Aarya Publishing House

Available Online at

http://www.aphinfo.com/ijmpbs

April-June 2024

INTERNATIONAL JOURNAL OF MEDICAL, PHARMACEUTICAL AND BIOLOGICAL SCIENCES

eISSN : 2832-787X, pISSN : 2832-7888

Research Article | Volume-4 | Issue-1 | Article ID: 0068

EVALUATION OF ANTIMICROBIAL ACTIVITY OF NANOGEL AGAINST PERIODONTAL DISEASE

Ashutosh Shukla, Rajeev Malviya* and Vishal Gupta

School of Pharmacy, Mansarovar Global University, Kolar Road, Bhopal (M.P.), India.

***Corresponding Author: Email:** rajeevrcp33@gmail.com

Received: 01 April 2024 / Revised: 23 May 2024 / Accepted: 20 June 2024 / Available online: 30 June 2024

ABSTRACT

Periodontal bacteria are the primary etiological agents responsible for periodontal disease, making antimicrobial therapy a rational treatment approach. Systemic antibiotics present several drawbacks, including erratic antibiotic concentration at the target site, low bioavailability, and drug resistance. Consequently, intra-pocket drug delivery systems have garnered significant interest for periodontal disease treatment. In this research, poloxamer 407 (P407) and carbopol 934P (CP 934P) were used as thermo-sensitive and pH-sensitive gelling polymers, respectively, for a dual-controlled release combination formulation containing doxycycline hyclate (DCN) and ketoprofen (KP). The aim of this study was to formulate and evaluate in situ gelling syringeable nanoemulgels and assess their antimicrobial activity against periodontal disease.

Keywords – *Nanogel, Periodontal Disease, Antimicrobial Activity, Poloxamer 407, Carbopol 934P, Doxycycline Hyclate, Ketoprofen, Intra-pocket Drug Delivery, Controlled Release.*

1. INTRODUCTION

Ketoprofen (KP), a member of the 2-arylpropionic acid class of non-steroidal anti-inflammatory drugs (NSAIDs), has demonstrated therapeutic value in treating periodontal disease by inhibiting the cyclooxygenase enzyme, which is responsible for prostaglandin biosynthesis [1]. Over recent years, there has been growing interest in using NSAIDs to prevent or control periodontal disease [2][3]. Topical applications of (S)-ketoprofen have been shown to reduce bone loss in the ligature model of periodontitis in beagles and primates [4]. An initial six-month preclinical pharmacology study demonstrated significant reductions in crevicular fluid prostaglandin E2 and leukotriene B4 in monkeys treated for ligature-induced periodontal bone loss with a 1.0% (w/w) KP cream formulation [5]. Subsequently, the pharmacokinetics and safety of KP in gel formulations were evaluated [6]. A local drug delivery system containing 1.5% KP gel, used in conjunction with scaling and root planning (SRP), was assessed for periodontal outcomes in subjects with chronic periodontitis [7]. Topically applied NSAIDs, such as KP, appear effective in reducing bone loss in the ligature model of periodontitis [8].

Doxycycline (DCN) exhibits broad-spectrum activity, being bacteriostatic at low concentrations and bactericidal at high concentrations. It binds to the soft and hard tissue walls of the periodontal pocket, creating a drug reservoir. DCN also has anti-collagenase activity against *Porphyromonas gingivalis*, human neutrophil collagenase, and human gingival fibroblasts, thereby reducing inflammation [9]. Eugenol, the principal component of clove oil (Eugenia aromatica), is well-known for its analgesic, local anesthetic, anti-inflammatory, and antibacterial effects. It is used in dental applications as cement, filler, and restorative material. Recognized as safe (GRAS) by the FDA, eugenol exhibits antibacterial activity against pathogens including *Escherichia coli, Listeria*

monocytogenes [10], and Helicobacter pylori [11], primarily by disrupting the cytoplasmic membrane [12]. Nanoemulsions are potential carriers for NSAID drugs like KP. They are pharmacokinetically stable liquid solutions with droplet diameters ranging from 10 to 100 nm. Nanoemulsions offer several advantages, including enhanced drug solubility, improved penetration into periodontal mucosa, reduced dosage, and fewer side effects compared to conventional formulations. Nanoemulsions can fuse with the outer membranes of microbes and disrupt them, effectively killing microorganisms. They have a broad spectrum of activity against bacteria such as *E. coli, S. typhi, and S. aureus* [13]. Due to their selective toxicity to microbes and non-irritant nature to mucous membranes, nanoemulsions are safe for periodontal treatment.

