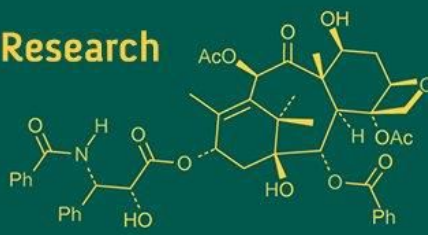
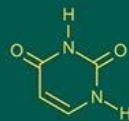
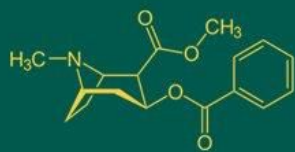


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(4): 438-444
www.biochemjournal.com
 Received: 01-02-2026
 Accepted: 05-03-2026

All author's name and
 affiliation are given below,
 after references.

In vitro synergistic antibacterial interaction of enrofloxacin and catechin against bacterial isolates from canine prostatitis

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i4f.8191>

Abstract

This study evaluated the *in vitro* antibacterial efficacy and synergistic interaction of enrofloxacin and catechin against bacterial isolates obtained from canine prostatitis. Bacterial isolates, including *Escherichia coli* and *Staphylococcus aureus*, were subjected to antimicrobial susceptibility testing, agar well diffusion assay, minimum inhibitory concentration (MIC) determination and fractional inhibitory concentration index (FICI) analysis.

Enrofloxacin exhibited strong antibacterial activity, whereas catechin demonstrated moderate activity when used alone. The combination of enrofloxacin and catechin produced significantly larger zones of inhibition compared with individual treatments. The zone of inhibition for the combination was 27.58 ± 0.24 mm against *E. coli* and 26.33 ± 0.27 mm against *Staphylococcus aureus*, which was higher than enrofloxacin alone.

MIC analysis revealed a significant reduction in inhibitory concentrations when both agents were used in combination. The MIC of enrofloxacin decreased from 0.286 ± 0.0489 $\mu\text{g/mL}$ to 0.0664 ± 0.0112 $\mu\text{g/mL}$ against *E. coli*, and from 0.306 ± 0.0515 $\mu\text{g/mL}$ to 0.0503 ± 0.0110 $\mu\text{g/mL}$ against *Staphylococcus aureus*. Similarly, catechin showed reduced MIC values in combination.

The FICI values ranged between 0.25 and 0.50, indicating a synergistic interaction between enrofloxacin and catechin. The enhanced antibacterial activity may be attributed to increased membrane permeability and inhibition of bacterial efflux mechanisms.

The findings suggest that catechin enhances the antibacterial efficacy of enrofloxacin and may serve as a potential adjunct in antimicrobial therapy.

Keywords: Enrofloxacin, catechin, MIC, FICI, synergism, canine prostatitis

Introduction

Canine bacterial prostatitis is an important inflammatory condition affecting sexually intact adult and geriatric male dogs, commonly resulting from ascending infections of the lower urinary tract. The condition is frequently associated with bacterial pathogens such as *Escherichia coli* and *Staphylococcus aureus*, leading to inflammation, disruption of prostatic architecture and impaired reproductive function^[12]. Owing to its persistent nature and complex pathophysiology, canine bacterial prostatitis presents significant therapeutic challenges.

Effective management of this condition is complicated by the presence of the blood-prostate barrier, which restricts the penetration of several antimicrobial agents into prostatic tissue and secretions, often resulting in subtherapeutic drug concentrations and poor clinical response^[3]. Additionally, increasing antimicrobial resistance among uropathogenic bacteria further limits the effectiveness of conventional antibiotic therapy^[4]. These limitations highlight the need for alternative or adjunct therapeutic strategies to improve treatment outcomes.

Combination therapy using natural bioactive compounds represents a promising approach to overcome antimicrobial resistance and enhance antibiotic efficacy^[5]. Enrofloxacin, a second-generation fluoroquinolone, is widely used in veterinary medicine due to its potent concentration-dependent bactericidal activity mediated through inhibition of bacterial DNA gyrase and topoisomerase IV^[6]. It exhibits favourable pharmacokinetic properties and good tissue penetration, including the prostate^[7]. However, emerging resistance among bacterial isolates necessitates strategies to enhance its antibacterial efficacy.

