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LOCAL MULTIFUNCTIONAL HYDROGEL THERAPY IN EXPERIMENTAL FIBRINOPURULENT PERITONITIS: ANTIBACTERIAL, FIBRINOLYTIC, AND REPARATIVE EFFECTS OF A NOVEL LEVOFLOXACIN-CUCUMAZINE-NA-CMC COMPOSITE

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ABSTRACT

Fibrinopurulent peritonitis (FPP) remains one of the most severe and life-threatening forms of intra-abdominal infection, with mortality rates ranging from 20% to 30% despite substantial advances in surgical source control, antimicrobial therapy, and intensive care management. The formation of extensive fibrinous deposits and bacterial biofilms within the peritoneal cavity creates a physical and biological barrier that significantly impairs the penetration and effectiveness of systemic antimicrobial agents, promotes persistent infection, enhances local inflammatory responses, and contributes to the development of postoperative adhesions and intra-abdominal abscesses. Objective: To develop and experimentally evaluate the therapeutic efficacy, biocompatibility, and reparative potential of a novel multifunctional composite hydrogel containing levofloxacin, cucumazine, and sodium carboxymethylcellulose (LCSC) for local treatment of fibrinopurulent peritonitis. Materials and Methods: The efficacy study was performed on 84 adult white rats with experimentally induced fibrinopurulent peritonitis using a modified distal cecal ligation and puncture (DCLP) model that closely reproduces the clinical and morphological features of diffuse peritonitis in humans. Animals were randomly assigned to three groups: control (saline lavage), levofloxacin (0.1% levofloxacin solution), and experimental (local application of LCSC hydrogel). Clinical, microbiological, histological, and morphometric assessments

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Keywords:
fibrinopurulent peritonitis, hydrogel, levofloxacin, cucumazine, sodium carboxymethylcellulose, local therapy, fibrinolysis, biofilms, morphology, adhesions, mesothelial regeneration.

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were performed on postoperative days 3, 7, 14, and 21. The evaluated parameters included mortality, bacterial contamination of peritoneal exudate, adhesion formation, serosal thickness, fibrosis area, inflammatory cell density, and degree of mesothelial epithelialization. Results: Local administration of the LCSC hydrogel resulted in improvements in the evaluated outcomes compared with standard treatment. Mortality decreased from 41.7% in the control group to 8.3% in the experimental group ($p < 0.05$). Bacterial contamination of the peritoneal exudate decreased from 10^5-10^6 CFU/mL to $\leq 10^4$ CFU/mL, while exudate volume was reduced by 30-40%. Morphometric analysis demonstrated a 46% reduction in fibrosis area, a 62% decrease in inflammatory cell infiltration, and an increase in epithelialization from 38 ± 5% to 72 ± 6% ($p < 0.001$). Histological examination revealed accelerated angiogenesis, early maturation of granulation tissue, and restoration of mesothelial integrity; no dense intra-abdominal adhesions were observed by day 21. Conclusions: The newly developed LCSC composite hydrogel demonstrated pronounced antibacterial, fibrinolytic, anti-inflammatory, anti-adhesive, and reparative properties in an experimental model of fibrinopurulent peritonitis. The combination of sustained local antimicrobial delivery, enzymatic fibrin degradation, and promotion of mesothelial regeneration provides a multifactorial pathogenetic effect that may represent a promising strategy for improving the outcomes of local treatment of severe intra-abdominal infections.

1. Introduction

Fibrinopurulent peritonitis (FPP) remains one of the most challenging and life-threatening complications in emergency abdominal surgery and continues to be associated with considerable morbidity and mortality worldwide. Despite substantial advances in surgical techniques, perioperative intensive care, and antimicrobial therapy, mortality rates in diffuse and generalized forms of peritonitis still range from 20% to 30%, while in patients with severe abdominal sepsis and multiple organ dysfunction syndrome they may exceed 40% [1,2,15-17]. Furthermore, survivors frequently experience serious postoperative complications, including intra-abdominal abscesses, adhesive disease, intestinal fistulas, and recurrent septic episodes, which can prolong hospitalization [13,19-25].

The pathogenesis of fibrinopurulent peritonitis is multifactorial and involves a complex interaction between microbial invasion, systemic inflammatory response syndrome (SIRS), microcirculatory dysfunction, and impaired local immune defense mechanisms [11,15,16]. One of the central morphological and pathogenetic events during the progression of peritonitis is the formation of extensive fibrinous deposits on the serosal surfaces of abdominal organs [11,14]. Initially, fibrin deposition serves as a protective mechanism aimed at localizing the infectious process and limiting bacterial dissemination within the abdominal cavity. However, persistent fibrin accumulation subsequently creates a favorable microenvironment for bacterial colonization, biofilm formation, and chronic inflammation [4,5,12].

Bacterial biofilms formed on fibrin matrices represent highly organized three-dimensional microbial communities embedded within an extracellular polymeric substance that protects microorganisms from host immune responses and antimicrobial agents [4-6,12,18]. Numerous studies have demonstrated that bacteria residing within biofilms exhibit antibiotic resistance levels that are 100 to 1000 times greater than those of planktonic bacterial forms [4,5,12]. Consequently, systemic antibiotic therapy often fails to achieve adequate drug concentrations within the infected fibrinous microenvironment,

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resulting in persistent infection, abscess formation, delayed tissue repair, and adhesion development [11,15].

The limitations of conventional treatment strategies have stimulated increasing interest in local therapeutic approaches capable of directly targeting the pathological substrate of fibrinopurulent inflammation [11]. Current concepts of local therapy emphasize the necessity of combining several pathogenetically important mechanisms, including enzymatic degradation of fibrin deposits, suppression of bacterial growth, adsorption of inflammatory mediators and tissue degradation products, and stimulation of mesothelial regeneration [6,8]. Such multifunctional therapeutic systems may simultaneously address several key components of peritoneal inflammation that remain inaccessible to systemic treatment alone.

Hydrogel-based drug delivery systems have emerged as one of the most promising directions in modern abdominal surgery and regenerative medicine [8-10]. Biocompatible polymers such as sodium carboxymethylcellulose (Na-CMC) and chitosan can form biodegradable matrices that provide sustained release of active compounds and may act as temporary physical barriers between injured surfaces [8,9,19,20]. Experimental and clinical studies have demonstrated that local hydrogel formulations may significantly improve antimicrobial efficacy, accelerate tissue regeneration, and reduce postoperative complications in purulent-inflammatory conditions [7-10].

Based on these considerations, a novel multifunctional composite hydrogel consisting of levofloxacin, cucumazine, and sodium carboxymethylcellulose (LCSC) was developed. The rationale behind this formulation was to combine prolonged local antibacterial activity provided by levofloxacin with the fibrinolytic properties of cucumazine and the biocompatible controlled-release matrix formed by Na-CMC [7,8]. Such a combination was expected to provide antibacterial, fibrinolytic, anti-inflammatory, anti-adhesive, and reparative effects within the peritoneal cavity.

The present study aimed to develop and experimentally evaluate the effectiveness of the LCSC composite hydrogel in the treatment of fibrinopurulent peritonitis using a clinically relevant modified distal cecal ligation and puncture (DCLP) model. Particular attention was paid to the assessment of mortality, bacterial contamination, adhesion formation, histological dynamics of peritoneal healing, and morphometric indicators of tissue repair in order to determine the therapeutic potential of this novel local treatment strategy.

