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Original Article

# Synthesis and Pharmacological Evaluation of Novel Formulation of Opiorphin and Lysozyme: An *in vitro* Study

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## ABSTRACT

**Background:** The antibacterial, anti-inflammatory and antioxidant properties of opiorphin and lysozyme are vital for health maintenance and disease prevention. Opiorphin reduces oxidative stress by neutralizing free radicals and boosting antioxidant enzymes. Lysozyme helps manage inflammation, supporting quicker healing in infections and injuries. These properties protect cellular components from damage, regulate inflammation and promote recovery. Understanding and utilizing these effects can enhance medical treatments and overall health. **Materials and Methods:** opiorphin is purchased in powder form and lysozyme is obtained from artificial saliva. Anti-inflammatory assay is performed with 0.2 mL of albumin and 3 mL of phosphate buffer in pH of 6.4+combination of opiorphin and lysozyme taken in different concentrations (20, 40, 60 ug/mL). Likewise, antioxidant assay with 28 mM of sodium phosphate, 0.6M sulphuric acid+, 4M of ammonium molybdate+opiorphin and lysozyme and anti-bacterial assay with freshly prepared nutrient broth of *Enterococcus faecalis* and *Streptococcus mutants*. **Results:** In anti-inflammatory assay, higher concentrations of the substance result in increased absorbance, indicating greater anti-inflammatory activity. Salicylic acid has the highest absorbance, suggesting it is the most effective in this assay. In an antioxidant assay, a minimal increase after increasing concentration is noted, measured by absorbance at 517 nm. Likewise, HC shows larger inhibition zones compared to LC. **Conclusion:** Lysozyme and opiorphin have numerous beneficial qualities, including antibacterial, anti-inflammatory and antioxidant ones. The findings are quite valuable. Although lysozyme and opiorphin alone have several advantages, when combined, they would improve or synergize the process. To create a medicine, research must be conducted in tandem to determine its pharmacophore and binding energy.

**Keywords:** Opiorphin, Lysozyme, Antioxidant property, Anti-inflammatory property, Antibacterial property.

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## INTRODUCTION

The anti-bacterial, anti-inflammatory and antioxidant properties of various compounds are integral to preserving health, preventing disease and innovating medical therapies.[1] These mechanisms are essential for combating infections, mitigating inflammatory responses and shielding cells from oxidative damage.[2] A deeper understanding and application of these attributes can pave the way for more holistic healthcare approaches, ultimately enhancing therapeutic efficacy and promoting optimal health outcomes.[1]

Opiorphin is a peptide synthesised in human saliva, blood, tears, sweat and all bodily excreting fluids, which has gathered interest due to its possible medical uses as an anti-inflammatory, antioxidant and antibacterial agent.[3] Its antioxidant properties may help fight oxidative stress, which is connected to aging and a number of ailments, while its anti-inflammatory properties may help treat chronic problems including arthritis and cardiovascular diseases.[4] Furthermore, opiorphin's antibacterial qualities show promise in the fight against infections, especially as antibiotic resistance becomes a more significant global issue.[5] Given its natural basis, there may be room for safer, biocompatible medicinal uses with fewer adverse effects.[6] Opiorphin research is particularly relevant to systemic and oral health.[7] Saliva contains it and may help prevent or treat oral diseases including gingivitis and dental cavities, while systemic absorption may have wider health advantages.[4] Opiorphin makes a strong argument for becoming a multipurpose medicinal substance when paired with its well-known pain-relieving properties.[8] Realizing its full potential may result in novel therapies for illnesses linked to oxidative stress, infections and inflammation.[3] The natural

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enzyme lysozyme, which is present in human secretions like saliva and tears, is crucial to its antibacterial qualities because it breaks down bacterial cell walls to prevent infections.[9] Its significance is increased by research into its antioxidant and anti-inflammatory qualities, which may aid in the management of inflammation and provide protection against oxidative stress, which is connected to aging and chronic illnesses.[2,10] The multifunctional potential of lysozyme provides a safe and natural option for treating infections, inflammation and oxidative damage in the face of antibiotic resistance, opening the door for novel therapeutic uses in biotechnology and medicine.[3,4]

