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Oxidative Stress Markers, Antioxidant Balance, and Protein Metabolism in Dogs with Acute Prostatitis

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ABSTRACT

The prostate gland in dogs is highly vulnerable to the action of negative pathogens due to its structure and topography. Among the numerous etiological factors in the development of prostatitis, inflammatory processes and oxidative stress play a predominant role, regardless of whether the condition is bacterial, viral, or autoimmune in origin. This study aimed to assess protein metabolism and redox balance indicators in the prostate tissue of dogs with acute prostatitis. For biochemical analyses, prostate tissue samples were taken from 24 mixed-breed dogs, including twelve animals that were considered healthy with no abnormalities of the genitourinary system (control group) and twelve animals with newly diagnosed acute prostatitis, from which samples were obtained via biopsy (experimental group). Following homogenization and sample preparation, all biochemical parameters in the prostate tissue were determined spectrophotometrically. The results of biochemical studies in dogs with acute prostatitis demonstrated a significant increase in the content of thiobarbiturate acid-reactive compounds by 102.2% and the level of lipid hydroperoxides by 35.7% compared to healthy dogs in the control group. In contrast, the total protein content was 32.9% lower than in the control group, while reduced glutathione levels decreased by 76.5%. Similar changes to the dynamics of oxidative stress markers were indicated by the activity of antioxidant enzymes, with glutathione peroxidase and catalase activities increasing by 61.3% and 21.8%, respectively, relative to the control group. These findings indicate the presence of oxidative stress in dogs with acute prostatitis. The biochemical changes observed in prostate tissue provide a foundation for future research aimed at developing therapeutic methods that incorporate anti-inflammatory, antibacterial, and antioxidant agents for the treatment of acute prostatitis in dogs.

Keywords: Biochemistry, Dog, Inflammation, Oxidative stress, Prostate

INTRODUCTION

The prostate gland is a large and unpaired organ composed of glandular tissue, smooth muscles, and connective tissue, functioning as an accessory sex gland (Krakowski et al., 2022; Zhao et al., 2023). The function of the prostate is primarily secretory, allowing it to synthesize plasma, which is a component of sperm and has a trophic and activating effect on male germ cells (Lea et al., 2022; Zhao et al., 2024). More importantly, the prostate is a gland that depends on hormone levels, such as dihydrotestosterone, and its anatomical location makes it easily exposed to pathogenic factors such as bacteria, viruses, and hypothermia, ultimately leading to prostatitis (Feng et al., 2021; Chen et al., 2024). Prostate epithelial cells are extremely vulnerable to external and internal stimuli that can induce DNA damage, cell pyroptosis, and abnormal proliferation (Mo et al., 2024; Wang et al., 2024). The etiology and pathogenesis of prostatitis are complex, involving infectious factors, autoimmune mechanisms, and endocrine imbalances. However, the manifestation of the disease is mainly accompanied by an inflammatory reaction, which contributes to the progression of the disease (Zhao et al., 2020; Ye et al., 2024). In males with prostatitis, reproductive disorders are mainly attributed to oxidative stress, which arises from the excessive formation of reactive oxygen species (ROS), resulting in damage to DNA, the lipid layer of the plasmalemma, and proteins in sperm cells (Ihsan et al., 2018; Koshevoy et al., 2021; Zhang et al., 2024a).

Experimental models of immune-mediated prostatitis and prostatitis associated with dietary or hormonal disruption in laboratory rodents (mice, rats) typically induce dorsolateral or ventral prostate inflammation (He et al., 2023). Typical changes in mice with simulated prostatitis included pronounced diffuse infiltration of leukocytes, increased levels of pro-inflammatory cytokines, and lipoperoxidation products (Chen et al., 2021). Development of oxidative stress was

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observed in mice with a model of prostatitis there was an increase in malondialdehyde (MDA) levels in prostate tissues, and a decrease in the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Fu et al., 2020).