MATERIALS AND METHODS

Study Design and Experimental Setting

This experimental study was conducted at the Department of Experimental Surgery and the Laboratory of Pathomorphology of the Republican Specialized Scientific and Practical Medical Center of Surgery named after Academician V. Vakhidov, Tashkent, Uzbekistan, between 2024 and 2025. The study was designed to evaluate the fibrinolytic activity, biocompatibility, antibacterial efficacy, and reparative potential of a newly developed multifunctional hydrogel composed of levofloxacin, cucumazine, and sodium carboxymethylcellulose (LCSC) in experimental fibrinopurulent peritonitis.

Ethical Approval

All experimental procedures involving laboratory animals were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and international guidelines for animal welfare and biomedical research. The study protocol was reviewed and approved by the Local Ethics Committee of the Republican Specialized Scientific and Practical Medical Center of Surgery named after Academician V. Vakhidov.

Experimental Animals

The study included 92 adult outbred white rats of both sexes. Of these, 84 underwent induction of fibrinopurulent peritonitis and eight intact rats were used for the biocompatibility assessment. The animals were aged 8-12 weeks and weighed between 220 and 250 g. Animals were housed under standard vivarium conditions with a controlled temperature of 21-23°C, relative humidity of 50-60%, and a 12-hour light/dark

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cycle. Standard laboratory chow and water were provided ad libitum throughout the experiment. Prior to the study, all animals underwent a seven-day acclimatization period.

Experimental Groups

The animals were randomly assigned to three equal groups consisting of 28 animals each:

1. **Control group (n=28):** Peritoneal lavage with warmed isotonic saline solution only.
2. **Levofloxacin group (n=28):** Peritoneal lavage followed by intraperitoneal instillation of 0.1% levofloxacin solution.
3. **LCSC group (n=28):** Peritoneal lavage followed by local application of the composite hydrogel containing levofloxacin (0.5%), cucumazine (0.01%), and sodium carboxymethylcellulose (Na-CMC) as a polymeric carrier matrix.

In addition, an independent biocompatibility group consisting of eight intact animals received intraperitoneal administration of the LCSC gel without induction of peritonitis to evaluate local tolerance and possible toxic effects.

Preparation of the LCSC Hydrogel

The LCSC hydrogel was developed as a multifunctional local therapeutic formulation combining antimicrobial, fibrinolytic, and reparative activities. The preparation consisted of:

- levofloxacin - 0.5%;
- cucumazine - 0.01%;
- sodium carboxymethylcellulose (Na-CMC) as a biodegradable polymeric matrix.

Levofloxacin served as a broad-spectrum antimicrobial agent, cucumazine provided proteolytic and fibrinolytic activity facilitating degradation of fibrin deposits and bacterial biofilms, while Na-CMC ensured prolonged local retention and controlled release of active components within the peritoneal cavity.

In Vitro Evaluation of Fibrinolytic Activity

The fibrinolytic activity of the hydrogel was evaluated using human fibrin clots obtained from freshly coagulated venous blood. After centrifugation at 3,000 rpm for five minutes, fibrin clots were washed with isotonic saline and divided into fragments weighing approximately 40 mg each.

Four experimental conditions were studied:

- control (saline only);
- LCSC gel containing 2 U of cucumazine;
- LCSC gel containing 5 U of cucumazine;
- LCSC gel containing 10 U of cucumazine.

Samples were incubated in 2 mL of physiological saline at 37°C, and clot weight was measured every 15 minutes for 240 minutes. Visual assessment of fragmentation and liquefaction was additionally performed.

Experimental Model of Fibrinopurulent Peritonitis

Fibrinopurulent peritonitis was induced using a modified distal cecal ligation and puncture (DCLP) technique based on the model originally described by Singleton and Wischmeyer [26]. Under inhalational anesthesia with sevoflurane vapor, a midline laparotomy measuring 2.5-3 cm was performed.

A distal 1-cm segment of the cecum was ligated together with its vascular supply to induce ischemia. Subsequently, the cecal wall was punctured twice using a sterile 0.6-mm needle, allowing controlled leakage of fecal contents into the abdominal cavity. The abdominal wall was closed in two layers using Vicryl and Prolene sutures.

This model provided reproducible induction of progressive fibrinopurulent peritonitis closely resembling the clinical course of diffuse peritonitis in humans.

Treatment Protocol

Following induction of peritonitis and abdominal lavage, the corresponding treatment protocol was applied according to group allocation.

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The LCSC hydrogel was administered locally into the peritoneal cavity during the early toxic phase of inflammation, when fibrin deposition and purulent exudation became macroscopically evident. The hydrogel was distributed evenly over affected serosal surfaces to ensure prolonged contact with the inflammatory substrate.

Clinical Evaluation

Animals were monitored daily for (Figure 1):

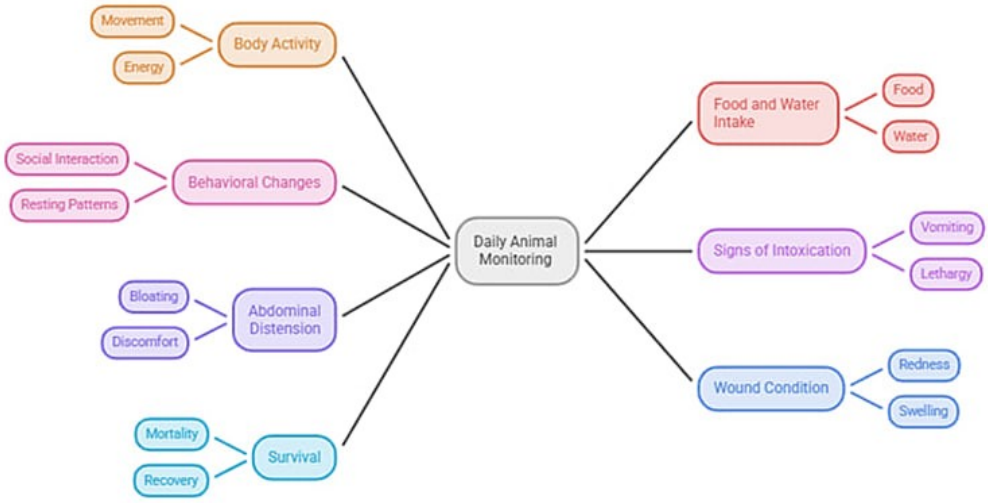


Figure 1. Daily animal monitoring parameters

Mortality was recorded throughout the observation period and analyzed as one of the primary outcome measures.

Macroscopic Assessment

At necropsy, the following parameters were evaluated:

- volume and character of peritoneal exudate;
- extent of fibrin deposition;
- severity of adhesions;
- presence of abscesses;
- intestinal distension and paralysis;
- restoration of anatomical relationships between abdominal organs.

Observations were performed on postoperative days 3, 7, 14, and 21.

Histological and Morphometric Analysis

Samples of peritoneum, omentum, and intestinal loops were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4-5 μm, and stained with hematoxylin and eosin.

Microscopic examination was performed using a DN-300M light microscope equipped with a digital imaging system. Morphometric measurements were obtained using ImageJ software.

