inflammatory prostatitis (IP), alternatively known as non-bacterial pelvic pain prostatitis, is a common urological disease that is manifested by incessant pelvic pains, symptoms of the lower urinary tract, and sexual dysfunction, which eventually lead to significant quality of life and psychological well-being losses. Although IP has a high prevalence, the etiology of the ailment is still unknown, and the present therapeutic measures often fail to achieve satisfactory results, which is why the necessity of new interventions is significant [1,2]. The fundamental basis of the IP pathogenesis has been a persistent inflammatory reaction in the prostate gland, which is characterized by inflammation with immune cells penetrating the gland and an imbalance in the cytokines. Activation of leukocytes leads to overproduction of reactive oxygen species (ROS) which intensifies oxidative stress (OS), damaging spermatozoa DNA, proteins, and lipids and thus causing infertility and decreased reproductive success [3,4]. Pro-inflammatory cascades propagated by cytokines including TNF- alpha, IL-1 beta, IL- 6 and IL-17, and pro-inflammatory cascades solved by IL-10 and TGF-B are a balance that is essential to the homeostasis of tissues 5, 6. The IL-6 and IL-17 among the intricate network of cytokines have an important role in the pathophysiology of IP. By activating the JAK/STAT and MAPK pathways, IL- 6 promotes the growth and proliferation of prostate epithelial cells and therefore in the progress of the epithelial hyperplasia and chronic inflammation 7. Contrastingly, IL-17 which is mostly produced by Th17 (T helper 17) cells help to infiltrate neutrophils and monocytes in prostate tissue aggravating the continued inflammation and tissue damage 8. These two cytokines have a positive feedback loop which is inflammatory and oxidative stress, which contributes to the persistence of IP [7,8].

The naturally occurring nano zeolite (ZEO), a crystalline aluminosilicate with a high surface area and strong ion-exchange capacity, has recently gained growing interest due to its antioxidant and immunomodulatory properties. The process of lipid peroxidation inhibition and re-establishment of redox balance by the ZEO is achieved through the activation of endogenous antioxidant enzymes and free radicals' adsorption. At the same time, it regulates cytokine production, inhibiting the production of pro-inflammatory mediators and enhancing the production of anti-inflammatory mediators [9,10]. These two mechanisms make ZEO a viable option when it comes to reducing pathologies of inflammation. Recently a review was conducted to assess the potential of zeolites, especially clinoptilolite, to promote gut health, alter microbiome composition and to provide detoxification, antioxidant, immunomodulatory and anti-inflammatory effects. The review also highlighted the peculiarity of zeolite in the control of neurological disorders that are related to inflammatory processes and oxidative stress 11. Recent science has drawn attention to the versatile nature of natural zeolites, in particular, clinoptilolite. Due to its strong detoxifying and ion-exchange characteristics, clinoptilolite isolates heavy metals and, in addition, reduces inflammatory processes and aids in the treatment of dermatologic illnesses by developing and regulating inflammation and enhancing antioxidant effects. The above features make clinoptilolite a promising and possibly useful product in terms of use in medicine and dermatology [12,13]. In spite of the therapeutic effects of clinoptilolite on inflammatory disorders being reported [9,10,14,15], there is a dearth of pre-clinical studies examining the ability of nano-zeolite to simultaneously mediate IL-6 and IL-17 signaling, inhibit tissue pathology, or reestablish oxidative homeostasis in inflamed prostate tissue. In addition, the models of carrageenan-induced chronic prostatitis, the proven gold standard in the provocation of chronic prostatitis inflammation have not yet been used in testing these effects 16-18. Such a gap in knowledge would in theory help increase the knowledge of the pathophysiological processes of IP, offer a standardized and reproducible model on testing anti-inflammatory interventions, and help to develop new interventions to treat patients with IP. The current study was the first study to use carrageenan induced IP rat model to investigate the prospective of ZEO in the alleviation of oxidative stress and inflammation in the prostate. Our hypothesis is that ZEO will prevent prostatic inflammation, decrease oxidative stress, as well as maintain reproductive functionality. And until now, no research has provided reports on the simultaneous occurrence of antioxidant and anti-inflammatory actions of zeolite on carrageenan-induced IP, as well as the effects on the IL-6 and IL-17. Due to the crucial importance of these cytokines in the process of prostate inflammation initiation and maintenance, the current study was aimed at addressing the gap and assessing the efficacy of zeolite as a new treatment method.

Methods

Natural Zeolite Nanoparticles

The natural zeolite used was sourced from Afrand Company (Tehran, Iran) and was a sodium/potassium clinoptilolite (CLN) with an initial particle size of approximately 5 µm. These nanoparticles were extracted from mines in Semnan Province, Iran, and, after processing, were utilized as the raw material for the present study. In this study, natural clinoptilolite zeolite was used as the starting material. Clinoptilolite, as the dominant mineral, constitutes approximately 95% of the composition of natural zeolite. The chemical composition of this zeolite, measured using X-ray fluorescence spectroscopy (XRF), includes SiO_2, CaO, Fe_2O_3, Al_2O_3, K_2O, MgO, Na_2O, TiO_2, MnO, and loss on ignition (LOI). The size of the nanoparticles was measured and confirmed using dynamic light scattering (DLS) analysis.