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Catechins are naturally occurring polyphenolic compounds known for their antimicrobial, antioxidant and anti-inflammatory properties [8]. Previous studies have demonstrated that catechins can enhance the activity of conventional antibiotics through mechanisms such as disruption of bacterial cell membrane integrity and inhibition of efflux pumps, thereby increasing intracellular antibiotic accumulation [9, 10]. These findings suggest their potential role as adjunct agents in antimicrobial therapy.

Despite these promising attributes, limited information is available regarding the synergistic interaction between enrofloxacin and catechin against bacterial isolates from canine prostatitis. Therefore, this study aimed to evaluate the *in vitro* antibacterial efficacy and synergistic interaction of enrofloxacin and catechin against clinical isolates using minimum inhibitory concentration (MIC) and fractional inhibitory concentration index (FICI) methods.

Materials and Methods

Bacterial Isolates

Bacterial isolates were obtained from urine samples of dogs clinically diagnosed with bacterial prostatitis presented to the Teaching Veterinary Clinical Complex, Mumbai Veterinary College, Parel, Mumbai. Samples were collected aseptically by catheterization or cystocentesis and subjected to bacteriological examination.

Isolation of *Escherichia coli* and *Staphylococcus aureus* was carried out using Eosin Methylene Blue (EMB) agar and Mannitol Salt Agar (MSA), respectively, followed by incubation at 37 °C for 18-24 h. Representative colonies were subcultured to obtain pure isolates and identified based on colony morphology, Gram staining and standard biochemical tests including Indole, Methyl Red, Voges-Proskauer and Citrate utilization tests [1].

Drugs and Chemicals

Analytical-grade enrofloxacin (98% purity) and catechin (>97% purity; TCI Chemicals, Shanghai) were used for *in vitro* antimicrobial studies. Catechin was dissolved in dimethyl sulfoxide (DMSO), while enrofloxacin was dissolved in sterile distilled water to prepare stock solutions. Fresh working solutions were prepared on the day of the experiment.

Preparation of Inoculum

Bacterial inoculum was standardized to 0.5 McFarland turbidity standard ($\sim 1 \times 10^8$ CFU/mL) and further diluted to obtain a final inoculum of approximately 5×10^5 CFU/mL for all assays².

Antibiotic Sensitivity Testing

Antimicrobial susceptibility of bacterial isolates was determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines³. Plates were incubated at 37 °C for 16-18 h and zones of inhibition were measured in millimeters.

Agar Well Diffusion Assay

The antibacterial activity of catechin, enrofloxacin and their combination was evaluated using the agar well diffusion method. Mueller-Hinton agar plates were inoculated with standardized bacterial suspension (0.5 McFarland standard). Wells of approximately 6 mm diameter were prepared using

a sterile cork borer.

Test solutions were added into respective wells and plates were incubated at 37 °C for 18-24 h. The diameter of zones of inhibition was measured in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of enrofloxacin, catechin and their combination was determined using the broth microdilution method in sterile 96-well microtiter plates, following CLSI guidelines [3]. Serial two-fold dilutions of enrofloxacin (0.125-64 µg/mL) and catechin were prepared in Mueller-Hinton broth.

A standardized bacterial inoculum (5×10^5 CFU/mL) was added to each well and plates were incubated at 37 °C for 18-24 h. The MIC was defined as the lowest concentration of the drug that completely inhibited visible bacterial growth.

Determination of Fractional Inhibitory Concentration Index (FICI)

The interaction between enrofloxacin and catechin was evaluated using the checkerboard microdilution method. The fractional inhibitory concentration index (FICI) was calculated using MIC values of individual drugs and their combination [4].

FICI values were interpreted as follows:

- ≤ 0.5 : Synergistic
- 0.5-1.0: Additive
- 1.0-4.0: Indifferent
- 4.0: Antagonistic

All experiments were performed in triplicate and included appropriate positive, negative and solvent controls.