The following parameters were evaluated:

- serosal thickness (μm);
- fibrosis area (mm^2);
- inflammatory cell density (cells per high-power field);
- epithelialization index (%).

Measurements were performed in ten randomly selected microscopic fields at magnifications of ×200 and ×400.

Microbiological Analysis

Peritoneal exudate samples were collected aseptically and cultured to determine bacterial contamination levels expressed as colony-forming units per milliliter (CFU/mL). Quantitative bacterial load was compared between groups during the observation period.

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Statistical Analysis

Statistical analysis was performed using standard biomedical statistical methods. Continuous variables were expressed as mean ± standard error of the mean (mean ± SEM). Intergroup comparisons were performed using Student's t-test. Differences were considered statistically significant at **$p < 0.05$**. Statistical calculations were performed using standard statistical software packages.

RESULTS

In Vitro Fibrinolytic Activity of the LCSC Hydrogel

One of the principal objectives in the development of the LCSC hydrogel was to provide not only effective local antibacterial activity but also targeted fibrinolytic action against fibrin deposits, which represent a major structural component of fibrinopurulent peritonitis and a critical substrate for bacterial biofilm formation. Therefore, the first stage of the investigation involved an in vitro assessment of the fibrinolytic properties of the developed formulation.

The experiments demonstrated a clear dose-dependent fibrinolytic activity of cucumazine incorporated into the hydrogel matrix (Table 1, Figure 2). The most pronounced fibrin degradation was observed in the formulation containing 10 U of cucumazine, where the fibrin clot mass decreased from 52.7 ± 1.2 mg to 25.2 ± 1.3 mg after 120 minutes of incubation, corresponding to a reduction of approximately 52.2% ($p < 0.001$ versus control).

The formulation containing 5 U of cucumazine produced a moderate but significant effect, reducing clot mass by 42.3% ($p < 0.01$ versus control), whereas the 2 U formulation demonstrated a weaker but still measurable fibrinolytic response with a reduction of 20.4% ($p < 0.05$ versus control).

In contrast, control samples exposed to physiological saline alone showed only a minimal decrease in clot mass (10.2%), which was attributable to physiological clot retraction processes rather than enzymatic fibrinolysis.

Table 1.

Dose-dependent fibrinolytic activity of the LCSC hydrogel in vitro

Group	Initial clot mass (mg)	120 min (mg)	Reduction (%)
Control	50.2 ± 1.3	45.1 ± 1.4	10.2
LCSC + 2 U cucumazine	51.9 ± 1.6	41.3 ± 1.6	20.4
LCSC + 5 U cucumazine	52.3 ± 1.4	30.2 ± 1.2	42.3
LCSC + 10 U cucumazine	52.7 ± 1.2	25.2 ± 1.3	52.2

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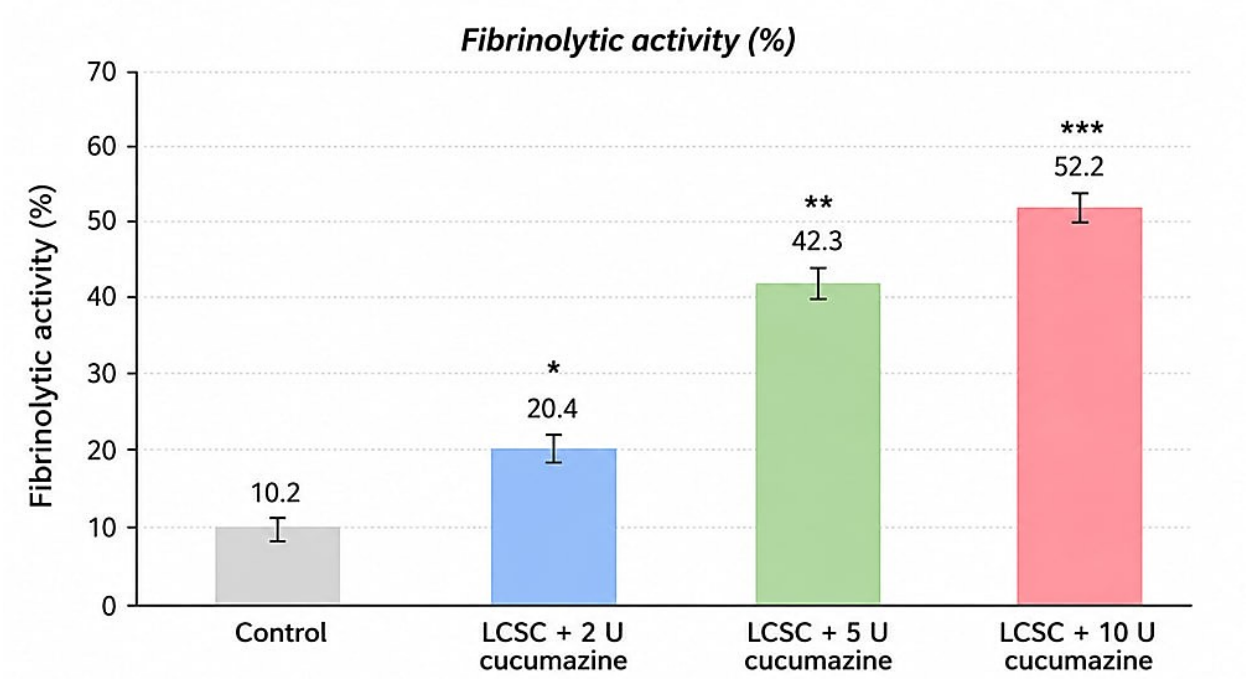


Figure 2. Dose-dependent fibrinolytic activity of the LCSC hydrogel in vitro. Data are presented as mean ± standard deviation (SD). *p < 0.05, **p < 0.01, ***p < 0.001 versus control.

These findings confirmed that the enzymatic component retained its biological activity after incorporation into the hydrogel matrix and provided effective degradation of fibrin structures under physiological conditions. Furthermore, the observed dose-dependent response supports the potential use of cucumazine as a local fibrinolytic agent for disrupting fibrin barriers and improving antimicrobial penetration into infected peritoneal tissues.

Biocompatibility of the LCSC Hydrogel

The safety profile of the developed formulation was evaluated in a separate group of intact animals receiving intraperitoneal administration of the hydrogel without induction of peritonitis.

During the entire observation period, no mortality, behavioral abnormalities, feeding disturbances, or signs of abdominal discomfort were observed. Macroscopic examination revealed no evidence of inflammatory reactions, serosal irritation, fibrin deposition, or adhesion formation. Histological assessment confirmed preservation of normal mesothelial architecture without inflammatory infiltration or vascular disturbances.

These findings demonstrated no detectable local toxicity under the study conditions, supporting further evaluation of the formulation in pathological conditions.

Clinical Course of Experimental Fibrinopurulent Peritonitis

The modified DCLP model produced highly reproducible progressive fibrinopurulent peritonitis closely resembling the clinical course of diffuse peritonitis in humans.

Day 1. Animals remained relatively active despite mild anorexia and decreased mobility. Laparotomy revealed localized inflammatory infiltration surrounding the ischemic cecal segment without evidence of diffuse contamination or generalized inflammation. These findings corresponded to the reactive phase of peritonitis.