Animal model

As experiment subjects, thirty male Sprague-Dawley rats, whose average body mass was between 200 and 230 grams with a postnatal age of seven weeks were used. To alleviate stress and enhance acclimatization, the animals were kept under one week of controlled environment (constant temperature, humidity, light and feeding regimes). They were given a commercial laboratory diet and allowed to ad libitum access to water.

Study design

The animals were assigned randomly into five groups, each consisting of six individuals. The groups included a mock group (Group I), a IP-control group (Group II), a IP-cernilton group (Group III), a IP-ZEO-IP group (Group IV), and a IP-ZEO-Oral group (Group V). For the experiments, we surgically observed the prostatic tissues of all the rats. In the IP-control, IP-ZEO-IP/Oral, and IP-cernilton groups, each rat received an injection of 100 µl of 1% carrageenan into the prostate [17]. The mock group rats received equal amounts of physiological saline (0.9% sodium chloride solution). After seven days, rats in the IP-cernilton and IP-ZEO-IP/Oral groups were administered cernilton (100 mg/kg) and ZEO (5 mg/kg) IP/orally for three weeks. Concurrently, the rats in both the mock and IP-control groups were given saline for an equivalent period. Every rat's overall physical condition was tracked constantly during the test period. The physical condition of each rat was continuously monitored throughout the testing period. After the final dose on day 28, the rats were fasted for 12 hours. They were then weighed and euthanized swiftly using the carbon dioxide (CO_2) inhalation method. The institutional ethics committee approved all stages of this research. Blood samples from the cardiac ventricles were collected to prepare serum for biochemical analyses. The animals were euthanized, and their prostate samples were then extracted, cleansed with PBS to remove any attached blood and fat tissues, and weighed to calculate the prostatic index (PI).

Evaluation of PSA, IL-17, and IL-6

Serum samples were obtained from blood collected after clotting and centrifuged at 3500 rpm for 15 min. The PSA, IL-6, and IL-17 levels were estimated using ELISA kits from MyBioSource (San Diego, California, USA) and DRG Diagnostics GmbH (Marburg, Germany). Measurements were performed according to the manufacturer's instructions.

Histopathological assessment

The prostate glands were fixed in 10% neutral formalin buffer in 24 h after which they were subjected to graded dehydration, clearing, and paraffin embedding. Thereafter, the preparation of the thin sections (5 µm) was followed by staining with hematoxylin and eosin (H&E) to enable distinguishing between the cytoplasm (purple) and the nuclei (blue).

Histological analysis was performed under a light microscope at a magnification of 400× by two trained and independent pathologist observers who were unaware of the identities of the treatment groups (blind assessment). Inflammation, hyperplasia, and hyperemia were evaluated in four random sections from each gland and scored using Nickel's method[18].

Statistical analysis

The analysis of descriptive data was performed with the help of SPSS software (Version 25). In the datasets that followed a normal distribution, one-way analysis of variance (ANOVA) was performed with a supplement of the honestly significant difference procedure by Tukey. On the other hand, non-normally distributed data were tested by Kruskal-Wallis test with Mann-Whitney U post hoc analysis. A p-value of less than 0.05 was deemed statistically significant.

Results

Particle size of zeolite

The particle size distribution of the sample exhibited two distinct peaks. The primary peak, with a mode of 495.2 nm, accounted for 90.42% of the intensity, indicating that most particles cluster around this size. A smaller secondary peak appeared at 107.68 nm, representing 9.58% of the intensity and suggesting a minority population of true nanoparticles. The distribution indices showed that 10% of particles (Di10%) were smaller than 375.23 nm, the median particle diameter (Di50%) was 495.2 nm, and 90% of particles (Di90%) were smaller than 568.88 nm, reflecting a relatively narrow size range. The average intensity-weighted diameter was 459.73 nm, aligning with the dominant peak. These findings imply a concentrated particle size distribution with a small nanoparticle fraction that could affect the material's properties. Given their size, most zeolite particles are unlikely to cross the intestinal barrier and will remain within the gastrointestinal tract, whereas the ~108 nm fraction falls within the absorption window for potential intestinal uptake (Figure 1).

PSA assessment

The findings of the present study indicated that the IP group exhibited the highest PSA level (0.086 ± 0.05), whereas the ZEO-Oral group had the lowest level (0.065 ± 0.05). Nonetheless, no statistically significant difference was identified among the groups ($p > 0.05$) (Figure 2A).

IL-6 assessment

The IP group had the highest level of IL-6 (109.82 ± 46.62), and the ZEO-Oral group had the lowest (70.08 ± 23.77). No statistically significant differences were found between the groups ($p > 0.05$) (Figure 2B).