Research on proteins and their encoding genes has facilitated the development of novel therapeutic strategies for prostatitis. The response of the immune system to self-antigens is a factor that contributes to the development of chronic prostatitis in both men and rodents, making it worth studying the etiological factors, immunological mechanisms, and molecular and biochemical changes in depth to develop pathogenetic therapies (Manuel and Vezina, 2024). Metabolomic analyses allow the assessment of peripheral blood metabolites that may reflect stable changes in the internal environment affected by diseases, among which proteins, nucleic acids, and amino acids are important (Meng et al., 2022). Thus, in a mouse model, inhibition of chemokine receptor type 4 (CXCR4) was shown to effectively alleviate prostatitis by reducing inflammatory infiltration, lowering the level of markers of DNA damage as well as toxic malondialdehyde (MDA), and mitigating apoptosis of prostate epithelial cells (Zhang et al., 2024b). According to Hua et al. (2024), sodium butyrate alleviates experimental autoimmune prostatitis in mice by inhibiting oxidative stress and suppressing the activation of the family pyrin domain-containing 3 (NLRP3) inflammasome.

The effects of antioxidant therapy using a resveratrol derivative in combination with shock wave therapy be positive for nonbacterial prostatitis in rats by Song et al. (2023), who observed relief of inflammation, fibrosis, and pro-inflammatory genes. The course of nonbacterial prostatitis in rats can be alleviated by administering plant polysaccharides that regulate the level of inducible nitric oxide synthase, MDA, and SOD in the inflamed prostate, thereby enhancing the activity of antioxidant defense (Liu et al., 2020). To find new and accurate biomarkers related to prostate inflammation, including acute and chronic prostatitis, benign prostatic hyperplasia, and cancer, ongoing research seeks to improve diagnosis and evaluate the effectiveness of treatment for prostate diseases (prostatitis, hyperplasia, etc), which are characterized by common pathogenetic links and macroscopic changes (Savoca et al., 2019; Bosma et al., 2022). Therefore, the present study aimed to determine changes in redox processes and protein content in the prostate tissue of dogs with acute prostatitis.

MATERIALS AND METHODS

Ethical approval

Experimental studies to investigate the role of oxidative stress in prostatitis in dogs were reviewed and approved by the Bioethics Committee of the State Biotechnological University, Ukraine (ethical approval No. 8-10, dated October 8, 2023). All manipulations with male dogs were carried out in accordance with the provisions set out in the General Ethical Principles of Animal Experiments (National Congress on Bioethics, Kyiv, Ukraine, 2001) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (2006).

Animal groups and their characteristics

The study included 24 dogs aged 7-9 years with a live weight of 15-20 kg. The animals in both control and experimental groups included mixed-breed dogs that had been referred to the veterinary clinic of LLC *Vetexpert* (Kharkiv, Ukraine). Animals in the experimental group (n = 12) were diagnosed with acute prostatitis based on clinical examination, anamnesis, prostate ultrasound, and biochemical and hematological blood tests (Domosławska et al., 2023). The control group (n = 12) consisted of animals that, after examination by a doctor, were considered healthy with no abnormalities of the genitourinary system.

Prostate tissue sampling and homogenate preparation

Twelve samples of tissue biopsy specimens from dogs with newly diagnosed acute prostatitis were obtained surgically (Holak et al., 2010). Age-related conditions not known or reported to affect the genitourinary tract were not considered exclusion criteria in accordance with the guidelines (Weinekötter et al., 2023). Similarly, twelve prostate samples from healthy dogs were obtained (Holak et al., 2010). Animal prostates were used to prepare tissue homogenates (Aggarwal et al., 2006). The isolated glands were cooled on ice for 5-6 minutes, after which they were perfused with saline (0.9% NaCl solution; chilled). After that, the gland was crushed with scissors, and 1 g of tissue was taken for further homogenization. The tissue (1 g) was placed in a glass cylinder with chilled 5 mM Tris-HCl buffer, pH 7.4 (9 ml). The tissue was then homogenized for 30-40 seconds by moving the glass cylinder up and down relative to the Teflon rotor of the homogenizer (MRTU-421505-63). During the homogenization process, the glass beaker with the tissue under study was cooled from the outside using a bag filled with crushed ice. Afterward, the homogenized tissue was filtered into a centrifuge tube using a double-layer gauze. The homogenates were centrifuged for 10 minutes at 3000 rpm (t: 0 ± 2°C) to precipitate cellular stromata. The supernatant was then collected from the tubes and used for the determination of the parameters.