Day 3. Signs of systemic intoxication became evident, including reduced activity, rough fur, and diminished responses to external stimuli. Macroscopically, inflammatory infiltrates involved approximately one-third of the intestinal loops and were associated with fibrin deposits and limited purulent exudation. Omental involvement and initial diffuse inflammatory changes became apparent (Figure 3A).

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Figure 3A. Macroscopic appearance of experimental fibrinopurulent peritonitis on postoperative day 3 showing localized fibrin deposition and inflammatory infiltration of intestinal loops.

Day 4. Progressive abdominal distension, anorexia, and behavioral suppression were observed. Laparotomy demonstrated increasing exudate volume, diffuse hyperemia, vascular congestion, and loss of normal serosal transparency, indicating transition to the toxic phase of peritonitis.

Days 5-7. Animals developed severe intoxication characterized by tachypnea, dehydration, and complete loss of appetite. The abdominal cavity contained abundant foul-smelling purulent exudate associated with extensive fibrin deposits, intestinal paralysis, and multiple intra-abdominal abscesses. Mortality reached approximately 30% during this period (Figure 3B).

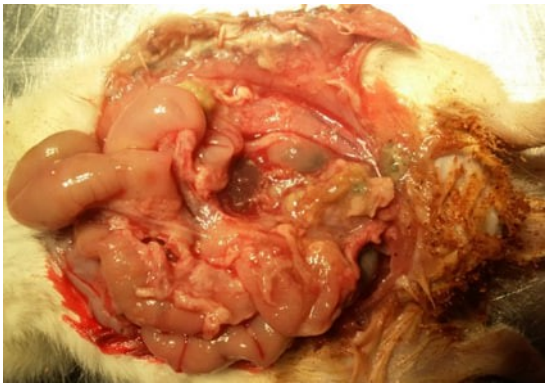


Figure 3B. Advanced fibrinopurulent peritonitis on postoperative day 7 characterized by diffuse fibrin deposits, purulent exudate, intestinal edema, and multiple intra-abdominal abscesses.

Clinical Efficacy of LCSC Therapy

Local administration of the LCSC hydrogel significantly improved survival and clinical outcomes compared with standard treatment protocols (Table 2, Figure 3C).

Mortality decreased significantly from 41.7% in the control group to 8.3% in LCSC-treated animals, corresponding to an approximately fivefold reduction in lethality ($p < 0.05$). Furthermore, treated animals demonstrated earlier recovery of spontaneous activity and feeding behavior, with normalization occurring approximately 2-3 days earlier than in untreated controls.

Table 2.

Clinical outcomes following treatment

Parameter	Control	LCSC group
Mortality	41.7%	8.3%
Adhesions	41.7%	0%
Exudate reduction	-	30-40%
Recovery acceleration	-	2-3 days

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The incidence of postoperative adhesions was reduced from 41.7% in the control group to 0% following LCSC treatment ($p < 0.05$). In addition, the volume of inflammatory exudate decreased by 30-40%, while the severity of systemic intoxication was markedly attenuated.

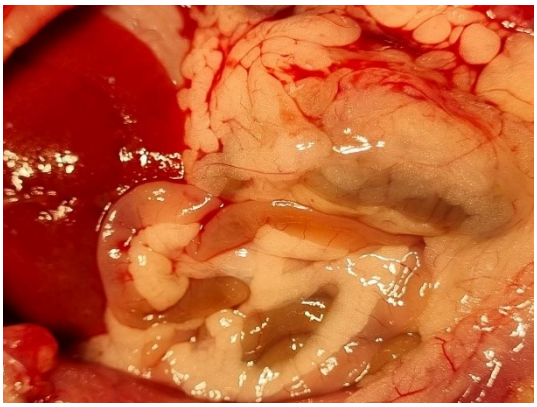


Figure 3C. Macroscopic appearance of the abdominal cavity following LCSC treatment demonstrating marked reduction of exudate, fibrin deposits, and inflammatory changes (Experimental group. Postoperative day 3).

Macroscopic examination of the abdominal cavity demonstrated a substantial reduction in fibrin deposits, inflammatory exudate, intestinal edema, and diffuse inflammatory changes in the experimental group compared with untreated animals (Figure 3C).

Comparative efficacy of local treatment strategies

To determine whether the observed therapeutic effects were attributable solely to local antibiotic administration or to the combined action of the composite hydrogel components, a comparative analysis between the control, levofloxacin, and LCSC groups was performed (Table 3).

Although local levofloxacin treatment resulted in moderate improvements in microbiological and inflammatory parameters, the LCSC hydrogel demonstrated greater improvements across the evaluated outcomes.

Mortality decreased from 41.7% in the control group to 25.0% in the levofloxacin group and further to 8.3% following LCSC treatment ($p < 0.05$). Similarly, bacterial contamination of peritoneal exudate decreased from 10^5-10^6 CFU/mL in untreated animals to 10^4-10^5 CFU/mL in the levofloxacin group and to $\leq 10^4$ CFU/mL in the LCSC group ($p < 0.01$).

The incidence of dense intra-abdominal adhesions decreased from 41.7% in controls to 16.7% after local levofloxacin administration and was not observed in animals treated with the LCSC hydrogel ($p < 0.05$).

Morphometric analysis demonstrated progressive improvements across treatment groups. Serosal thickness decreased from 980 ± 38 μm in controls to 900 ± 36 μm in the levofloxacin group and to 820 ± 35 μm following LCSC therapy ($p < 0.001$). Similarly, fibrosis area was reduced from 2.8 ± 0.2 mm² to 2.1 ± 0.2 mm² and 1.5 ± 0.1 mm², respectively ($p < 0.01$), while inflammatory cell density decreased from 52 ± 4 cells/HPF to 34 ± 3 cells/HPF and 20 ± 2 cells/HPF ($p < 0.001$).

In contrast, mesothelial epithelialization progressively increased from 38 ± 5% in untreated animals to 56 ± 5% following levofloxacin treatment and reached 72 ± 6% in the LCSC group ($p < 0.001$).

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Table 3.

Comparative efficacy of different local treatment strategies in experimental fibrinopurulent peritonitis

Parameter	Control	Levofloxacin	LCSC Hydrogel	p-value
Mortality (%)	41.7	25.0	8.3	<0.05
Bacterial contamination (CFU/mL)	10^5-10^6	10^4-10^5	$\leq 10^4$	<0.01
Dense adhesions (%)	41.7	16.7	0	<0.05
Exudate volume reduction (%)	-	15-20	30-40	<0.05
Recovery acceleration (days)	-	1-2	2-3	<0.05
Serosal thickness (μm)	980 ± 38	900 ± 36	820 ± 35	<0.001
Fibrosis area (mm^2)	2.8 ± 0.2	2.1 ± 0.2	1.5 ± 0.1	<0.01
Inflammatory cells/HPF	52 ± 4	34 ± 3	20 ± 2	<0.001
Epithelialization (%)	38 ± 5	56 ± 5	72 ± 6	<0.001

The comparative analysis demonstrated that although local levofloxacin administration improved bacterial control and partially reduced inflammatory activity, the multifunctional LCSC hydrogel produced greater therapeutic benefits. These findings indicate that the superior outcomes observed in the experimental group were attributable not only to antimicrobial activity but also to the combined fibrinolytic, anti-adhesive, and reparative effects provided by cucumazine and the Na-CMC hydrogel matrix.