Statistical Analysis

The data obtained from antimicrobial assays were expressed as mean $\pm$ standard error. Statistical analysis was performed using standard methods, and differences were considered significant at $p < 0.05$.

Results

Antibiotic Susceptibility Profile

The antimicrobial susceptibility pattern of bacterial isolates was evaluated using the disc diffusion method. Ciprofloxacin exhibited the highest antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*, with mean zones of inhibition of 22.25 ± 0.88 mm and 22.33 ± 0.71 mm, respectively. Cefotaxime and gentamicin also showed considerable antibacterial activity, whereas amoxicillin-clavulanate and tetracycline demonstrated comparatively lower efficacy.

Agar Well Diffusion Assay

Catechin exhibited moderate antibacterial activity, producing zones of inhibition of 13.17 ± 0.30 mm against *E. coli* and 12.08 ± 0.25 mm against *Staphylococcus aureus*.

Enrofloxacin demonstrated strong antibacterial activity, with zones of inhibition of 24.58 ± 0.37 mm against *E. coli* and 22.83 ± 0.31 mm against *Staphylococcus aureus*.

The combination of enrofloxacin and catechin produced the largest zones of inhibition, measuring 27.58 ± 0.24 mm against *E. coli* and 26.33 ± 0.27 mm against *Staphylococcus aureus*.

The comparative antibacterial activity is presented in Fig. 1.

Comparative Antibacterial Activity

The combination treatment exhibited significantly greater antibacterial activity compared with individual agents ($p<0.05$), as evidenced by increased zones of inhibition against both bacterial species.

Minimum Inhibitory Concentration (MIC)

A marked reduction in MIC values was observed when enrofloxacin was used in combination with catechin. The reduction in MIC values is illustrated in Fig. 2 and Fig. 3.

Escherichia coli

- Enrofloxacin alone: 0.286 ± 0.0489 $\mu\text{g/mL}$
- Catechin alone: 1.188 ± 0.163 mg/mL

- Enrofloxacin (combination): 0.0664 ± 0.0112 $\mu\text{g/mL}$
- Catechin (combination): 0.240 ± 0.0343 mg/mL

Staphylococcus aureus

- Enrofloxacin alone: 0.306 ± 0.0515 $\mu\text{g/mL}$
- Catechin alone: 0.639 ± 0.0845 mg/mL
- Enrofloxacin (combination): 0.0503 ± 0.0110 $\mu\text{g/mL}$
- Catechin (combination): 0.132 ± 0.0243 mg/mL

Fractional Inhibitory Concentration Index (FICI)

The FICI values ranged between 0.25 and 0.50, indicating a synergistic interaction between enrofloxacin and catechin against both *E. coli* and *Staphylococcus aureus*.

Table 1: Minimum inhibitory concentration (MIC) and fractional inhibitory concentration index (FICI)

Organism	Enro MIC ($\mu\text{g/mL}$)	Catechin MIC (mg/mL)	Enro MIC (combo) ($\mu\text{g/mL}$)	Catechin MIC (combo) (mg/mL)	FICI	Interpretation
<i>E. coli</i>	0.286 ± 0.0489	1.188 ± 0.163	0.0664 ± 0.0112	0.240 ± 0.0343	0.45	Synergistic
<i>S. aureus</i>	0.306 ± 0.0515	0.639 ± 0.0845	0.0503 ± 0.0110	0.132 ± 0.0243	0.37	Synergistic

Table 2: Zone of inhibition (mm)

Organism	Enro MIC ($\mu\text{g/mL}$)	Catechin MIC (mg/mL)	Enro MIC (combo) ($\mu\text{g/mL}$)
<i>E. coli</i>	0.286 ± 0.0489	1.188 ± 0.163	0.0664 ± 0.0112
<i>S. aureus</i>	0.306 ± 0.0515	0.639 ± 0.0845	0.0503 ± 0.0110