IL-17 assessment

The IP group exhibited the highest levels of IL-17 (60.41 ± 14.39), whereas the Cernilton group showed the lowest (48.54 ± 31.75). Both forms of ZEO (oral and injectable) led to a decrease in IL-17 levels compared to the IP group. Nevertheless, the reduction was more pronounced in the Cernilton group. No statistically significant differences were found among the groups ($p > 0.05$). Oral ZEO demonstrated greater effectiveness than its injectable counterpart (Figure 2C).

Histopathological assessment

Histopathological scoring revealed that the IP group exhibited the highest severity of inflammation (mean rank 3.25, $p = 0.0024$), hyperemia (mean rank 3.0, $p = 0.0022$), and hyperplasia (mean rank 3.5, $p = 0.0034$) compared to controls. The ZEO-IP and Cernilton groups showed the lowest inflammation ranks (2.25, $p = 0.011$). Meanwhile, the ZEO-IP group had the lowest hyperemia rank (1.25, $p = 0.034$), and both the ZEO-IP and Cernilton groups had the lowest hyperplasia rank (1.5, $p = 0.013$) (Table 1). Examination of the microscopic slides revealed structural improvement in prostate tissue in the treatment groups, indicating a positive effect of the treatments on prostate tissue condition (Figure 3). The microscopic appearance of IP-related complications (inflammation, hyperplasia, and hyperemia) in prostate tissue is shown in Figure 4.