Microbiological Findings

Quantitative microbiological analysis demonstrated a substantial reduction in bacterial contamination within the peritoneal cavity following local hydrogel treatment (Table 4).

In untreated animals, bacterial contamination remained high throughout the observation period, reaching concentrations of 10^5-10^6 CFU/mL by postoperative day 7. Local administration of levofloxacin reduced bacterial counts to approximately 10^4-10^5 CFU/mL, whereas animals treated with the LCSC hydrogel exhibited bacterial loads not exceeding 10^4 CFU/mL, corresponding to at least a tenfold reduction in microbial burden compared with untreated controls ($p < 0.01$).

These findings indicate that the sustained local release of levofloxacin from the Na-CMC matrix, combined with enzymatic disruption of fibrin barriers by cucumazine, significantly enhanced antibacterial efficacy within the infected peritoneal cavity.

Table 4.

Bacterial contamination of peritoneal exudate

Group	Bacterial load (CFU/mL)
Control	10^5-10^6
Levofloxacin	10^4-10^5
LCSC	$\leq 10^4$

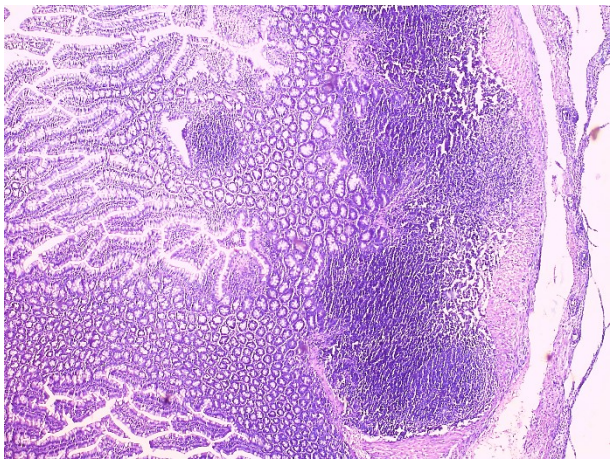
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The microbiological findings suggest that the superior antibacterial effect of the LCSC hydrogel was not solely attributable to local antibiotic administration but also to improved penetration of antimicrobial agents following enzymatic fibrinolysis and disruption of bacterial biofilm-associated fibrin matrices.

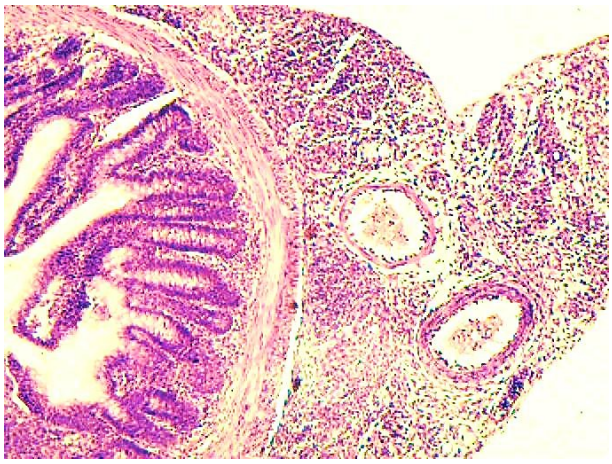
Morphological Findings

Histological examination demonstrated substantial differences in the dynamics of peritoneal tissue repair between the control and LCSC-treated groups (Figures 4-7).

Postoperative Day 3. On postoperative day 3, control animals exhibited extensive fibrinopurulent exudation characterized by diffuse neutrophilic infiltration, mesothelial destruction, vascular congestion, and early fibroblast proliferation (Figure 4A). In contrast, LCSC-treated animals demonstrated significantly thinner fibrin deposits, predominance of macrophages over neutrophils, and early development of granulation tissue (Figure 4B), indicating attenuation of the acute inflammatory response and initiation of reparative processes.



A: Control group demonstrating extensive fibrin deposition, diffuse neutrophilic infiltration, vascular congestion, and mesothelial destruction. Hematoxylin and eosin staining, ×100.



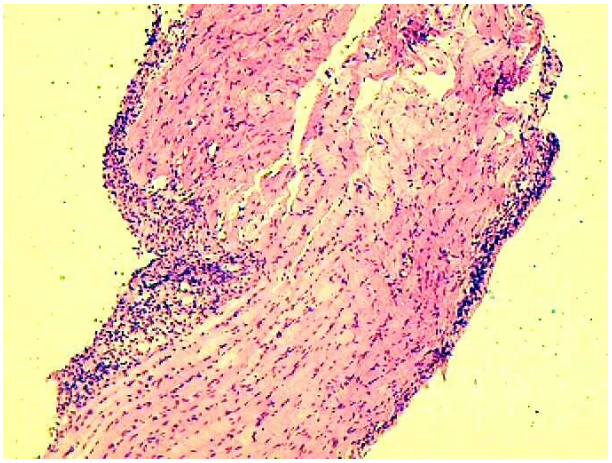
B: LCSC-treated group showing reduced inflammatory infiltration and early granulation tissue formation. H&E, ×100.

Figure 4. Representative histological sections on postoperative day 3.

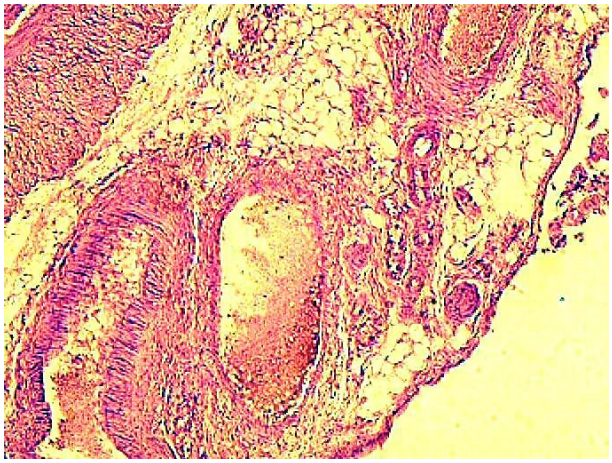
Postoperative Day 7. By postoperative day 7, control specimens continued to demonstrate extensive inflammatory infiltration and disorganized immature connective tissue. Conversely, LCSC-treated tissues exhibited active angiogenesis, orderly fibroblast orientation, and early mesothelial regeneration, suggesting accelerated maturation of reparative tissue and improved organization of the healing process.

Postoperative Day 14. On postoperative day 14, mature granulation tissue with well-developed capillary networks and regularly oriented collagen fibers predominated in the LCSC group (Figure 5B). Nearly complete restoration of the mesothelial lining was observed, whereas control animals continued to exhibit chronic inflammatory infiltration and progressive fibrosis (Figure 5A).