By neutralizing free radicals, opiorphin can lessen oxidative stress in cells. This aids in preventing oxidative damage to lipids, proteins and DNA, among other biological constituents.[9] To further support its protective properties, it might increase the activity of endogenous antioxidant enzymes including catalase and Superoxide Dismutase (SOD). The anti-inflammatory properties of lysozyme may help control chronic inflammation.[9] Lysozyme helps speed up healing and lessens problems in situations like infections and accidents by regulating acute inflammation.[9] When used together, opiorphin and lysozyme may have synergistic effects as a multipurpose treatment strategy. Lysozyme's significant antibacterial activities and possible anti-inflammatory and antioxidant functions are enhanced by opiorphin, which is well-known for its pain-relieving, anti-inflammatory, antioxidant and antibacterial qualities.[9] By successfully fighting infections, lowering inflammation and guarding against oxidative stress, they may work in concert to improve the body's defenses. This combination has potential uses in systemic treatments, wound healing and oral health. Lysozyme fights bacterial infections and promotes general oral cleanliness, while opiorphin may ease pain and inflammation in the oral cavity.[3] By lowering inflammation and the bacterial load while minimizing oxidative damage, the combination may fasten tissue repair in wound healing.[11] Moreover, opiorphin and lysozyme are natural, biocompatible compounds that may provide safer substitutes for manufactured medications, which makes them an excellent starting point for the creation of novel, multi-targeted therapies.[3,9]

## MATERIALS AND METHODS

### Sample collection

Initially opiorphin and lysozyme were collected separately; opiorphin is purchased in a powder form and diluted in nuclease free water as per instruction and lysozyme was collected from artificial saliva via ultrafiltration method. Both materials were equally quantified and prepared for three different assays (i.e.; anti-bacterial, antioxidant and anti-inflammatory values). Anti-inflammatory assay: Primary part was to prepare reaction solution, i.e.; 0.2 mL of albumin with 3 mL of phosphate buffer in pH of 6.4 and combination of opiorphin and lysozyme taken in different concentrations (20, 40, 60 ug/mL) under absorbance at 660 nm. Sulphuric acid was taken as a control. Then inflammation inhibition rate was calculated, Inhibition(%)=100x(Vt/Vc-1). For antioxidant assay: Reaction solution for the assay is 28 mM of sodium phosphate, 0.6 M sulphuric acid and 4M of ammonium molybdate with opiorphin and lysozyme under absorbance at 517 nm. Increased absorbent shows increased antioxidant property. Ascorbic acid was taken as a control. Antibacterial assay is done in a freshly prepared nutrient broth *Enterococcus faecalis* and streptococcus, where opiorphin and lysozyme were cultured. Results were obtained by the amount of zone formation.

## RESULTS

The antibacterial properties of three compounds Antibiotics (Abs), Opiorphin and lysozyme in High Concentration (HC) and in lower Concentration (LC) (Abs, HC and LC) against *Streptococcus mutans* and *Enterococcus faecalis* (Figure 1). Bacterial growth is effectively inhibited by the clear zones surrounding HC and LC, with HC exhibiting bigger inhibition zones than LC (Figure 2).

The anti-inflammatory assay shows higher concentrations of the substance that result in increased absorbance, indicating greater anti-inflammatory activity. Salicylic acid, control group has the highest absorbance, suggesting it is the most effective in this assay (Figure 3).

$$\text{Inhibition } (\%)=100\times(Vt/Vc\text{-}1)$$

Antioxidant property was analysed by DPPH assay, in which ascorbic acid is taken as a control. Here opiorphin and lysozyme are taken in different concentrations like 4 uL/mL and 8 uL/mL. By increasing the concentration, it is showing better antioxidant values. This was measured by absorbance at 517 nm (Figure 4).