Determination of total protein content and oxidative stress markers

Total protein content in tissue homogenates was quantified using the Lowry Method via Simko LTD kits (Lviv, Ukraine), following the protocol described by Lowry et al. (1951). Absorbance measurements were taken with a spectrophotometer (Unico 1205, USA). The concentration of lipid hydroperoxides was determined via protein precipitation with trichloroacetic acid and lipid extraction with ethanol. Upon the addition of ammonium thiocyanate to the ethanol lipid extracts, a colorimetric reaction occurred. The absorbance of the resulting coloured product was measured using a spectrophotometer at a wavelength of 480 nm. The concentration of lipid hydroperoxides was calculated by subtracting the absorbance values of the control prostate homogenates from the experimental ones and expressed in units per 1 g of tissue (SU/g of tissue). Subsequently, the number of compounds that reacted with thiobarbituric acid was determined by the reaction of malondialdehyde with thiobarbituric acid in an acidic medium and at an elevated temperature of 100°C. This reaction led to a colour change. The absorbance of the resulting coloured product was measured using a spectrophotometer at the two wavelengths of 535 nm for lipid hydroperoxides content and 580 nm for thiobarbituric acid reactive compounds. The concentration of thiobarbituric acid reactive compounds was then calculated as MDA per gram of tissue (nmol/g tissue). All the methods for determining total protein content and oxidative stress markers were implemented as outlined in the manual by Vlizlo et al. (2012).

Evaluation of antioxidant protection indicators

The measurement of reduced glutathione (GSH) concentration is based on the formation of a thionitrophenyl anion (coloured product) when the 2-nitrobenzoic acid binds to the SH group of the GSH molecule. The GSH level was determined by the intensity of colour change. The absorbance of the coloured product was then determined spectrophotometrically at 412 nm. The GSH concentration was then determined, expressed in mmol per gram of tissue (mmol GSH/g tissue). Glutathione peroxidase (GSH-Px) activity was assessed by measuring the rate of GSH oxidation in the presence or absence of tert-butyl hydroperoxide. This method is based on the oxidation of SH groups of GSH tripeptide after adding a 2-nitrobenzoic acid to the medium. As a result of the corresponding oxidation reaction, a coloured compound (dinitrophenyl anion) was formed, the optical density of which was measured spectrophotometrically (λ=412 nm). Glutathione peroxidase activity was expressed in nmol GSH per minute per milligram of protein (nmol/min × mg prot.). Catalase activity was determined by the reaction of molybdenum salts with hydrogen peroxide, resulting in the formation of a coloured product. The optical density of the coloured product was measured spectrophotometrically at a wavelength of λ=410 nm. Catalase activity was calculated in mmol per minute per milligram of protein (mmol/min×mg prot.) All methods for determining the content of antioxidants were performed as described in the manual by Vlizlo et al. (2012).

Statistical analysis

Mathematical and statistical analyses were conducted using the Statistical Package for Social Science (SPSS), version 22. An ANOVA test was performed to compare data from control and experimental prostate samples, with normality determined using the Shapiro-Wilk test. Significant differences between groups were confirmed by the Tukey test. Statistical significance was considered to be a P-value less than 0.05.

RESULTS AND DISCUSSION

Biochemical evaluation in prostate tissue from dogs with acute prostatitis and healthy controls revealed significant alterations in markers of oxidative stress, antioxidant enzyme activity, reduced glutathione content, and total protein ($p < 0.05$). Figure 1 shows the differences in total protein content, lipid hydroperoxides, and thiobarbiturate acid-reactive compounds between the control and experimental groups.

In dogs with acute prostatitis, a decrease in total protein content in prostate tissue was by 32.9% (4.21 ± 0.17 mg/kg, $p < 0.05$) compared to control animals (6.27 ± 0.29 mg/kg). At the same time, the intensification of free radical oxidation processes of lipids and proteins in prostate tissue was determined. Whereas the level of lipid hydroperoxides in healthy dogs was 0.42 ± 0.02 SU/g in the prostate tissue, it was significantly higher by 35.7% (0.57 ± 0.02 SU/g, $p < 0.05$) in the experimental group. The concentration of thiobarbiturate acid-reactive compounds in the prostate tissue of dogs in the experimental group was 2.71 ± 0.12 nmol/g, which was 102.2% higher than in the control group ($p < 0.05$). The presence of oxidative stress in the inflamed prostate is further confirmed by the reduced antioxidant defense, as illustrated in Figure 2.