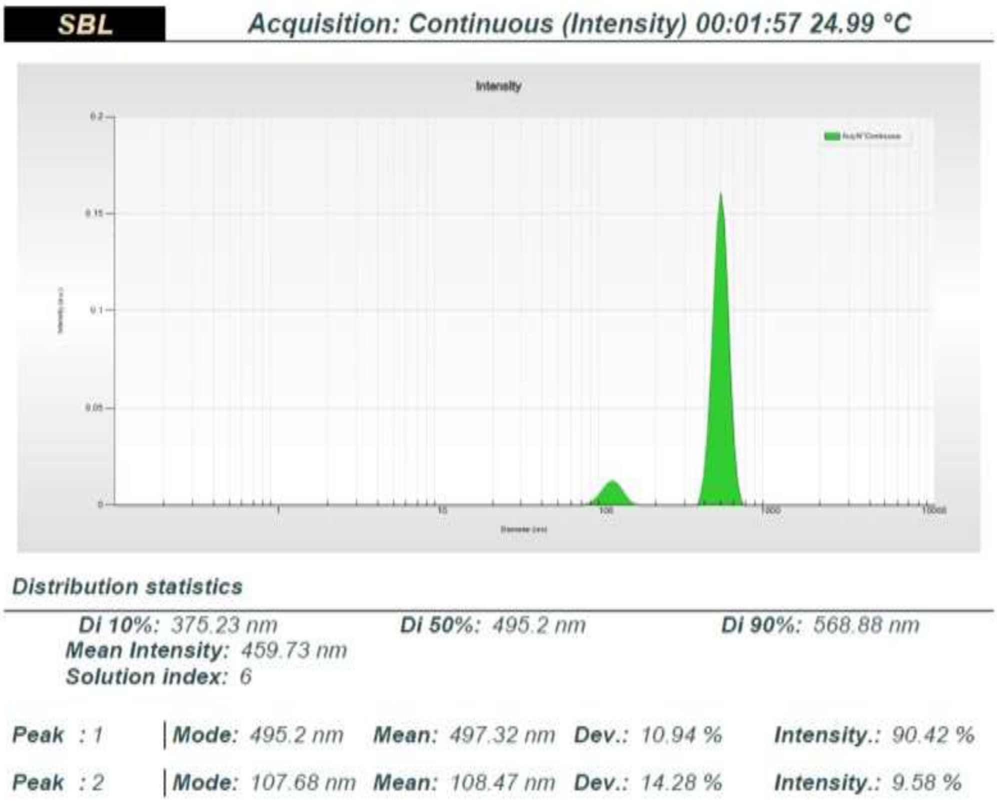

Figure 1. Dynamic light scattering (DLS) analysis

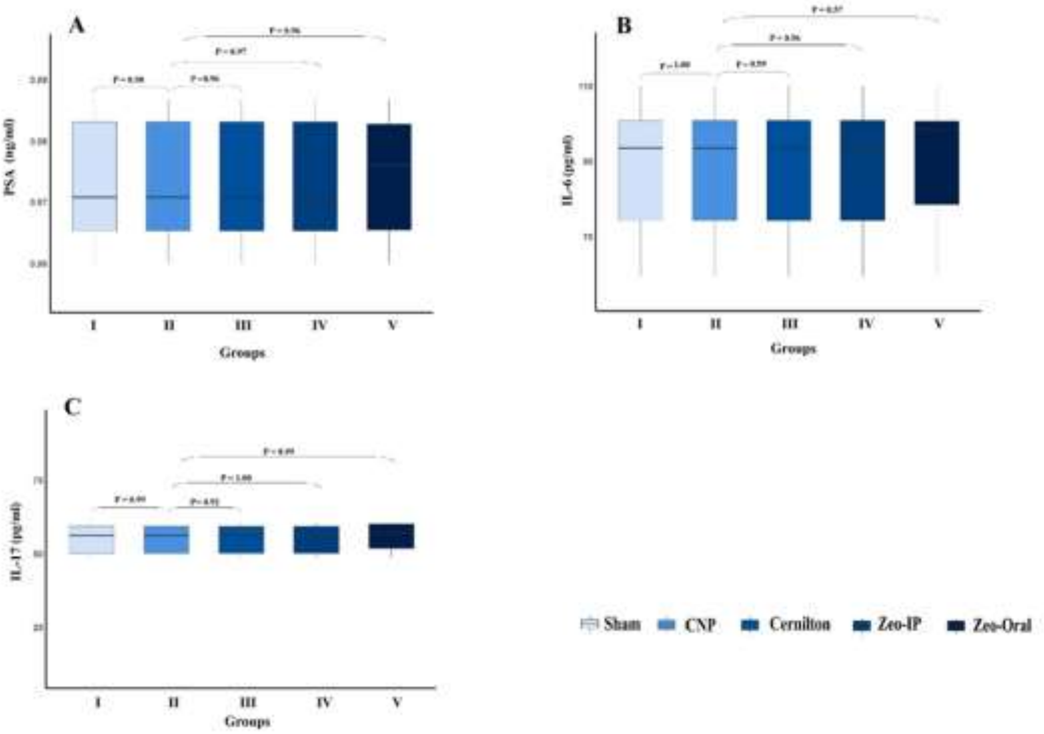

Figure 2. Box plots illustrating PSA (A), IL-6 (B), and IL-17 (C) levels in the studied group; I: Sham, II: IP, III: Cernilton, IV: ZEO-IP, V: ZEO-Oral. Similar letters denote the absence of a significant difference. The X-axis represents the studied groups, and the Y-axis represents (A): PSA (ng/ml), (B): IL-6 (pg/ml), and (C): IL-17 (pg/ml) levels.

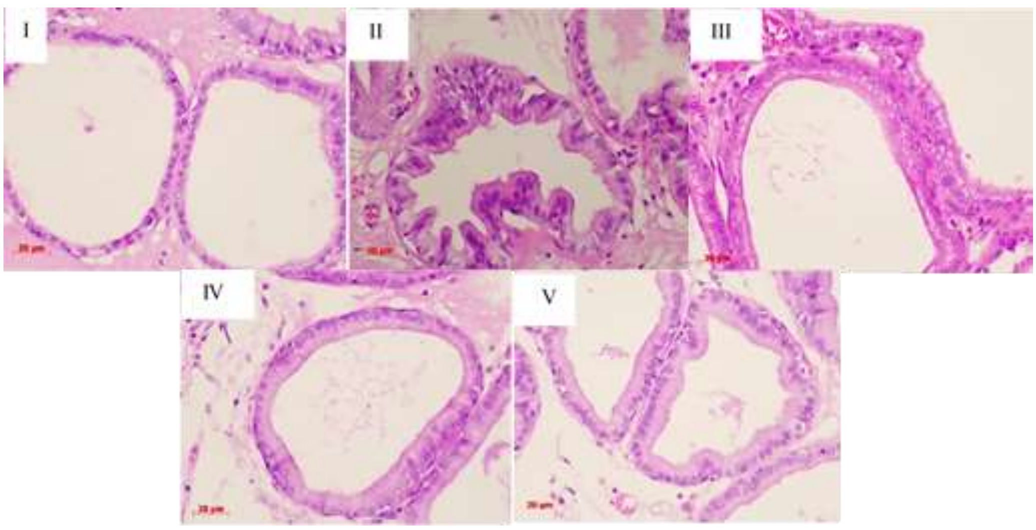

Figure 3. The comparison of the histopathology of IP effects on prostate tissue in the studied groups. I: Sham, II: IP, III: Cernilton, IV: ZEO-IP, V: ZEO-Oral (Hematoxylin-eosin staining, magnification: 400x).

Figure 4. Microscopic appearance of chronic prostatitis effects on prostate tissue, Inflammatory cells, Hyperplasia, and Hyperemia (H&E staining, magnification: 400x).

Table 1. Comparison of tissue changes in the prostate among the study groups based on the Mean-Rank index

Prostate tissue changes	Sham	IP	Cernilton	ZEO-IP	ZEO-Oral
Inflammation	2.25	3.5	1.5	1.5	1.5
Hyperplasia	2	3.5	1.5	1.5	1.75
Hyperemia	1.75	3	1.75	1.25	1.25

Discussion

In this study, carrageenan induced Inflammatory Prostatitisin 30 male Sprague Dawley rats, which were then treated with Cernilton or natural nano zeolite (ZEO) via injection or orally. Treatment with ZEO demonstrated a trend towards reduced levels of PSA, IL-6, and IL-17 compared to untreated IP rats; however, these differences were not statistically significant and should be regarded as preliminary findings. Histopathological results indicated that ZEO may play a role in restoring the architecture of prostate tissue and reducing inflammation, hyperplasia, and hyperemia, such that these changes were qualitatively similar to those observed in the control group .