## DISCUSSION

Opiorphin's primary function is to reduce pain by preventing the breakdown of endogenous enkephalins, which are naturally occurring analgesics.[3] Its analgesic effects are complemented by additional antibacterial, anti-inflammatory and antioxidant properties. Opiorphin treats several facets of tissue damage and pain by lowering oxidative stress, inflammation and infection.[13] These characteristics broaden its potential applications beyond pain to include infections, chronic inflammatory illnesses and healing problems.[12,13] Opiorphin when used in combination with another protein will synergise and provide effective results.[14] In our study, we have taken opiorphin in combination with lysozyme. Lysozymes have been taken because of its antimicrobial resources. Additionally, they neutralize free radicals, minimize oxidative damage and help reduce inflammation, aiding the healing process. These combined effects make opiorphin and lysozymes valuable in maintaining health and addressing various biological challenges.

Previous study done by Subhadeep Chakrabarti *et al.*, stated bioactive peptides derived from dietary proteins have been assessed for their positive benefits, such as antioxidant and anti-inflammatory properties, which are important in the treatment of chronic illnesses associated with inflammation and oxidative stress.[14] Another study done by Giada Meogrossi *et al*, stated structural changes of Antimicrobial Peptides (AMPs) increase their effectiveness and resistance to proteases, giving them a flexible tool against bacteria[15] Peptides of marine origins, such those found in oyster soft tissue, have shown strong anti-inflammatory and antioxidant properties, underscoring the potential of peptides as a treatment for inflammation and oxidative stress was proposed by Bingjun Qian *et al.*[16]

In our study, Antibiotics (Abs), opiorphin and lysozyme, both at High Concentrations (HC) and at Lower Concentrations (LC), have antibacterial qualities against *Streptococcus mutans* and *Enterococcus faecalis*. The clear zones surrounding HC and LC, with HC showing larger inhibition zones than LC, efficiently limit bacterial growth. Abs do not exhibit any observable antibacterial qualities. The anti-inflammatory assay demonstrates that greater absorbance at higher doses of the drug indicates stronger anti-inflammatory activity. With the maximum absorption, salicylic acid, the control group, appears to be the most successful in this test. Ascorbic acid was used as a control in the DPPH experiment, which was used to analyze antioxidant properties. Lysozyme and opiorphin are taken at varying concentrations here,

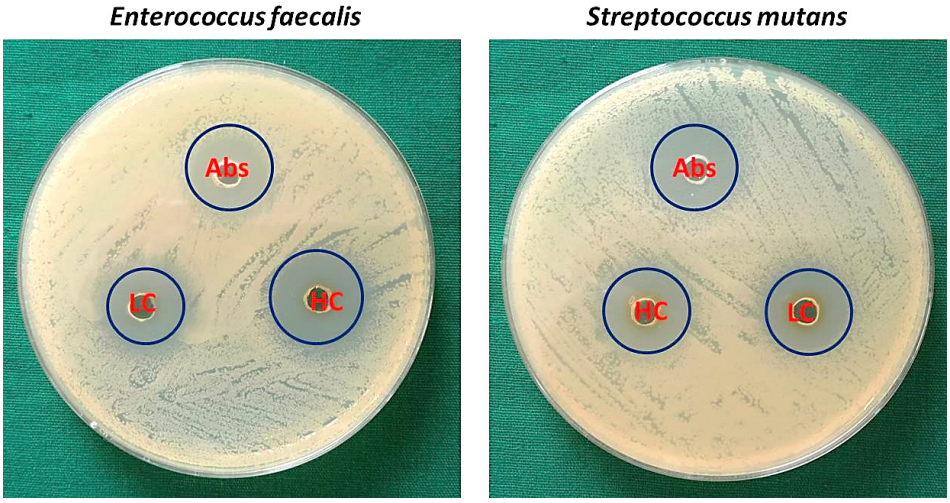


**Figure 1:** An indication of the antibacterial activity of a treatment against the tested bacterial strain (*Enterococcus faecalis* in the left and *Streptococcus mutans* in the right side) is the size of the clear zone (zone of inhibition) surrounding each sample. i.e, control group (Abs), Higher Concentration (HC) and Lower Concentration (LC).