Data in Figure 2 indicate significant alterations in reduced glutathione levels and the activity of glutathione peroxidase and catalase in dogs with acute prostatitis compared to control animals. The content of glutathione in the prostate tissue of dogs in the control group was 0.17 ± 0.007 mmol/g, while it was only 0.04 ± 0.003 mmol/g in the

animals in the experimental group, which was lower by 76.5% ($p < 0.05$). Glutathione peroxidase activity in dogs with prostatitis decreased to 24.3 ± 1.2 nmol GSH/min×mg protein, which was 61.3% lower than the control values (62.8 ± 2.7 nmol GSH/min×mg protein, $p < 0.05$). While the catalase activity in healthy dogs was 234.4 ± 11.7 mmol H_2O_2/min×mg protein, it decreased to 183.3 ± 9.4 mmol H_2O_2/min×mg protein with the development of prostatitis, which is 21.8% lower than the value for the control group ($p < 0.05$).

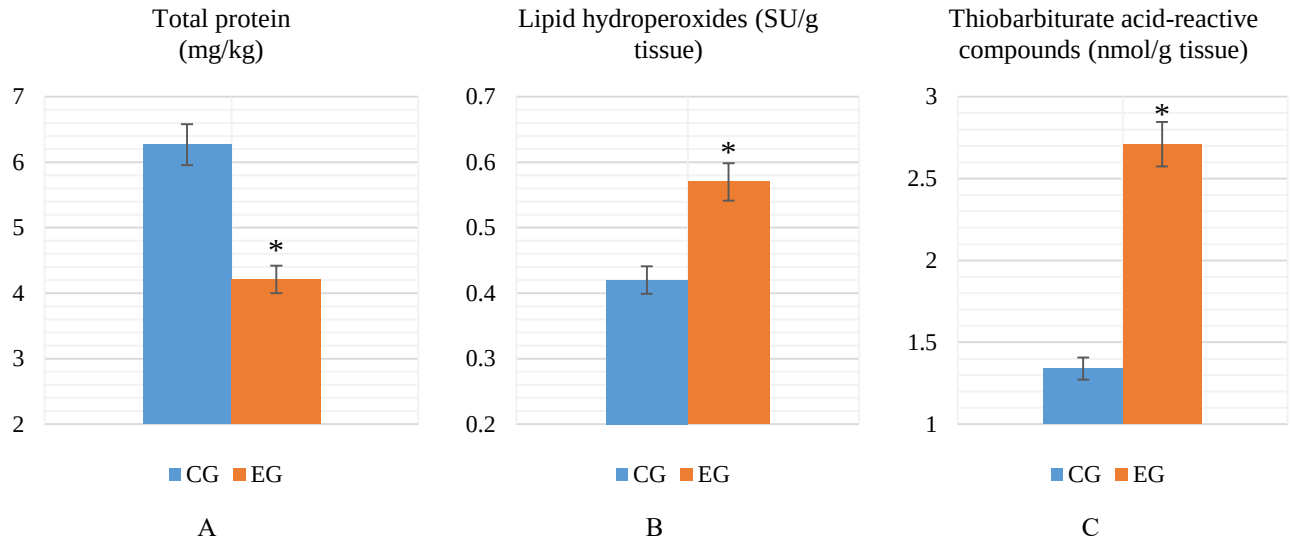

Figure 1. Total protein content (A), lipid hydroperoxide levels (B), and thiobarbiturate acid-reactive compounds (C) in prostate tissue of dogs. CG: Control group, EG: Experimental group. Significant differences ($p < 0.05$) between groups are marked with * in the figures.