DLS showed a bimodal, intensity-weighted distribution with a dominant peak centered at ≈495 nm (≈90% of measured intensity) and a smaller peak at ≈108 nm (≈9.6% intensity). We acknowledge that particles in the ~400–500 nm range are generally too large for efficient trans-epithelial uptake in the intestine and therefore systemic bioavailability of the bulk material is likely limited. Importantly, intensity-weighted DLS preferentially emphasizes larger scatterers, so the actual number-based fraction of sub-200 nm particles may be greater than the intensity percentages reported here. Even so, several non-mutually-exclusive explanations can account for the observed treatment effects despite limited systemic uptake: (i) the small nanoparticle fraction (~100 nm) may be preferentially absorbed (via Peyer's patches/M-cells or endocytosis) and deliver biologically active material systemically; (ii) orally administered zeolite may act locally in the gastrointestinal tract (adsorbing toxins, modulating luminal oxidative stress, or altering the microbiome), with downstream immunomodulatory effects on distant organs including the prostate; and (iii) zeolite may release soluble ions or low-molecular-weight species that are bioavailable and contribute to antioxidant or anti-inflammatory actions. We therefore interpret the present findings as preliminary and exploratory. While histopathology suggests tissue-level improvement, the lack of statistically significant changes in circulating PSA, IL-6, and IL-17, together with the measured size distribution, indicates that further pharmacokinetic and biodistribution studies are required. Prostatitis refers to the increased number of inflammatory cells within the prostate gland. Suppose the infectious agent of acute prostatitis is not entirely eradicated. In that case, the inflammation may persist chronically, producing excessive pro-inflammatory cytokines and reactive oxygen and nitrogen species. OS is one of the key mechanisms in the development of chronic prostate diseases. Therefore, antioxidants may be vital in treating bacterial and non-bacterial prostatitis. Furthermore, due to the anti-inflammatory properties of antioxidants, these compounds may help prevent the progression of inflammation and reduce the risk of prostate cancer [19].

In a study examining the effects of metformin on prostate treatment, it was reported that the PSA levels in patients with prostatitis increased, and the use of metformin was able to reduce PSA levels by 20% [20]. In a study examining the effect of Pentoxifylline on IP rats, it was reported that carrageenan injection increased PSA levels. At the same time, the administration of Pentoxifylline led to a decrease in PSA levels in IP rats. Additionally, it was reported that PSA is one of the crucial markers for assessing prostatic hyperplasia, which is associated with the severity of prostatic inflammation. Oral administration of Cernilton and Pentoxifylline significantly reduced PSA levels compared to untreated- IP rats [21]. In the current study, the consumption of ZEO resulted in a relative decrease in PSA; however, this change was not statistically significant. Therefore, this finding should be interpreted with caution and requires further investigation in studies with higher statistical power.

In this study, ZEO intake led to a slight decrease in PSA, but the change wasn't statistically significant and should be viewed cautiously, as it needs to be confirmed in larger studies with stronger statistical power.

In patients with IP, inflammatory cytokines such as TNF-α and IL-17 are expressed [8,22]. Elevated IL-6 has been reported in severe infectious diseases such as sepsis and viral infections [23,24]. Increased inflammation, along with elevated levels of OS and ROS, has also been observed in patients with prostatitis [6]. The innate immune system responds to infections by recognizing pathogens through pattern recognition receptors, which detect bacterial cell wall components and trigger the secretion of cytokines. This process attracts immune cells to the site of infection, where neutrophils accumulate first, followed by lymphocytes and macrophages. The activation of the immune system triggers a respiratory burst that generates reactive oxygen species (ROS), which are crucial in causing inflammation and tissue injury 19. OS creates a positive feedback loop between inflammation and ROS by activating immune cells and stimulating pro-inflammatory cytokines such as IL-6 and TNF-α. This process leads to the persistence of chronic inflammation and tissue damage in various organs [25]. Free radicals can destroy prostate tissue by damaging DNA, proteins, and cell membranes, further activating inflammatory pathways and exacerbating inflammation 3. In the current study, the level of IL-17 and IL-6 in IP rats has increased, which may be due to inflammation caused by the prostate. Treatment with ZEO showed a tendency to reduce these indices; however, these effects were not statistically confirmed and are therefore reported as potential trends. A study examining the effect of ZEO on ROS removal under laboratory conditions reported that ZEOs can effectively prevent damage caused by OS, suggesting potential benefits for extracorporeal medical applications such as heart and lung transplantation or hemodialysis [14]. A study has examined ZEO's role in managing inflammatory bowel disease and reported that consuming ZEO may improve inflammatory markers in Crohn's disease [15]. Alzheimer's and Parkinson's disease have both been linked to oxidative damage, higher levels of lipid peroxidation, and reduced activity of key antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, along with lower glutathione levels. Research in animal models and humans has demonstrated that ZEOs function as potent antioxidants, neutralizing free radicals, enhancing antioxidant enzyme activity, and mitigating oxidative stress in these conditions [11]. ZEO has been recognized as the most effective substance for absorbing reactive ROS. In a laboratory model designed to simulate ROS production in mitochondria, treatment with ZEO significantly reduced mitochondrial ROS and decreased cell death [26]. In animal studies, ZEO has been shown to increase the activity of hepatic antioxidant enzymes, including catalase, glutathione peroxidase, and superoxide dismutase, enhance overall antioxidant capacity, and reduce levels of malondialdehyde as well as decrease the activity of nitric oxide synthase [27]. The exact mechanism of ZEOs' antioxidant effect is not yet fully understood. However, in addition to directly trapping ROS, ZEOs may contribute to this effect by releasing metal ions in their structure, which act as cofactors in activating the body's antioxidant enzymes [9]. In the present study, ZEO has demonstrated antioxidant and anti-inflammatory properties, successfully reducing the levels of IL-6 and IL-17, alleviating the severity of inflammation, and improving immune system balance.