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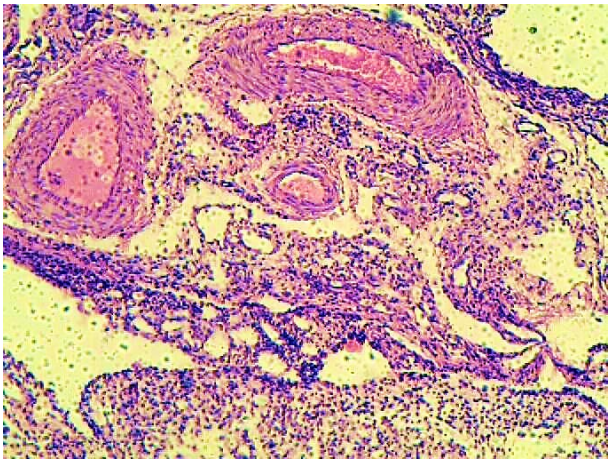
A: Chronic inflammatory changes and fibrosis in controls. H&E, ×100.



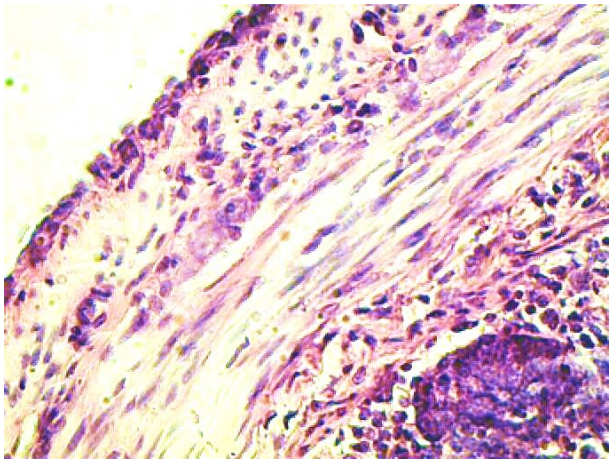
B: Mature granulation tissue and advanced mesothelial regeneration in the LCSC-treated animals. H&E, ×200.

Figure 5. Histological appearance on postoperative day 14.

Postoperative Day 21. By postoperative day 21, control animals developed dense fibrous adhesions and scar formation associated with incomplete epithelial recovery (Figure 6A). In contrast, the LCSC group demonstrated complete mesothelial regeneration, restoration of normal vascular architecture, and absence of inflammatory infiltrates or adhesions (Figure 6B). Histologically, the peritoneal surface closely resembled that of healthy animals.



A: Dense fibrous adhesions and incomplete epithelial recovery in controls. H&E, ×100.



B: Complete mesothelial restoration and normal peritoneal architecture in the LCSC group. H&E, ×200.

Figure 6. Histological appearance on postoperative day 21.

Collectively, the histological findings demonstrated that local administration of the LCSC hydrogel significantly accelerated the transition from the inflammatory phase to the reparative phase of healing, promoted early mesothelial regeneration, reduced fibrosis, and no adhesions were observed within the peritoneal cavity.

Morphometric Analysis

Quantitative morphometric analysis objectively confirmed the histological findings and demonstrated significant improvements in peritoneal healing following LCSC treatment (Table 5, Figure 7).

Table 5.

Morphometric parameters after treatment

Parameter	Control	LCSC	p-value
Serosal thickness (μm)	980 ± 38	820 ± 35	<0.001
Fibrosis area (mm²)	2.8 ± 0.2	1.5 ± 0.1	<0.01
Inflammatory cells/HPF	52 ± 4	20 ± 2	<0.001
Epithelialization (%)	38 ± 5	72 ± 6	<0.001

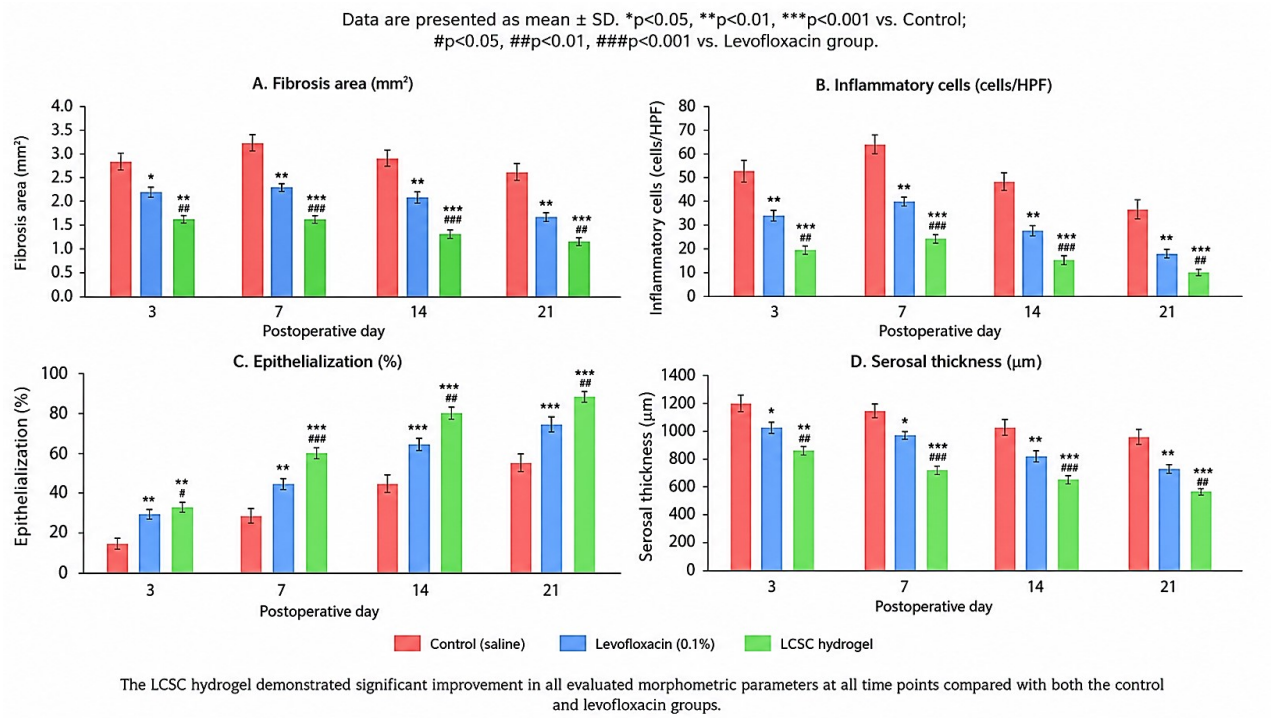


Figure 7. Comparative morphometric parameters of peritoneal healing following local treatment of experimental fibrinopurulent peritonitis. ***(A)*** Fibrosis area (mm²); ***(B)*** Inflammatory cell density (cells/high-power field, HPF); ***(C)*** Degree of mesothelial epithelialization (%); and ***(D)*** Serosal thickness (μm). Data are presented as mean ± standard deviation (SD). *p < 0.05, **p < 0.01, ***p < 0.001 versus the control group; #p < 0.05, ##p < 0.01, ###p < 0.001 versus the Levofloxacin group.

Compared with the control group, animals treated with the LCSC hydrogel exhibited a significant reduction in serosal thickness from 980 ± 38 μm to 820 ± 35 μm ($p < 0.001$), indicating attenuation of inflammatory edema and restoration of normal peritoneal architecture.

Similarly, the fibrosis area decreased by 46%, from 2.8 ± 0.2 mm² in controls to 1.5 ± 0.1 mm² in the LCSC group ($p < 0.01$). The density of inflammatory cells was reduced from 52 ± 4 cells/HPF to 20 ± 2 cells/HPF, corresponding to a 62% decrease in inflammatory infiltration ($p < 0.001$).