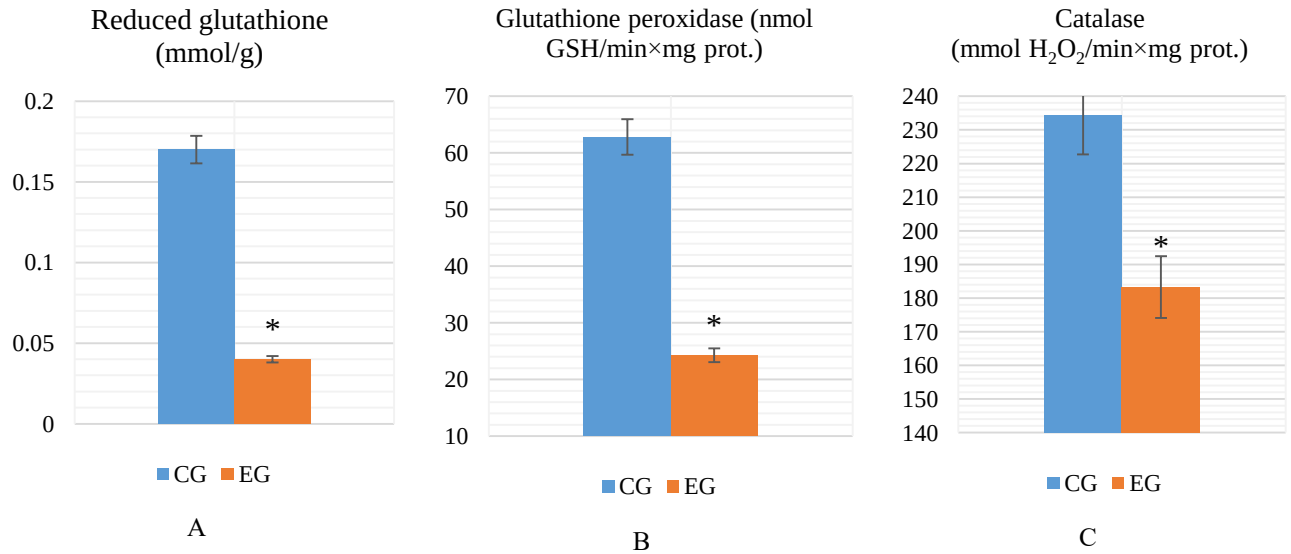

Figure 2. Reduced glutathione content (A), glutathione peroxidase activity (B), and catalase activity (C) in canine prostate tissue. CG: Control group. EG: Experimental group. Significant differences ($p < 0.05$) between groups are marked with * in the figures.

The prostate gland is the only accessory sex gland in dogs (Delaude et al., 2021; Khanbazi et al., 2021). Pathological processes in the prostate occur in older males but often remain undiagnosed, although it has been established that pathomorphological examination of the prostate in most dogs reveals morphological abnormalities with pronounced signs of inflammation (Angrimani et al., 2020; Palmieri et al., 2022). In dogs, prostatitis is a poly-etiological pathology for which there are various causes. It is accompanied by diverse clinical signs, most of which are non-specific (i.e., characteristic of several disorders of the genitourinary system) (Ryman-Tubb et al., 2022). Early diagnostic tests for disorders in the canine reproductive system, including the prostate gland, are carried out by biomarkers of reproductive function, which can be determined in the serum of male offspring, sperm, or prostate secretion, and gonadal tissues

(Koshevoy et al., 2022; Mogielnicka-Brzozowska and Cichowska, 2024). Many of these biomarkers are proteins such as albumins, clusterin, lactotransferrin, and metalloproteinases, with prostate-specific esterase being particularly important in assessing reproductive potential in male dogs (Holst et al., 2021). The present study determined a decrease in the content of total protein in prostate tissue, which confirmed the development of a pathological process. Several studies have shown that the release of proteins from the prostate is evidence of damage to this organ. These studies suggest that the presence of some proteins, particularly prostate-specific esterase, is a diagnostic tool for detecting prostate pathologies (Bucci et al., 2023; Gibson and Culp, 2024).

According to Paulis (2018), the production of ROS, mainly superoxide anion and hydroxyl radical, during acute prostatitis is aimed at destroying the bacteria that caused the infection. This is especially important for older dogs (7-10 years old) as age-related hormonal changes activate a chronic inflammatory reaction in the prostate gland, which leads to oxidative stress, DNA damage, and prostatic hyperplasia (Azadeh et al., 2022; Domosławska-Wyderska et al., 2024). The present study confirmed the development of oxidative stress in the prostate tissue of dogs during acute prostatitis. A significant increase in TBA-RC was noted in the prostate tissue of dogs in the experimental group, which is evidence of increasing free radical damage processes under acute prostate inflammation. Among the main TBA-RC metabolites, the majority consists of toxic malondialdehyde, whose accumulation in tissue indicates the destruction of prostate cell membranes and damage to lipid and protein components of the cytoskeleton (Šutulović et al., 2021; Zhang et al., 2025). The current findings revealed an increase in the content of lipid hydroperoxides in dogs with prostatitis compared to those with a healthy prostate. In general, these changes indicate the need for complex prostatitis therapy, which, along with anti-inflammatory and antibacterial effects, can neutralize toxic metabolites of oxidative stress (Park et al., 2022; Porato et al., 2023).