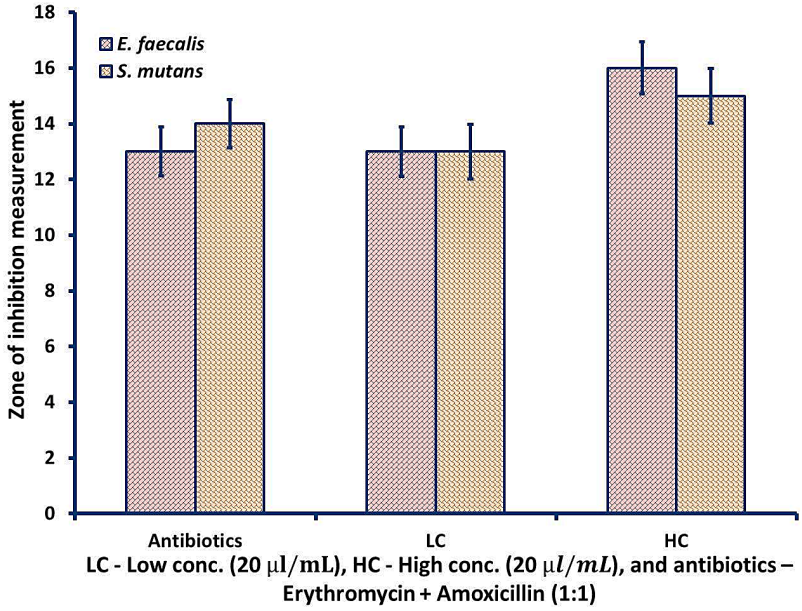


**Figure 2:** Table showing results of zone formation in mm when bacteria like *Enterococcus faecalis* and *streptococcus mutans* come in contact with opiorphin and lysozyme.

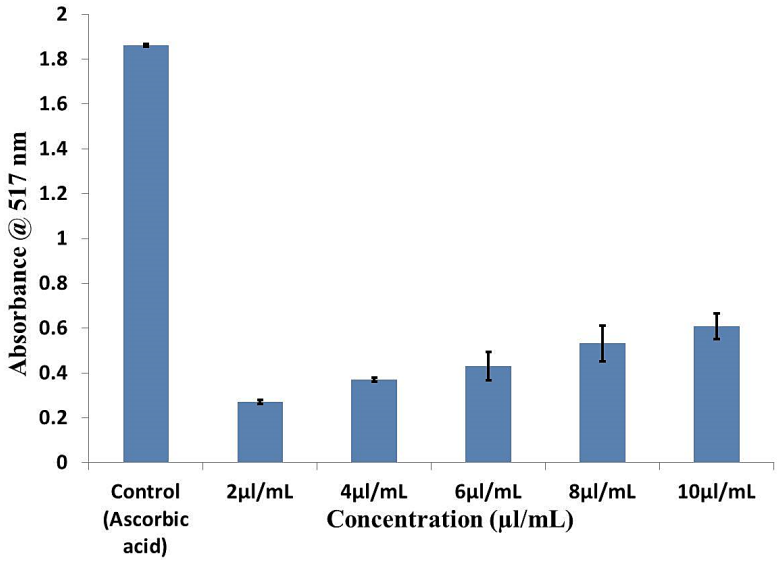


**Figure 3:** The graph shows the anti-inflammatory activity of different concentrations (2, 4, 6, 8 and 10 µL/mL) of a substance compared to ascorbic acid, measured by absorbance at 660 nm during BSA denaturation.