In contrast, mesothelial epithelialization increased significantly from 38 ± 5% in untreated animals to 72 ± 6% following hydrogel administration ($p < 0.001$), indicating accelerated regeneration of the peritoneal surface.

These quantitative findings support the pronounced anti-inflammatory, anti-fibrotic, and reparative effects of the LCSC hydrogel in experimental fibrinopurulent peritonitis (Table 5, Figure 7).

DISCUSSION

The present study demonstrates that local administration of the newly developed levofloxacin-cucumazine-sodium carboxymethylcellulose (LCSC) hydrogel provides a significant therapeutic advantage in the management of experimental fibrinopurulent peritonitis by simultaneously targeting several key pathogenetic mechanisms involved in the progression of intra-abdominal infection. The obtained results indicate that the combination of prolonged local antimicrobial delivery, enzymatic fibrin

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degradation, suppression of inflammatory activity, and stimulation of mesothelial regeneration contributes to substantial improvements in both clinical and morphological outcomes (Tables 3-5, Figures 3-7).

Fibrin deposition is recognized as one of the earliest and most important protective responses of the peritoneum during bacterial contamination of the abdominal cavity [19,20]. Initially, fibrin formation serves to localize the infectious process and limit microbial dissemination. However, persistent fibrin accumulation subsequently becomes a major pathogenic factor contributing to the development of bacterial biofilms, chronic inflammation, impaired tissue repair, and adhesion formation [4,5,19,20]. Biofilm-associated matrices can limit antimicrobial penetration into infected tissues, thereby reducing treatment efficacy and facilitating microbial persistence [4,5,18].

Previous experimental and clinical studies have demonstrated that bacteria embedded within biofilms exhibit resistance to antimicrobial agents that may exceed that of planktonic bacteria by several hundred-fold [4-6,12,18]. Consequently, even high-dose systemic antibiotic therapy frequently fails to eradicate microorganisms located within biofilm-associated material and necrotic tissue fragments [4,12]. This phenomenon explains the persistent incidence of postoperative abscesses, recurrent infections, and adhesive complications despite apparently adequate antimicrobial treatment and surgical source control [15-17,19,20].

The concept underlying the development of the LCSC hydrogel was based on overcoming these limitations through simultaneous local intervention at multiple stages of the pathological process. The antibacterial component, levofloxacin, provides broad-spectrum antimicrobial activity directly within the inflammatory focus, achieving local concentrations substantially higher than those attainable through systemic administration without increasing systemic toxicity [7,8]. This mechanism likely explains the at least a tenfold reduction in bacterial contamination observed in the experimental group (Table 4) as well as the significant reduction in mortality and adhesion formation (Table 3).

Importantly, although local administration of levofloxacin alone improved microbiological and inflammatory parameters, its therapeutic effects remained consistently inferior to those achieved with the multifunctional LCSC hydrogel (Table 3). This finding indicates that the superior outcomes observed in the experimental group cannot be attributed solely to local antibiotic delivery but rather to the combined antimicrobial, fibrinolytic, anti-adhesive, and reparative effects.

The second essential component of the formulation is cucumazine, a proteolytic enzyme possessing fibrinolytic activity. The in vitro experiments demonstrated a clear dose-dependent ability of cucumazine to induce fibrin degradation (Table 1, Figure 2), with the highest concentration resulting in more than a 50% reduction in clot mass within two hours of incubation. These findings strongly support the hypothesis that local enzymatic destruction of fibrin deposits may improve antibiotic penetration, reduce bacterial protection within biofilms, and facilitate physiological clearance mechanisms [6,12]. The rapid disappearance of fibrin deposits and absence of dense adhesions in the treated animals (Table 3, Figure 3C) provide further in vivo confirmation of this mechanism.

The role of sodium carboxymethylcellulose as a polymeric matrix should also be emphasized. In addition to functioning as a biocompatible carrier system for active substances, Na-CMC provides prolonged retention of therapeutic components at the site of inflammation and creates a temporary protective barrier over damaged serosal surfaces [8-10]. Such local retention may be particularly important during the early phases of peritoneal inflammation, when exudation, fibrin formation, and bacterial proliferation occur most intensively. Furthermore, temporary physical barriers may contribute to adhesion prevention by mechanically separating injured serosal surfaces during the critical phases of peritoneal healing [3,19-21].

One of the most important findings of the present study is the marked improvement in reparative processes within the peritoneum. Histological analysis demonstrated accelerated angiogenesis, earlier maturation of granulation tissue, and rapid restoration of mesothelial integrity in animals treated with the

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LCSC hydrogel (Figures 5-7). By postoperative day 14, nearly complete mesothelial recovery was observed in the experimental group (Figure 5B), whereas control animals continued to exhibit chronic inflammatory infiltration and active fibrosis (Figure 5A). By day 21, treated animals demonstrated complete restoration of normal peritoneal architecture without evidence of pathological adhesions or residual inflammation (Figure 6B).

The morphometric findings objectively confirmed these histological observations (Table 5, Figure 7). The 46% reduction in fibrosis area, 62% decrease in inflammatory cell infiltration, and nearly twofold increase in epithelialization provide quantitative evidence supporting the histological findings and indicate a profound influence of the hydrogel on tissue remodeling and regeneration.

The anti-adhesive effect observed in this study deserves particular attention. Adhesive disease remains one of the most common long-term complications following diffuse peritonitis and abdominal surgery, with reported incidences ranging from 40% to 60% [3,19-25]. Adhesions are responsible for a substantial proportion of postoperative bowel obstructions, chronic abdominal pain syndromes, infertility, and repeated surgical interventions [20-25]. In the present investigation, dense adhesions developed in 41.7% of untreated animals, whereas no dense adhesions were observed following LCSC treatment (Table 3). This finding suggests that simultaneous fibrin degradation and mesothelial preservation may be substantially more effective than mechanical anti-adhesion barriers alone [14,19,21].

The reduction in mortality from 41.7% to 8.3% represents perhaps the most clinically relevant outcome of the study (Table 3). Mortality in diffuse peritonitis is determined not only by bacterial contamination but also by the severity of systemic inflammatory response syndrome, endotoxemia, and multiple organ dysfunction [1,2,15-17]. By reducing bacterial burden, accelerating elimination of fibrinous exudates, and limiting the duration of local inflammation, the LCSC hydrogel appears to influence several determinants of survival simultaneously.

Several limitations of the study should be acknowledged. First, the investigation was conducted exclusively in an experimental animal model and therefore cannot fully reproduce the complexity of human diffuse peritonitis [15,16]. Second, the study focused primarily on morphological and microbiological outcomes without detailed assessment of cytokine profiles or molecular pathways involved in inflammation and tissue repair. Third, long-term evaluation beyond 21 days was not performed, which limits conclusions regarding very late adhesive complications or chronic inflammatory changes.

Despite these limitations, the present findings provide experimental evidence supporting the use of multifunctional local hydrogel systems in the treatment of severe intra-abdominal infections [8-10]. The combination of antimicrobial, fibrinolytic, anti-inflammatory, anti-adhesive, and reparative effects within a single formulation represents a promising direction in modern abdominal surgery and may contribute to improved outcomes in patients with complicated peritonitis.