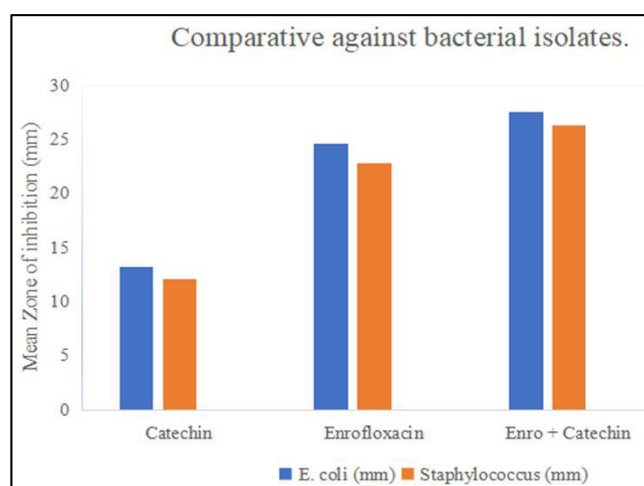


Fig 1: Comparative antibacterial activity of catechin, enrofloxacin and their combination against bacterial isolates.

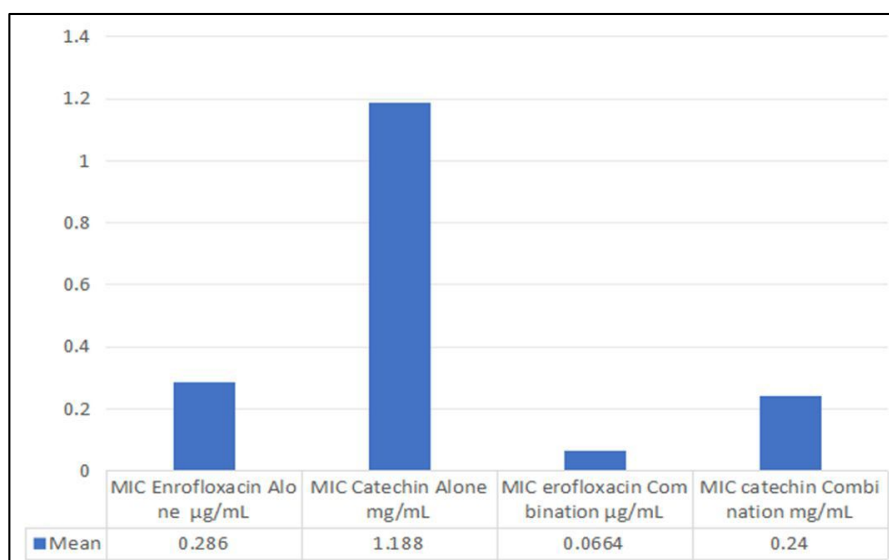


Fig 2: MIC values of enrofloxacin, catechin and their combination against *E. coli*

