

## Microbiological researches of rectal suppositories for the treatment of benign diseases of the prostate gland

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### ABSTRACT

**Background and Aim:** Prostatitis is one of the most common and difficult to diagnose and treat conditions in urological practice. Nowadays, the most common types of prostatitis are bacterial and infectious, characterized by acute and chronic cyclic flow with phases of exacerbations and remissions, where particular importance is the involvement of microorganisms, fungi, and protozoa in the pathological process. For the treatment and prevention of benign prostatic diseases, we developed the rectal suppositories with indole-3-carbinol and meloxicam. The aim of this work was to conduct microbiological studies of developed suppositories. **Methods:** To study the antimicrobial action, six experimental compositions were made. As test cultures, reference strains of bacteria and fungi were used, obtained in the Ukrainian Collection of Microorganisms of the Institute of Microbiology and Virology of D.K. Zabolotny (Kyiv, Ukraine): *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, and *Candida albicans* ATCC 10231. The study of the specific antimicrobial action of the medicinal product was conducted *in vitro* by diffusion in nutrient agar in the modification of “wells.” To determine antibacterial activity, Muller-Hinton agar was used, while studying antifungal action used Saburo dextrose agar. **Results:** Studies have shown that the presence of meloxicam and excipient Montanox 80 in the composition of suppositories does not significantly affect the degree of the antibacterial and antifungal effects. Suppositories with indole-3-carbinol and the combination of indole-3-carbinol and meloxicam have a pronounced antibacterial effect in relation to Gram-positive and Gram-negative bacteria and antifungal action. **Conclusion:** The conducted microbiological research has established the possibility and feasibility of using developed rectal suppositories with indole-3-carbinol and meloxicam in the treatment of bacterial and infectious prostatitis.

**KEY WORDS:** Antifungal effect, Antimicrobial action, Bacterial and infectious prostatitis, Indole-3-carbinol, Meloxicam, Rectal suppositories

### INTRODUCTION

Diseases of the pelvic organs, including the prostate, are referred to as diseases of civilization. One of the most dangerous ailments of the stronger sex is prostatitis – the inflammation of the prostate gland. If earlier, it was believed that prostatitis is a disease for most of the older men; today, this pathology “gets younger” and it increasingly begins to appear in men under 30 years old.<sup>[1-3]</sup>

Problems with the health of men in the genitourinary area depend on many factors - it can be infection,

incorrect lifestyle, poor nutrition and ecology, and weak immunity. Additional “provocateurs” of the disease are stress, hypothermia, inactivity, and bad habits. All this leads to stagnant phenomena in the small pelvis, disruption of blood supply, and outflow of the prostate gland secretion.<sup>[4]</sup>

The trigger mechanism for the development of prostatitis is, as a rule, the microbial factor - pathogenic or conditionally pathogenic microorganisms are able to penetrate the prostate gland in various ways: Urinogenic, that is, with urine - while passing through the urethra; hematogenous, that is, with blood - in the presence of any focus of infection in the body; lymphogenous, that is, with a current of lymph, into which the microbes came from neighboring organs. In this regard, bacterial and infectious prostatitis has

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been and remains one of the most common diseases in men, taking the leading place among inflammatory diseases of the male sexual system. These types of prostatitis are more common in young men (from 20 to 40 years) and are dangerous because the process in addition to the prostate gland also covers nearby organs.<sup>[5,6]</sup>

Bacterial prostatitis is caused by various microorganisms - *Escherichia coli*, *Klebsiella*, *Enterococci*, and *Pseudomonas aeruginosa*. The role of other microorganisms - staphylococci, anaerobic bacteria, ureaplasma, chlamydia, and viruses - remains controversial. The role of Gram-positive bacteria and anaerobes is debated for several decades and still remains unclear, which is due to the complexities of the method of prostatic secretion culture test.<sup>[7,8]</sup>

According to the latest data, in 95% of cases, a mixed bacterial infection is isolated from the secretion of the prostate caused by both aerobic and anaerobic microorganisms. Anaerobes predominate in the etiologic structure of bacterial prostatitis. They are found in 100% of patients, staphylococci are isolated from the secretion of the prostate in 83% of patients, other bacteria are recorded in 71% of cases.<sup>[9-11]</sup>

Infectious prostatitis is caused by pathogenic microorganisms, most often *E. coli*. The clinical picture of infectious prostatitis is similar to the one of bacterial. The difference between infectious and bacterial prostatitis is that the first is caused by bacteria, and the development of the second (which is much less common) can be triggered by other pathogens, for example, fungi and protozoa.<sup>[4,12,13]</sup>