Eugenol has been selected as the oil phase in the development of the nanoemulsion. The clinical efficacy of such treatments depends on the drug release and mechanical properties of the formulation. Ideal formulations should be easily inserted into the periodontal pocket, show controlled release of the drug into the crevicular fluid, retain within the pocket for the desired period (without mechanical bonding to tooth surfaces), and be biodegradable, non-toxic, and non-irritant. Carbopol 934P (CP 934P) and Poloxamer 407 (P407) have been selected for periodontal delivery. CP 934P interacts with mucin-coated epithelial and tooth surfaces through specific interfacial forces, a process known as mucoadhesion [14].

Thermoreversible and in situ gelling systems are considered a technical approach to delivering NSAID drugs directly into the periodontal pocket for controlled/sustained release. This delivery system not only helps sustain effective NSAID delivery but also avoids first-pass metabolism and dose-related side effects.

This study describes the development and characterization of nanoemulgels (NEG) loaded with KP and DCN, designed for application to the periodontal pocket for the treatment of periodontitis.

2. MATERIALS AND METHODS

2.1 Materials

Doxycycline Hydrochloride was obtained as a gift sample from Ranbaxy Research Laboratories Ltd, Gurgaon, India. Ketoprofen (Matrix Laboratories Ltd. Hyderabad, India) and Eugenol oil (Arora and Company, New Delhi, India) used in the study were procured from authenticated source. All the chemicals and regents used were of laboratory grade.

2.2 Preparation of nanoemulgel (NEG)

To prepare the formulation, CP934P (0.25%, 0.50%, and 1% w/w) was initially dissolved in distilled water using a mechanical stirrer to ensure complete dissolution. Afterward, P407 (15%, 20%, and 25% w/w) was added to the gel mixture, and the mixture was stored at 4°C for 12 hours to ensure complete wetting of P407 using the cold method. Following storage, the mixture was stirred thoroughly to ensure uniform mixing of the two components. The optimized nanoemulsion loaded with the drug was then added dropwise to the sol system with continuous magnetic stirring. Finally, the mixture was neutralized with triethanolamine (TEA) and stored at 4°C for 24 hours to ensure stability and uniformity of the final formulation.

2.3 Evaluation of Antimicrobial Activity

The antibacterial activity of eugenol gel and NEG loaded with KP containing eugenol as oil phase was evaluated using the agar diffusion method (cup plate method). Staphylococcus aureus and Escherichia coli bacteria were used for the study. Nutrient agar media served as the culture medium for the antibacterial assay. Actively growing broth cultures of both microbes were prepared, and the turbidity was adjusted to contain approximately 10^8 CFU/mL. Sterilized molten nutrient agar was poured into sterilized Petri dishes and allowed to solidify. The plates were then swabbed with 100 µL of the microbial cultures. Uniform-sized cups of 6 mm diameter were aseptically punched into the seeded agar medium using a sterilized well borer at equidistant positions. The prepared

gel samples were filled into the cylinder cups and incubated at 37±0.5°C for 48 hours. The antibacterial activity was assessed by measuring the diameter (mm) of the zone of growth inhibition around each well. All tests were conducted in triplicate (n=3).

3. RESULTS AND DISCUSSION

The zone of growth inhibition for *Staphylococcus aureus and Escherichia coli* was studied using the agar-cup diffusion method. The NEG loaded with KP and DCN showed a more significant ($p<0.07$) antibacterial effect on S. aureus and E. coli, with zones of growth inhibition measuring 31.7 ± 0.27 mm and 30.6 ± 0.23 mm, respectively. In contrast, the zones of growth inhibition for eugenol gels alone were 8.7 ± 0.24 mm for S. aureus and 7.4 ± 0.22 mm for E. coli. The NEG containing eugenol as the oil phase demonstrated greater antibacterial activity compared to eugenol alone because, in NEG, eugenol was in nano droplet form, which can easily fuse with the outer membrane of the microbes. The surfactants in the NEG disrupt the external membrane, leading to the death of the microbes. The NEG formulation containing both drugs (KP and DCN), with eugenol present as one of the oils, showed synergistic antibacterial activity.