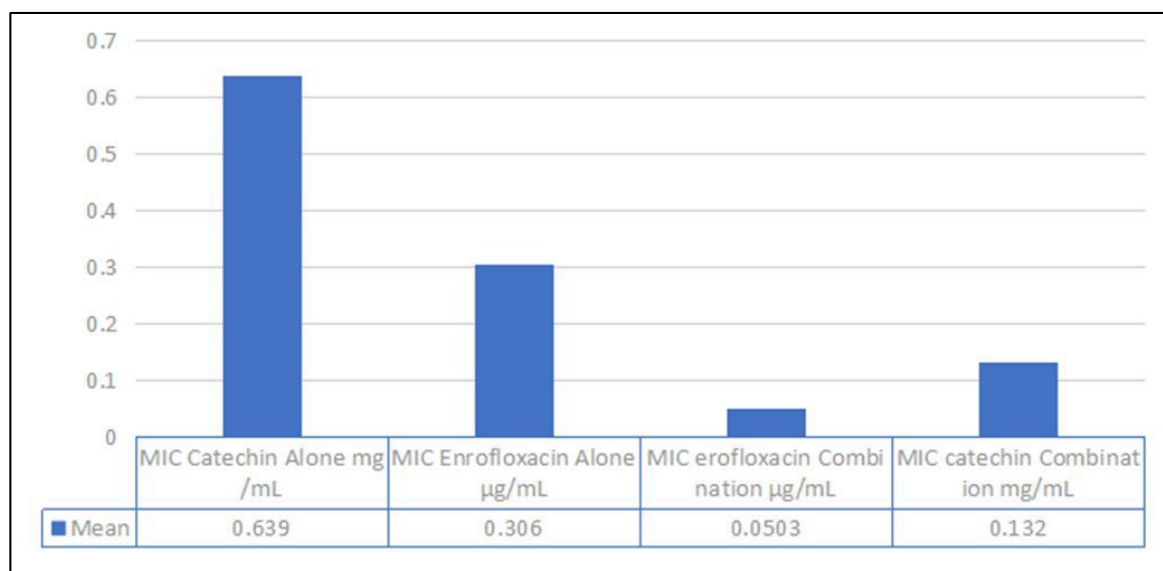


Fig 3: MIC values of enrofloxacin, catechin and their combination against *Staphylococcus aureus*.



Fig 4: Collection of Urine from Dog with Canine Prostatitis condition

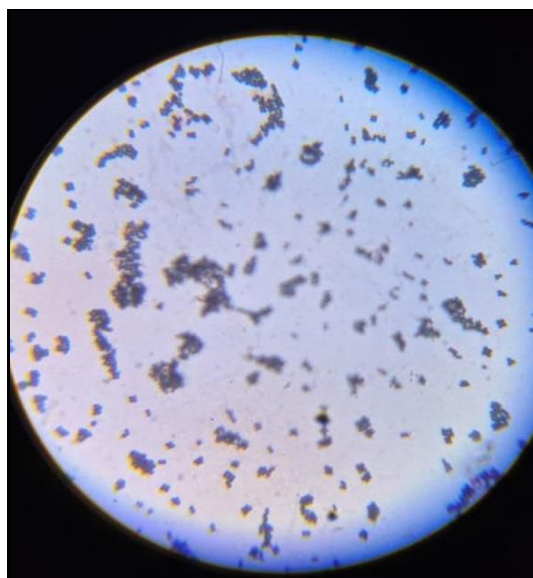


Fig 6: Microscopic Examination of *S. aureus* isolated from Urine Sample.

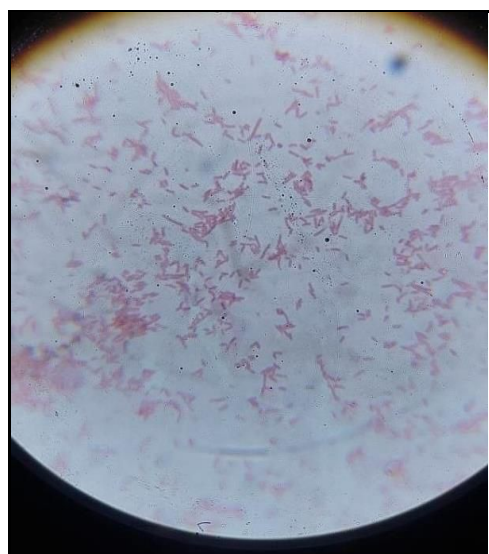


Fig 5: Microscopic Examination of *E. coli* isolated from Urine Sample.



Fig 7: Isolation of *E. coli* on EMB Agar.



Fig 8: Isolation of *S. aureus* on MSA.

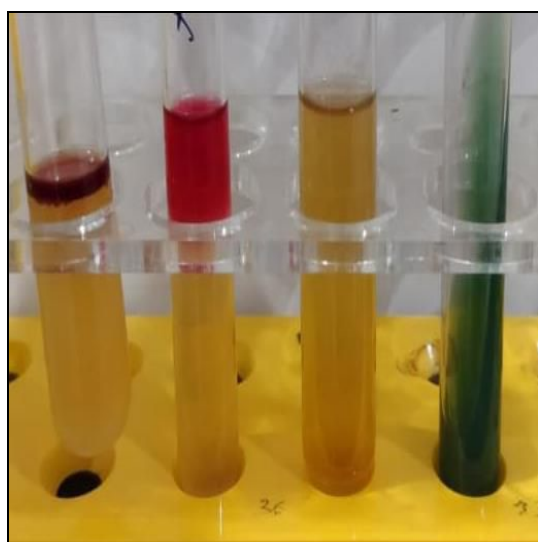


Fig 9 (a): *S. aureus* showing biochemical IMViC test, (Indole - Negative, Methyl Red -Positive, Voges-Proskauer Positive, Citrate test -Negative)