In the histopathological results of prostate tissue, increased inflammation, hyperplasia, and hyperemia were observed in IP rats. Apoptosis and inflammation play significant roles in regulating cell growth and maintaining tissue balance, and disruptions in the apoptotic machinery are associated with benign prostatic hyperplasia [28]. Studies on molecules and cellular structures in the prostate have indicated that IL-6 is involved in benign prostatic hyperplasia. This protein is implicated in the regulation of epithelial cell growth. Specifically, this protein acts in two ways: paracrine, meaning that surrounding cells can produce IL-6 and influence the epithelial cells through it, and autocrine, where epithelial cells produce IL-6 themselves, and this protein affects the same cells. Overall, this regulatory cycle can lead to abnormal growth and an increase in the number of epithelial cells in the prostate, contributing to the development of benign prostatic hyperplasia [7]. Prostate inflammation is frequently observed in patients with benign prostatic hyperplasia, possibly due to cytokine secretion from inflammatory cells. ROS, generally produced by inflammatory cells due to internal or external injury, may play an essential role in benign prostatic hyperplasia [29]. Research shows that ongoing inflammation can promote growth processes and post-translational modifications in prostate tissue due to oxidative stress [30]. In simpler terms, continuous damage to the tissue and the oxidative stress that accompanies it may trigger compensatory cell growth, heightening the chances of hyperplasia or potentially neoplastic alterations [31]. The administration of pentoxifylline reduced hyperplasia and inflammation, resulting in the prostate tissue of rats affected by IP resembling that of healthy rats [21]. The results of the present study are consistent with those of previous studies. In the present study, ZEO's antioxidant and anti-inflammatory properties reduced inflammatory cytokine levels, decreasing inflammation, hyperplasia, and hyperemia in IP rats. The results indicated that Oral ZEO absorption performed better than IP ZEO. This result may be due to the more excellent absorption of ZEO through the intestine and its entry into the bloodstream, leading to enhanced anti-inflammatory and antioxidant effects of ZEO in IP. Therefore, the oral administration of ZEO demonstrated its therapeutic properties more effectively than the injection method. A comparison of the routes of administration indicated that the oral absorption of ZEO appears to be more effective than intraperitoneal injection, likely due to better absorption from the gastrointestinal tract. However, only a small percentage of ZEO entered the bloodstream, which may explain the lack of statistically significant differences. If the ZEO used is completely nanostructured (100%), a higher percentage will likely be absorbed, resulting in more pronounced anti-inflammatory and antioxidant effects.

Although the results of this study in the IP animal model show promising potential, the translation of these findings to humans requires more thorough examination and further research. Therefore, it is recommended that future studies be conducted with larger samples, various doses, and the use of 100% nanostructured particles to evaluate the effects of ZEO more accurately. The first step in initiating clinical trials is to determine the human-equivalent dose based on animal data, taking into account pharmacological differences such as absorption and metabolism. Additionally, a comprehensive understanding of the safety and toxicity profile of nanozeolite in humans is crucial. Although ZEO is generally recognized as a safe substance and has been used in some food and pharmaceutical products, a thorough assessment of potential side effects at high doses and its long-term effects on various organs and systems is essential. From a regulatory perspective, any use of nanozeolite as a complementary treatment or new drug requires approval from regulatory agencies such as the Iranian Food and Drug Administration (IR.FDA), which reviews comprehensive preclinical and clinical data to ensure safety and efficacy before granting authorization. Therefore, future research should focus on completing these evaluations to facilitate the translation of promising preclinical results into practical clinical applications for patients with IP.