To date, there are many ways to treat acute and chronic forms of bacterial and infectious prostatitis. On one hand, they are aimed at the destruction of pathogenic microorganisms - the main cause of the disease. On the other hand - to restore the functions of the prostate gland.<sup>[14]</sup>

The main methods of treatment are as follows:

- Antibacterial therapy - synthetic antibacterial drugs (fluoroquinolones) and antibiotics are used. They should be prescribed by a doctor, based on the results of preliminary studies, the individual characteristics of the patient and the severity of the course of the disease;
- Anti-inflammatory and analgesic therapy - non-steroidal anti-inflammatory drugs are used, usually in the form of suppositories (Diclofenac, Efferalgan, etc.);
- Specific drug therapy - there is a number of drugs whose effect is aimed directly at suppressing the processes of inflammation and restoration of prostate functions (Vitaprost, Prostatilen, Afala, etc.);

- Phytotherapy with the use of prostatoprotective agents of plant origin (Prostamol<sup>®</sup> Uno, Prostaplant, Permixon, ProstaFit, etc.). Preparations of this group have anti-inflammatory, antiproliferative, antioxidant, and antimicrobial actions.<sup>[15,16]</sup>

However, the rapidly increasing resistance of microorganisms to antibiotics and the low effectiveness of antibiotic therapy of bacterial and infectious prostatitis dictate the need to further improve the treatment of this disease and develop new domestic medicines.

We are developing the rectal suppositories of complex action for the treatment and prevention of benign prostatic diseases. As active pharmaceutical ingredients, it has been proposed to use a combination of indole-3-carbinol and meloxicam, which possess anti-inflammatory, analgesic, antibacterial properties, and also contributes to the restoration of hormonal background in men with age-related changes and activation of regeneration of the injured organ.<sup>[17]</sup>

Considering the above, the aim of this work was to conduct microbiological studies of developed suppositories with indole-3-carbinol and meloxicam for the treatment of prostatitis and BPH.

## MATERIALS AND METHODS

In the experimental studies, as the objects were used substances of meloxicam (Boehringer Ingelheim GmbH, Germany) and indole-3-carbinol (Sigma-Aldrich Co., USA), as well as prototype samples of suppositories of the following composition:

- Sample 1 - polyethylene oxide (PEO) base - A mixture of PEO-1500 and PEO-400 (at a ratio of 95: 5); Montanox 80 (Polysorbate-80, Tween-80);
- Sample 2 - PEO-base; Montanox 80; indole-3-carbinol 0.2 g/1 suppository;
- Sample 3 - PEO-base; Montanox 80; meloxicam 0.0075 g/1 suppository;
- Sample 4 - PEO-base; Montanox 80; indole-3-carbinol (0.2 g/1 suppository); meloxicam (0.0075 g/1 suppository);
- Sample 5 - PEO-base; indole-3-carbinol (0.2 g/1 suppository);
- Sample 6 - PEO-base; indole-3-carbinol (0.2 g/1 suppository); meloxicam (0.0075 g/1 suppository).

To study the antimicrobial activity as test cultures, reference strains of bacteria and fungi were used, obtained in the Ukrainian Collection of Microorganisms of the Institute of Microbiology and Virology of D.K. Zabolotny (Kyiv, Ukraine): *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231.

Test strains of bacteria were grown at a temperature of about 35°C from 18 to 24 h on soy-casein agar. Test organism *C. albicans* was grown on Saburo-dextrose agar at a temperature of about 35°C from 24 to 48 h.

Before the development of the experiment, the purity of each culture and its typical properties were checked for morphological, cultural, and tinctorial (for bacteria) characteristics.

To prepare a suspension of microorganisms, a pharmacopoeial standard sample of turbidity (PSS SPU) of 10 IU was used.

The study of the specific antimicrobial action of the medicinal product was conducted *in vitro* by diffusion in nutrient agar in the modification of “wells” in accordance with the recommendations.<sup>[18-21]</sup>

To determine antibacterial activity, Muller-Hinton agar was used, while studying antifungal action used Saburo-dextrose agar. Thick nutrient media melted, cooled to a temperature of about 45°C and inoculated with suspensions of test microorganisms. Microbial loading was about  $1 \times 10^7$  colony forming units/ml of molten nutrient medium. By 20 ml of inoculated with microorganisms, medium was added to Petri dishes and left to solidify the medium. In a layer of nutrient agar, the wells were prepared using a sterile punch of diameter 8 mm.