The results of this study suggest that the LCSC hydrogel should be considered not merely as a local antimicrobial carrier but as a pathogenetically oriented therapeutic platform capable of modifying the biological environment of the inflamed peritoneal cavity and promoting physiological tissue recovery. Such an approach may represent an important step toward the development of next-generation local treatment strategies for fibrinopurulent peritonitis and other severe purulent-inflammatory diseases of the abdominal cavity.

CONCLUSIONS

1. The modified distal cecal ligation and puncture (DCLP) model demonstrated high reproducibility and reliably reproduced the principal clinical, microbiological, and morphological features of fibrinopurulent peritonitis, including extensive fibrin deposition, purulent exudation, bacterial contamination, and progressive systemic intoxication.

2. The newly developed levofloxacin-cucumazine-sodium carboxymethylcellulose (LCSC) hydrogel exhibited pronounced dose-dependent fibrinolytic activity in vitro and was associated with

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reduced fibrin deposits in vivo, supporting improved local sanitation of the inflammatory focus (Table 1, Figure 2).

3. Local administration of the LCSC hydrogel significantly improved clinical outcomes, reducing mortality from 41.7% to 8.3%, decreasing exudate volume by 30-40%, and accelerating recovery of spontaneous activity and feeding behavior by approximately 2-3 days compared with untreated controls (Tables 2 and 3, Figure 3C).

4. The hydrogel demonstrated substantial antimicrobial efficacy, reducing bacterial contamination of peritoneal exudate from 10^5-10^6 CFU/mL to $\leq 10^4$ CFU/mL, corresponding to at least a tenfold reduction in microbial burden (Table 4).

5. Morphological and morphometric analyses demonstrated marked acceleration of reparative processes, including a 46% reduction in fibrosis area, a 62% decrease in inflammatory cell infiltration, enhancement of mesothelial epithelialization, and no dense intra-abdominal adhesions observed by postoperative day 21 (Figures 4-6, Table 5, Figure 7).

6. The LCSC hydrogel demonstrated no detectable local toxic effects under the study conditions, supporting further preclinical investigation for severe intra-abdominal infections.

REFERENCES

1. Sartelli M, Coccolini F, Kluger Y, Abu-Zidan FM, Ansaloni L, Bala M, et al. WSES/GAIS/SIS-E/WSIS/AAST global clinical pathways for patients with intra-abdominal infections. World J Emerg Surg. 2021;16:49.
2. Ceresoli M, Coccolini F, Fugazzola P, Tomasoni M, Sartelli M, Catena F. Enhanced perioperative care in emergency general surgery: the WSES position paper. World J Emerg Surg. 2023;18:47.
3. Ouaïssi M, Gaujoux S, Veyrie N, Denève E, Brigand C, Castel B, et al. Postoperative adhesions after digestive surgery: their incidence and prevention: review of the literature. J Visc Surg. 2012;149(2):e104-e114.
4. Bi Y, Li J, Yu Y, Wang Y, Zhang X, Chen H. Therapeutic strategies against bacterial biofilms. Fundam Res. 2021;1(2):193-212.
5. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. Nat Rev Microbiol. 2016;14(9):563-575.
6. Fleming D, Chahin L, Rumbaugh K. Glycoside hydrolases degrade polymicrobial bacterial biofilms in wounds. Antimicrob Agents Chemother. 2017;61(2):e01998-16.
7. Ishida H, Ishida Y, Kurosaka Y, Otani T, Sato K, Kobayashi H. In vitro and in vivo activities of levofloxacin against biofilm-producing Pseudomonas aeruginosa. Antimicrob Agents Chemother. 1998;42(7):1641-1645.
8. Elviri L, Bianchera A, Bergonzi MC, Bettini R. Controlled local drug delivery strategies from chitosan hydrogels for wound healing. Expert Opin Drug Deliv. 2017;14(7):897-908.
9. Liang X, Huang C, Liu H, Chen H, Shou J, Cheng H, Liu G. Natural hydrogel dressings in wound care: design, advances, and perspectives. Chin Chem Lett. 2024;35(10):109442.
10. Alberts A, Moldoveanu ET, Niculescu AG, Grumezescu AM. Hydrogels for wound dressings: applications in burn treatment and chronic wound care. J Compos Sci. 2025;9(3):133.
11. Andresen AK, Felding MS, Sacramento MMA, Nielsen LH. Hydrogel-based drug delivery systems for localized eradication of bacterial biofilms: advances, challenges and future directions. J Control Release. 2026;390:114509.
12. Mirghani R, Saba T, Khaliq H, Mitchell J, Do L, Chambi L, et al. Biofilms: formation, drug resistance and alternatives to conventional approaches. AIMS Microbiol. 2022;8(3):239-277.
13. Singh B, Dahiya M, Kumar V, Ayyagari A, Chaudhari DN, Ahire JJ. Biofilm and antimicrobial resistance: mechanisms, implications, and emerging solutions. Microbiol Res. 2025;16(8):183.

14. Fang CC, Chou TH, Lin GS, Yen ZS, Lee CC, Chen SC. Peritoneal infusion with cold saline decreased postoperative intra-abdominal adhesion formation. World J Surg. 2010;34(4):721-727.
15. Mazuski JE, Tessier JM, May AK, et al. The Surgical Infection Society revised guidelines on the management of intra-abdominal infection. Surg Infect. 2017;18(1):1-76.
16. Solomkin JS, Mazuski JE, Bradley JS, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. Clin Infect Dis. 2010;50(2):133-164.
17. Montravers P, Dupont H, Leone M, Constantin JM, Mimoz O, Veber B, et al. Guidelines for management of intra-abdominal infections. Anaesth Crit Care Pain Med. 2015;34(2):117-130.
18. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2(2):95-108.
19. Hellebrekers BWJ, Kooistra T. Pathogenesis of postoperative adhesion formation. Br J Surg. 2011;98(11):1503-1516.
20. Arung W, Meurisse M, Detry O. Pathophysiology and prevention of postoperative peritoneal adhesions. World J Gastroenterol. 2011;17(41):4545-4553.
21. Diamond MP, Wexner SD, diZerega GS, Korell M, Zmora O, Van Goor H, Kamar M. Adhesion prevention and reduction: current status and future recommendations of a multinational interdisciplinary consensus conference. Surg Innov. 2010;17(3):183-188.
22. ten Broek RPG, Issa Y, van Santbrink EJP, Bouvy ND, Kruitwagen RFPM, Jeekel J, et al. Burden of adhesions in abdominal and pelvic surgery: systematic review and meta-analysis. BMJ. 2013;347:f5588.
23. Van Goor H. Consequences and complications of peritoneal adhesions. Colorectal Dis. 2007;9(Suppl 2):25-34.
24. Parker MC, Wilson MS, Menzies D, et al. Clinical and economic consequences of postoperative adhesions. Eur J Surg Suppl. 2001;577:5-9.
25. Attard JA, MacLean AR. Adhesive small bowel obstruction: epidemiology, biology and prevention. Can J Surg. 2007;50(4):291-300.
26. Singleton KD, Wischmeyer PE. Distance of cecum ligated influences mortality, tumor necrosis factor-alpha and interleukin-6 expression following cecal ligation and puncture in the rat. Eur Surg Res. 2003;35(6):486-491.

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