Aligning with the present study, Dearakhshandeh et al. (2019) reported reduced serum levels of glutathione peroxidase and superoxide dismutase (SOD) in dogs with prostatitis compared to control animals. Oxidative stress in the prostate tissue of experimental dogs developed against the background of a decrease in the activity of antioxidant defense. Thus, the content of reduced glutathione in the prostate tissue of dogs with acute prostatitis was lower than that in the control group. A substantial decrease in this compound indicates heightened oxidative stress in the prostate gland, as it represents the most active glutathione form in the animal body (Woolcock et al., 2020). Reduced glutathione content negatively affects reparative processes in the damaged prostate, as it is known to promote the induction of apoptosis and reduce ferroptosis in the hyperplastic prostate (Li et al., 2023; Zhou et al., 2024).

At the same time, a decrease in the activity of glutathione peroxidase was observed. Glutathione peroxidase is an enzyme that catalyses the reduction of lipid hydroperoxides to the corresponding alcohols and the reduction of hydrogen peroxide to water (Kendall et al., 2017). The present study also found lower catalase activity in the prostate tissue of experimental dogs compared to controls. As a result, the neutralization of hydrogen peroxide, a toxic peroxidation by-product, was significantly impaired in prostatitis-affected dogs (Aiudi et al., 2022). Current evidence suggests that catalase not only serves an antioxidant function by protecting prostate cells from damage but may also reduce apoptosis in damaged cells, thereby prolonging the recovery period, as observed in a human prostate cancer model (Giginis et al., 2023).

The findings of the present study are consistent with those of Domosławska et al. (2022), who assessed total antioxidant activity in the serum of dogs of various breeds with prostatic hyperplasia. Their study found significantly lower antioxidant levels in affected dogs than in healthy controls, with oxidative biomarkers in proteins and lipids showing a trend toward oxidative imbalance, though statistical significance was not observed. Their study also demonstrated that in prostatic hyperplasia, decreased antioxidant protection and increased oxidative modification of proteins in the prostatic fluid and semen support the pathogenetic role of oxidative stress (Domoslawska et al., 2022). The present study further confirmed the pathogenetic role of oxidative stress in acute prostatitis in dogs. Previous research by the authors of this article supported the use of redox-active nanoparticles for treating reproductive disorders in male rabbits, suggesting a potential application for dogs with prostatitis (Koshevoy et al., 2022). It is proposed that therapeutic and preventive measures for canine prostatitis should include metal nanoparticle-based treatments, which offer antibacterial, antiviral, anti-inflammatory, and antioxidant properties (Naumenko et al., 2023; Xing et al., 2024). Anti-inflammatory and antibiotic drugs, combined with phytobiotics or hormonal drugs, could enhance the effectiveness of prostate inflammation treatment and prevent reproductive complications in affected dogs (Socha et al., 2018; Clerc-Renaud et al., 2021; Tverdokhlib et al., 2024).

CONCLUSION

This study identified typical changes in biochemical processes in acute prostatitis in dogs. A significant increase in lipid hydroperoxides and thiobarbiturate acid-reactive compounds was observed in prostate tissue, alongside a decrease in the

activity of antioxidant enzymes (glutathione peroxidase and catalase), a reduction in glutathione levels, and a reduction in total protein content. These findings confirmed the key role of oxidative stress in the pathogenesis of acute canine prostatitis. The authors propose further research on changes in sperm quality in dogs with prostatitis and the development of a comprehensive treatment approach based on nanotechnology.

DECLARATIONS

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Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

Volodymyr Serhiienko, Vsevolod Koshevoy, and Svitlana Naumenko created the idea, developed the research design, and conducted the experimental part, and analysis of the results obtained, Bohdan Kotyk and Oksana Ilina participated in the preparation of prostate tissue samples, homogenization, and biochemical studies, Yuriy Shchepetilnikov and Diana Makhotina performed mathematical and statistical data processing, literature review on the research issues, while Ihor Marakhovskyi assisted in the selection of material for research, clinical examination of animals, etc. All authors took part in discussing the results and writing the article and agreed on the final version. The authors confirmed that the final edition of the manuscript of this article for publication in this journal has been reviewed and approved by all authors.

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Competing interests

All authors declare no conflict of interest regarding the publication of this manuscript.

Ethical considerations

The authors of this article, while performing the work and preparing the manuscript, complied with the requirements of current regulations to prevent ethical violations, including plagiarism, double posting, and/or submission and redundancy, fabrication, or falsification of data.

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