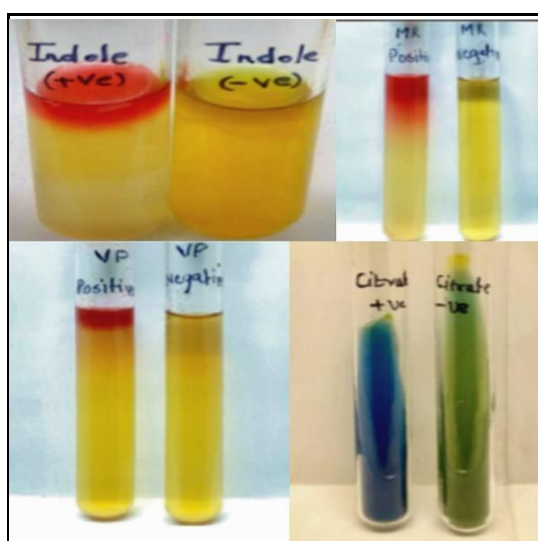


Fig 9. (b): *E. coli* showing biochemical IMViC test, (Indole - Positive, Methyl Red -Positive, Voges-Proskauer Negative, Citrate test -Negative)



Fig 10: AST of Enrofloxacin alone, Catechin alone and both in combination against *E. coli* using Agar Well Diffusion method.



Fig 11: AST of Enrofloxacin alone, Catechin alone and both in combination against *S. aureus* using Agar Well Diffusion method.

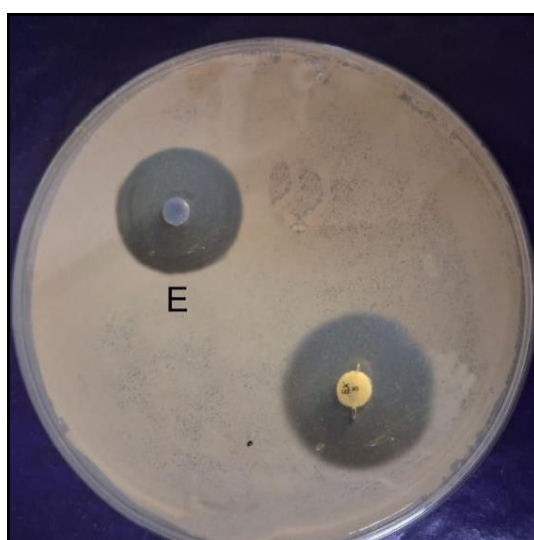


Fig 12: Comparative AST assesment of Enrofloxacin at a same dose using Agar gel well diffusion method and Disc diffusion method.

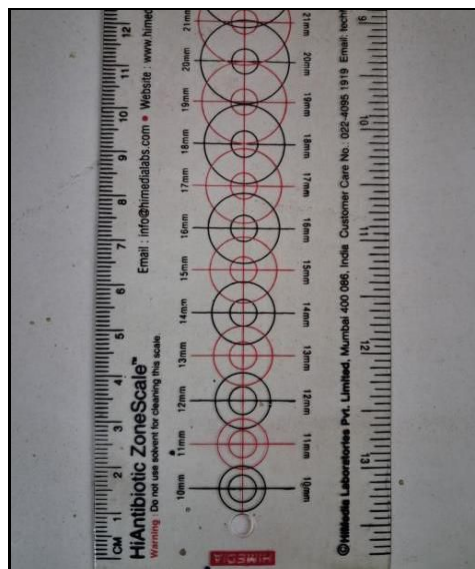


Fig 13: Antibiotic Zone Scale.

Discussion

The present study evaluated the *in vitro* antibacterial efficacy and synergistic interaction of enrofloxacin and catechin against bacterial isolates obtained from canine prostatitis. The findings demonstrated that the combination therapy exhibited enhanced antibacterial activity compared with either agent used alone.