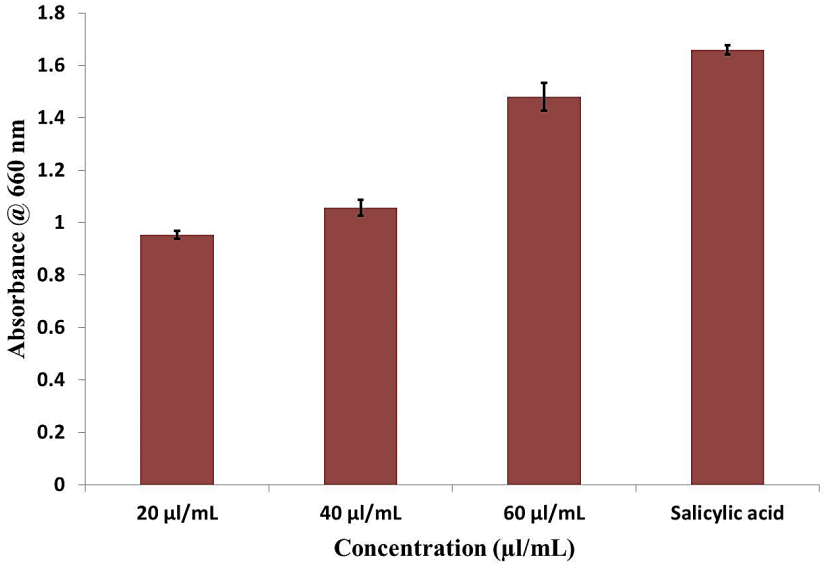


**Figure 4:** The graph shows antioxidant property at the concentration of 4 uL/mL, 8 uL/mL showing a minimal increase after increasing the concentration, measured by absorbance at 517 nm.

such as 4 and 8 uL/mL. It offers a straightforward comparison of their effectiveness and backs up the findings with tests like the DPPH assay, inhibitory zone and anti-inflammatory absorbance. Together by synergistic it could exhibit a better treatment value, As the values show better anti-inflammatory, antibacterial and antioxidant value when it comes in combination. Study done by Sahib *et al*, with MgO nanoparticles showed good results by inhibiting bacteria (*S. epidermidis, B. subtilis*). Opiorphin neutralizes free radicals, thereby reducing oxidative stress and protecting cellular components, while its modulation of pro-inflammatory cytokines helps manage inflammation and promote tissue healing. Though primarily known for pain modulation, opiorphin also shows potential antibacterial effects by disrupting bacterial cell membranes. Lysozyme, a key player in immune defense, excels in breaking down bacterial cell walls, especially in Gram-positive bacteria, leading to cell lysis and death. It also possesses antioxidant capabilities that protect against oxidative damage and anti-inflammatory properties that modulate cytokine production and regulate immune responses.

## CONCLUSION

Opiorphin and lysozyme serve many valuable properties like anti-bacterial, anti-inflammatory and antioxidant properties. The results show significant value. Opiorphin and lysozyme by themselves have many properties but in combination it would

enhance or synergise the reaction. Research must be done in combination to know its binding energy and pharmacophore to fabricate as a drug.

## LIMITATIONS

More bacteria can be evaluated to know the mechanism and resistance of the drug combination. Levels of concentration can be increased to get better values. Research can be made of opiorphin with some other combination and evaluate the better properties out of it.

## CONFLICT OF INTEREST

The authors declare no Conflict of interest.

## ABBREVIATIONS

**LC:** Low concentration; **HC:** High concentration; **Abs:** Antibiotics; **BSA:** Bovine serum albumin; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl assay; **AMPs:** Antimicrobial peptides.

## SUMMARY

Opiorphin and lysozyme exhibit antibacterial, anti-inflammatory and antioxidant properties crucial for health and disease prevention. Lysozyme helps with inflammation management and repair, while opiorphin neutralizes free radicals to lower oxidative stress. In this work, the biological activities of lysozyme (from artificial saliva) and opiorphin (in powder form) were evaluated utilizing assays for antibacterial, antioxidant and anti-inflammatory properties. Higher doses improved anti-inflammatory efficacy, according to the results, but salicylic acid was still the most effective. At greater dosages, antibacterial tests showed bigger inhibition zones, while antioxidant activity increased only slightly. The potential advantages of combining opiorphin and lysozyme for enhanced therapeutic effects are highlighted by these studies. Investigating their pharmacological characteristics and potential for medication development will require more study.

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