Thank you for providing the original version with the superscript reference style (e.g., cancer 19, by 20% 20, disease15, conditions11). I understand now that you want the English edition to preserve this exact formatting.

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In this study, carrageenan induced inflammatory prostatitis (IP) in 30 male Sprague-Dawley rats, which were then treated with Cernilton or natural nano-zeolite (ZEO) via injection or orally. Treatment with ZEO demonstrated a trend towards reduced levels of PSA, IL-6, and IL-17 compared to untreated IP rats; however, these differences were not statistically significant and should be regarded as preliminary findings. Histopathological results indicated that ZEO may play a role in restoring the architecture of prostate tissue and reducing inflammation, hyperplasia, and hyperemia, such that these changes were qualitatively similar to those observed in the control group.

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In a study examining the effects of metformin on prostate treatment, it was reported that PSA levels in patients with prostatitis increased, and the use of metformin was able to reduce PSA levels by 20% 20. In a study examining the effect of pentoxifylline on IP rats, it was reported that carrageenan injection increased PSA levels, while the administration of pentoxifylline led to a decrease in PSA levels in IP rats. Additionally, it was reported that PSA is one of the crucial markers for assessing prostatic hyperplasia, which is associated with the severity of prostatic inflammation. Oral administration of Cernilton and pentoxifylline significantly reduced PSA levels compared to untreated IP rats 21. In the current study, ZEO consumption resulted in a relative decrease in PSA; however, this change was not statistically significant. Therefore, this finding should be interpreted with caution and requires further investigation in studies with higher statistical power.

In patients with IP, inflammatory cytokines such as TNF-α and IL-17 are expressed [8,22]. Elevated IL-6 has been reported in severe infectious diseases such as sepsis and viral infections [23,24]. Increased inflammation, along with elevated levels of OS and ROS, has also been observed in patients with prostatitis 6. The innate immune system responds to infections by recognizing pathogens through pattern recognition receptors, which detect bacterial cell wall components and trigger the secretion of cytokines. This process attracts immune cells to the site of infection, where neutrophils accumulate first, followed by lymphocytes and macrophages. Activation of the immune system triggers a respiratory burst that generates reactive oxygen species (ROS), which are crucial in causing inflammation and tissue injury [19]. OS creates a positive feedback loop between inflammation and ROS by activating immune cells and stimulating pro-inflammatory cytokines such as IL-6 and TNF-α. This process leads to the persistence of chronic inflammation and tissue damage in various organs [25]. Free radicals can destroy prostate tissue by damaging DNA, proteins, and cell membranes, further activating inflammatory pathways and exacerbating inflammation [3]. In the current study, IL-17 and IL-6 levels in IP rats increased, which may be due to inflammation caused by the prostate. Treatment with ZEO showed a tendency to reduce these indices; however, these effects were not statistically confirmed and are therefore reported as potential trends.

A study examining the effect of ZEO on ROS removal under laboratory conditions reported that zeolites can effectively prevent damage caused by OS, suggesting potential benefits for extracorporeal medical applications such as heart and lung transplantation or hemodialysis [14]. A study has examined ZEO's role in managing inflammatory bowel disease and reported that consuming ZEO may improve inflammatory markers in Crohn's disease [15]. Alzheimer's and Parkinson's disease have both been linked to oxidative damage, higher levels of lipid peroxidation, and reduced activity of key antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, along with lower glutathione levels. Research in animal models and humans has demonstrated that zeolites function as potent antioxidants, neutralizing free radicals, enhancing antioxidant enzyme activity, and mitigating oxidative stress in these conditions [11]. ZEO has been recognized as an effective substance for absorbing reactive ROS. In a laboratory model designed to simulate ROS production in mitochondria, treatment with ZEO significantly reduced mitochondrial ROS and decreased cell death 26. In animal studies, ZEO has been shown to increase the activity of hepatic antioxidant enzymes, including catalase, glutathione peroxidase, and superoxide dismutase, enhance overall antioxidant capacity, and reduce levels of malondialdehyde as well as decrease the activity of nitric oxide synthase [27]. The exact mechanism of ZEO's antioxidant effect is not yet fully understood. However, in addition to directly trapping ROS, ZEO may contribute to this effect by releasing metal ions from its structure, which act as cofactors in activating the body's antioxidant enzymes [9]. In the present study, ZEO demonstrated antioxidant and anti-inflammatory properties, successfully reducing IL-6 and IL-17 levels, alleviating the severity of inflammation, and improving immune system balance.