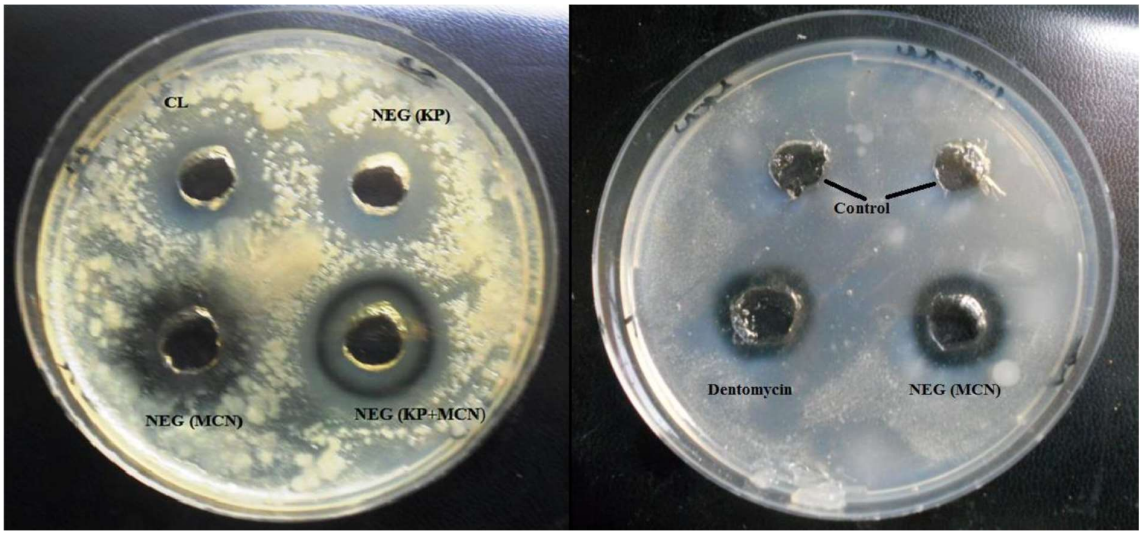

Fig. 1: Photographs showing comparative antibacterial activity of different optimized and marketed periodontal formulations on *S. aureus* and *E. coli*

Table 1: Antibacterial Efficacy of Different Periodontal Formulations on S. aureus and E. coli

S. No.	Formulation	Zone of Inhibition (mm) ± S.D.	
1	Eugenol gel (CL)	7.7 ± 0.24	6.4 ± 0.22
2	NEG1 without KP (Eugenol as oil phase)	10.7 ± 0.47	9.2 ± 0.27
3	NEG1 loaded with KP	10.6 ± 0.34	9.1 ± 0.36
4	NEG2 loaded with DCN	16.7 ± 0.62	13.2 ± 0.73
5	NEG loaded with DCN + KP	30.7 ± 0.27	29.6 ± 0.23
6	Dentomycin (marketed formulation)	17.7 ± 0.29	16.4 ± 0.87

[Eugenol oil showed synergistic antibacterial effect with DCN drug in NEG loaded with DCN + KP. Zone of Inhibition (mm) of NEG loaded with (DCN + KP) combination > sum of the NEG1 loaded with KP + NEG2 loaded with DCN]

The findings revealed that the nanoemulsion gel (NEG) loaded with ketoprofen (KP) and doxycycline (DCN) showed a significant antibacterial effect on S. aureus and E. coli, with notable zones of growth inhibition. In comparison to eugenol gels alone, the NEG formulations displayed superior antibacterial activity due to the nano-droplet form of eugenol, which facilitated its fusion with microbial membranes and the subsequent disruption of these membranes by surfactants. The synergistic effect of KP, DCN, and eugenol within the NEG further enhanced its antimicrobial efficacy.