The antibiotic susceptibility profile indicated that fluoroquinolone antibiotics, particularly ciprofloxacin, exhibited strong antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*. This observation is consistent with the known mechanism of action of fluoroquinolones, which inhibit bacterial DNA gyrase and topoisomerase IV [11]. However, variability in susceptibility among isolates highlights the emerging concern of antimicrobial resistance.

The agar well diffusion assay revealed that catechin exhibited moderate antibacterial activity, whereas enrofloxacin demonstrated strong antibacterial effects against both Gram-negative and Gram-positive isolates. The significantly larger zones of inhibition observed in the combination treatment indicate an enhanced antibacterial effect, suggesting a potential synergistic interaction between the two agents.

The minimum inhibitory concentration (MIC) analysis further confirmed these findings. Enrofloxacin exhibited low MIC values, indicating high antibacterial potency, whereas catechin showed comparatively higher MIC values when used alone. However, a marked reduction in MIC values was observed in the combination treatment. The approximately four-to six-fold decrease in MIC values suggests a substantial enhancement of antibacterial activity when both agents were used together.

The fractional inhibitory concentration index (FICI) values ranged between 0.25 and 0.50, confirming a synergistic interaction between enrofloxacin and catechin. According to standard interpretative criteria, FICI values ≤ 0.5 indicate synergy, demonstrating that the combined effect of the two agents is greater than the sum of their individual effects [12].

The observed synergistic effect may be attributed to multiple complementary mechanisms. Catechins are known to disrupt bacterial cell membrane integrity, leading to increased permeability and leakage of intracellular components [13]. This membrane-disrupting effect may facilitate enhanced penetration of enrofloxacin into bacterial cells, thereby

increasing its intracellular concentration and antibacterial efficacy.

In addition, catechins have been reported to inhibit bacterial efflux pumps, which play a crucial role in antimicrobial resistance by actively extruding antibiotics from bacterial cells [14]. Inhibition of efflux mechanisms may result in increased intracellular accumulation of enrofloxacin, thereby enhancing its antibacterial activity. Furthermore, catechins may interfere with bacterial enzyme systems and virulence factors, contributing to reduced bacterial survival.

From a pharmacodynamic perspective, fluoroquinolones exhibit concentration-dependent antibacterial activity, and their efficacy is closely associated with pharmacokinetic-pharmacodynamic indices such as C_{max}/MIC and AUC/MIC ratios [15]. The reduction in MIC values observed in the present study may improve these indices, thereby enhancing antibacterial efficacy and potentially reducing the likelihood of resistance development.

However, the findings of the present study are limited to *in vitro* conditions and require further *in vivo* validation. Overall, the results demonstrate that the combination of enrofloxacin and catechin exhibits superior antibacterial activity compared with either agent alone, suggesting that catechin may serve as a promising adjunct to conventional antimicrobial therapy.

Conclusion

The present study demonstrated that the combination of enrofloxacin and catechin exhibits significantly enhanced antibacterial activity compared with either agent alone. The observed reduction in MIC values and FICI values below 0.5 confirm a synergistic interaction between the two compounds.

These findings suggest that catechin may serve as a potential adjunct to conventional antimicrobial therapy, enhancing the antibacterial efficacy of enrofloxacin and potentially reducing the risk of antimicrobial resistance. Further *in vivo* and pharmacokinetic-pharmacodynamic studies are warranted to validate the clinical applicability of this combination in the management of bacterial infections in dogs.

Ethical Approval

The study was conducted in accordance with the ethical standards for veterinary clinical research. The experimental protocol was reviewed and approved by the Institutional Ethics Committee for Veterinary Clinical Research (IEC-VCR), Mumbai Veterinary College, Parel, Mumbai, under Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur (Approval No. IEC-VCR/01/2026).

All procedures involving animals were carried out under veterinary supervision, ensuring minimal stress and discomfort.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgement

The authors are thankful to the Department of Veterinary Pharmacology and Toxicology, Mumbai Veterinary College, Parel, Mumbai, under Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur, for providing the necessary facilities to carry out the research work.

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