In the histopathological results of prostate tissue, increased inflammation, hyperplasia, and hyperemia were observed in IP rats. Apoptosis and inflammation play significant roles in regulating cell growth and maintaining tissue balance, and disruptions in the apoptotic machinery are associated with benign prostatic hyperplasia [28]. Studies on molecules and cellular structures in the prostate have indicated that IL-6 is involved in benign prostatic hyperplasia. This protein is implicated in the regulation of epithelial cell growth. Specifically, IL-6 acts in two ways: paracrine (surrounding cells produce IL-6 and influence epithelial cells) and autocrine (epithelial cells produce IL-6 themselves, and this protein affects the same cells). Overall, this regulatory cycle can lead to abnormal growth and an increase in the number of epithelial cells in the prostate, contributing to the development of benign prostatic hyperplasia [7]. Prostate inflammation is frequently observed in patients with benign prostatic hyperplasia, possibly due to cytokine secretion from inflammatory cells. ROS, generally produced by inflammatory cells due to internal or external injury, may play an essential role in benign prostatic hyperplasia [29]. Research shows that ongoing inflammation can promote growth processes and post-translational modifications in prostate tissue due to oxidative stress [30]. In simpler terms, continuous tissue damage and the accompanying oxidative stress may trigger compensatory cell growth, increasing the chances of hyperplasia or potentially neoplastic alterations [31]. The administration of pentoxifylline reduced hyperplasia and inflammation, resulting in the prostate tissue of IP-affected rats resembling that of healthy rats [21]. The results of the present study are consistent with those of previous studies. In the present study, ZEO's antioxidant and anti-inflammatory properties reduced inflammatory cytokine levels, decreasing inflammation, hyperplasia, and hyperemia in IP rats.

The results indicated that oral ZEO administration performed better than intraperitoneal (IP) ZEO injection. This outcome may be due to the superior absorption of ZEO through the intestine and its entry into the bloodstream, leading to enhanced anti-inflammatory and antioxidant effects of ZEO in IP. Therefore, oral administration of ZEO demonstrated its therapeutic properties more effectively than the injection method. A comparison of the routes of administration indicated that oral absorption of ZEO appears to be more effective than intraperitoneal injection, likely due to better absorption from the gastrointestinal tract. However, only a small percentage of ZEO entered the bloodstream, which may explain the lack of statistically significant differences. If the ZEO used were completely nanostructured (100%), a higher percentage would likely be absorbed, resulting in more pronounced anti-inflammatory and antioxidant effects.

Although the results of this study in the IP animal model show promising potential, the translation of these findings to humans requires more thorough examination and further research. Therefore, it is recommended that future studies be conducted with larger samples, various doses, and the use of 100% nanostructured particles to evaluate the effects of ZEO more accurately. The first step in initiating clinical trials is to determine the human-equivalent dose based on animal data, taking into account pharmacological differences such as absorption and metabolism. Additionally, a comprehensive understanding of the safety and toxicity profile of nano-zeolite in humans is crucial. Although ZEO is generally recognized as a safe substance and has been used in some food and pharmaceutical products, a thorough assessment of potential side effects at high doses and its long-term effects on various organs and systems is essential. From a regulatory perspective, any use of nano-zeolite as a complementary treatment or new drug requires approval from regulatory agencies such as the Iranian Food and Drug Administration (IR.FDA), which reviews comprehensive preclinical and clinical data to ensure safety and efficacy before granting authorization. Therefore, future research should focus on completing these evaluations to facilitate the translation of promising preclinical results into practical clinical applications for patients with IP.

Conclusion

For the first time, our study demonstrated that natural ZEO may have anti-inflammatory and antioxidant effects on IP. We found that ZEO improves the tissue structure of the prostate and reduces inflammation, hyperplasia, and hyperemia by decreasing OS and regulating levels of inflammatory cytokines such as IL-6 and IL-17. Ultimately, this study indicates that ZEO may reduce tissue damage caused by chronic inflammation. However, these findings are preliminary, and further research is essential to confirm these effects. Future studies are recommended to involve larger sample sizes, varied dosages, and the use of 100% nanoscale particles to better understand the therapeutic potential and mechanisms of ZEO. Additionally, translating these promising preclinical results into clinical applications will require more thorough investigations, including pharmacokinetic studies, safety assessments, and obtaining regulatory approvals. Given its proven anti-inflammatory and antioxidant properties, ZEO has the potential to be used as a complementary or alternative treatment for patients with IP. Continued research in this area may lead to the development of new and effective therapies for chronic prostatitis inflammation.

Acknowledgments

The authors thank all participants for their cooperation and sample contribution. Semnan University, Semnan, Iran, supported this research.

Conflict of Interest Disclosures

The authors declare no conflict of interest.

Funding Sources

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' Contributions

N.D. methodology, data curation, H.R.M. conceptualization, methodology, project administration, formal analysis, H.S. supervision, H.T. methodology, study consultation, M.A.H. conceptualization, project administration. All Authors write, edit, read, and approve the final manuscript.

Ethical Statement

All protocols of this study were conducted by ethical principles approved by the Semnan Veterinary College Ethics Committee (IR.SU.REC.1403.03).

Declaration of Generative AI and AI-assisted technologies

During the preparation of this work the authors used Chatgpt in order to writing the manuscript. After using this service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the published article.

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