Preparation of the prototype samples was carried out as indicated below. Prepared working solutions of the substances IC and M in purified water, preparation of the samples was carried out at heating in a water bath (temperature from 45°C to 50°C), using thorough shaking. The concentration of the working solutions was 1000 µg/ml.

The suppositories were chopped, transferred to a suitable sterile glassware and held in a water bath (temperature from 45°C to 50°C) to melt and then during the experiment.

Working solutions of substances and prepared samples of suppositories were injected of 0.1 ml into wells, prepared in a dense nutrient medium, using dispensers. After the samples were placed, the Petri dishes were kept at room temperature for 1 h and then placed in a thermostat and incubated for 18–24 h at a temperature of about 35°C. At the end of the incubation, the diameter of the lack of growth zone around the wells with the prototype samples was measured to an accuracy of 0.1 mm. Each sample was tested in six repetitions.

Statistical processing of the results was carried out using generally accepted methods using Student's criterion. In the paper, the probability level  $P < 0.05$  is accepted.<sup>[22]</sup>

The activity of the prototypes and the sensitivity of the microorganisms to them was evaluated according to the following criteria:<sup>[18,20,21]</sup>

- Absence of zones of growth inhibition - absence of activity and sensitivity;
- Zones diameter <15 mm - low activity and sensitivity;
- Diameter of zones from 15 mm to 25 mm - moderate activity and sensitivity;
- Zone diameter >25 mm - highly pronounced activity and sensitivity.

## RESULTS AND DISCUSSION

The results of a comparative study of the antimicrobial activity of suppository samples by the method of diffusion in agar [Table 1] in relation to the reference strains of bacteria and fungi showed that Sample 1, which includes Montanox 80 and PEO-base, does not exhibit antimicrobial action against *B. subtilis* ATCC 6633, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6538, and *C. albicans* ATCC 10231. There were small growth inhibition zones of the test microorganism *E. coli* ATCC 8739, whose diameter was  $13.27 \pm 0.36$  mm, indicating a poorly expressed antimicrobial activity against the *E. coli*.

In spite of the lack of a clear antimicrobial action of the indole-3-carbinol substance, suppositories with indole-3-carbinol (Sample 2) showed antimicrobial effect in relation to all test microorganisms that were used in experimental studies. The most pronounced activity was observed in relation to the *S. aureus* - the growth inhibition zone of *S. aureus* ATCC 6538 was  $38.60 \pm 0.99$  mm, indicating a high level of antibacterial activity. High antibacterial activity was also observed in relation to the Gram-positive *B. subtilis* ATCC 6633 - the diameter of the growth inhibition zones of this test microorganism was  $27.83 \pm 0.77$  mm. Gram-negative bacteria showed moderate sensitivity to the action of Sample 2, the diameter of the growth inhibition zones of the intestinal and synovial sticks was <20 mm and comprised for *E. coli* ATCC 8739 was  $18.52 \pm 0.77$  mm, for *P. aeruginosa* ATCC 9027 –  $16.35 \pm 0.43$  mm. In relation to the strain of the yeast-like fungus *C. albicans* ATCC 10231, Sample 2 showed a moderate antifungal effect - the diameter of the growth inhibition zones was  $22.43 \pm 0.33$  mm.

The degree of expressiveness of the antibacterial and antifungal action of the suppositories with indole-3-carbinol, which contained no Montanox 80 (Sample 5), did not significantly differ from the degree of expressiveness of the antimicrobial activity of suppositories containing Montanox 80 (Sample 2). The diameter of the growth inhibition zones of each of the test organisms *B. subtilis* ATCC 6633, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *S. aureus*

**Table 1: Antimicrobial activity of investigated samples of suppositories (according to the method of diffusion in agar)**

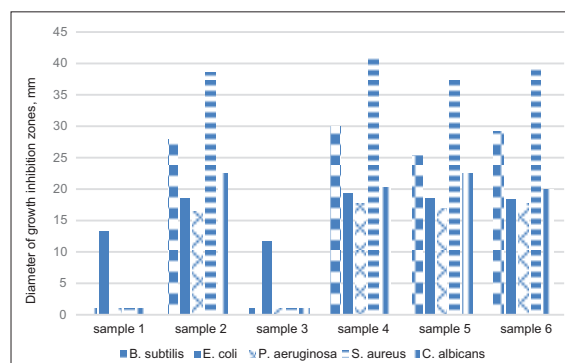
Test microorganism	The diameter of zones of growth inhibition, mm (M±m) at n=6					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
<i>B. subtilis</i> ATCC 6633	Zones are absent	27.83±0.77	Zones are absent	29.96±0.69	25.38±1.20	29.22±0.51 <sup>3</sup>
<i>E. coli</i> ATCC 8739	13.27±0.36	18.52±0.26	11.68±0.39 <sup>1</sup>	19.27±0.61	18.50±0.21	18.38±0.33
<i>P. aeruginosa</i> ATCC 9027	Zones are absent	16.35±0.43	Zones are absent	17.75±0.34 <sup>2</sup>	16.87±0.45	17.65±0.31
<i>S. aureus</i> ATCC 6538	Zones are absent	38.60±0.99	Zones are absent	40.80±0.42	37.43±0.78	39.53±0.53
<i>C. albicans</i> ATCC 10231	Zones are absent	22.43±0.33	Zones are absent	20.27±0.48 <sup>2</sup>	22.43±0.57	19.95±0.26 <sup>3</sup>