Overall, this study underscores the promising application of nanoemulsion-based drug delivery systems in periodontal therapy. The ability of nanoemulsions to effectively deliver therapeutic agents directly to the periodontal pocket, along with their enhanced antimicrobial properties, positions them as a superior alternative to traditional treatment methods. Future research should focus on optimizing these formulations and conducting clinical trials to further validate their efficacy and safety in human subjects.

4. CONCLUSION

This research work highlights the significant potential of nanoemulsion based gel as a drug delivery system for the treatment of periodontal disease. Nanoemulsions are pharmacokinetically stable liquid solutions with droplet diameters ranging from 10 to 100 nm, offering several advantages over conventional formulations. These advantages include enhanced drug solubility, improved penetration into periodontal mucosa, reduced dosage amounts, and minimized side effects. The ability of nanoemulsions to fuse with the outer membrane of microbes and disrupt them with surfactants makes them highly effective in killing microorganisms. This study demonstrated the broad-spectrum antibacterial activity of nanoemulsions against bacteria such as *Escherichia coli, Salmonella typhae, and Staphylococcus aureus*.

REFERENCES:

1. Malyadri T. Formulation development and evaluation of Luliconazole Topical Emulgel. Int. J. Indig. Herbs Drugs. 2021;6:79–87.
2. Pagano C, Baiocchi C, Beccari T, et al. Emulgel loaded with flaxseed extracts as new therapeutic approach in wound treatment. Pharmaceutics. 2021;13:1107.
3. Permana AD, Utami RN, Layadi P, et al. Thermosensitive and mucoadhesive in situ ocular gel for effective local delivery and antifungal activity of itraconazole nanocrystal in the treatment of fungal keratitis. Int J Pharm. 2021;602:120623.
4. Javed H, Shah SN, Iqbal FM. Formulation development and evaluation of diphenhydramine nasal nano-emulgel. AAPS pharmscitech. 2018;19:1730–43.
5. da Silva Campelo M, Melo EO, Arrais SP, et al. Clove essential oil encapsulated on nanocarrier based on polysaccharide: A strategy for the treatment of vaginal candidiasis. Colloids Surf A Physicochem Eng Asp. 2021;610:125732.
6. Ramasamy M, Lee JH, Lee J. Direct one-pot synthesis of cinnamaldehyde immobilized on gold nanoparticles and their antibiofilm properties. Colloids Surf B Biointerfaces. 2017;160:639–48.
7. Dario MF, Oliveira CA, Cordeiro LR, et al. Stability and safety of quercetin-loaded cationic nanoemulsion: In vitro and in vivo assessments. Colloids Surf A Physicochem Eng Asp. 2016;506:591–9.
8. Torregrosa A, Ochoa-Andrade AT, Parente ME, et al. Development of an emulgel for the treatment of rosacea using quality by design approach. Drug Dev Ind Pharm. 2020;46:296–308.
9. Ammanage A, Rodriques P, Kempwade A, et al. Formulation and evaluation of buccal films of piroxicam co-crystals. Future J Pharm Sci. 2020;6:16.

10. Liu S, Jin MN, Quan YS, et al. Transdermal delivery of relatively high molecular weight drugs using novel self-dissolving microneedle arrays fabricated from hyaluronic acid and their characteristics and safety after application to the skin. Eur J Pharm Biopharm. 2014;86:267–76.
11. Draize JH. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther. 1944;82:377–90.
12. Ahad A, Al-Saleh AA, Al-Mohizea AM, et al. Pharmacodynamic study of eprosartan mesylate-loaded transfersomes Carbopol® gel under Dermaroller® on rats with methyl prednisolone acetate-induced hypertension. Biomed Pharmacother. 2017;89:177–84.
13. Dario MF, Oliveira CA, Cordeiro LR, et al. Nanoemulsions as Drug Delivery Systems: A Review of the Fundamentals, Challenges and Applications. Int J Nanomedicine. 2019;14:6135-6153.
14. Ahad A, Raish M, Al-Mohizea AM, et al. Formulation and evaluation of eprosartan mesylate-loaded transfersomes for transdermal delivery: Box-Behnken design and pharmacokinetic study. Artif Cells Nanomed Biotechnol. 2018;46(Suppl 3):725-736.