<sup>1</sup>P<0.05 in comparison with Sample number 1, <sup>2</sup>P<0.05 in comparison with Sample number 2, <sup>3</sup>P<0.05 in comparison with Sample number 5. *C. albicans*: *Candida albicans*. *S. aureus*: *Staphylococcus aureus*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *E. coli*: *Escherichia coli*, *B. subtilis*: *Bacillus subtilis*

ATCC 6538, and *C. albicans* ATCC 10231 for Sample 5 was not significantly different from the values obtained for Sample 2 and was  $25.38 \pm 1.20$  mm,  $18.50 \pm 0.21$  mm,  $16.87 \pm 0.45$  mm,  $37.43 \pm 0.78$  mm, and  $22.43 \pm 0.57$  mm, respectively.

Suppositories with meloxicam (Sample 3), as well as Sample number 1, did not show antimicrobial activity against the test microorganisms *B. subtilis* ATCC6633, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6538, and *C. albicans* ATCC 10231 - zones of growth inhibition were absent. In relation to *E. coli* ATCC 8739, as well as for Sample 1, there was a poorly expressed activity - the diameter of the growth inhibition zones was  $11.68 \pm 0.39$  mm and was significantly lower than the diameter of the growth retardation zones of the *E. coli* for Sample 1.

The antimicrobial activity of the suppository samples containing indole-3-carbinol and meloxicam was not significantly different from the activity of suppositories containing only indole-3-carbinol. It should be noted that some tendency to increase the severity of antibacterial activity in the presence of meloxicam in the suppositories was observed [Figure 1], but the difference in the diameters of growth inhibition zones was reliable only in two cases - for Samples 2 and 4 in relation to *P. aeruginosa* ATCC 9027 and for Samples 5 and 6 in relation to *B. subtilis* ATCC 6633. In contrast, the antifungal activity of the suppositories containing indole-3-carbinol and meloxicam in relation to *C. albicans* ATCC 10231 was significantly lower than that of the suppositories containing only indole-3-carbinol. There was no statistically significant difference in the diameter of the growth inhibition zones for samples containing and not containing Montanox 80. For Samples 4 and 6, the diameters of the growth inhibition zones of *B. subtilis* ATCC 6633, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6538, and *C. albicans* ATCC 10231 were, respectively,  $29.96 \pm 0.69$  mm and  $29.22 \pm 0.51$  mm,  $19.27 \pm 0.61$  mm and  $18.38 \pm 0.33$  mm,  $17.75 \pm 0.34$  mm and  $17.65 \pm 0.31$  mm,  $40.80 \pm 0.42$  mm and  $39.53 \pm 0.53$  mm, and  $20.27 \pm 0.48$  mm and  $19.95 \pm 0.26$  mm.



**Figure 1:** Antimicrobial activity of the studied suppository samples (according to the method of diffusion in agar)

## CONCLUSION

1. The substance of indole-3-carbinol at a concentration of 1000 µg/ml exhibits a moderately pronounced antifungal effect in relation to *C. albicans* and does not exhibit antibacterial activity.
2. The substance of meloxicam at a concentration of 1000 µg/ml does not show a marked antimicrobial action.
3. Suppositories with indole-3-carbinol and the combination of indole-3-carbinol and meloxicam have a pronounced antibacterial effect in relation to Gram-positive and Gram-negative bacteria and antifungal action.
4. The presence of meloxicam in the composition of suppositories does not significantly affect the degree of the antibacterial effect severity, but insignificantly, but reliably reduces antifungal activity.
5. The presence in suppositories of the excipient Montanox 80 does not affect the degree of the antibacterial and antifungal effects severity.
6. The revealed features of antimicrobial action open prospects for the use of suppositories of the combined composition with indole-3-carbinol and meloxicam, which are being developed, for the treatment of not only abacterial but also bacterial (infectious) forms of prostatitis.

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