



RESEARCH ARTICLE

EFFECT OF TETRACYCLINE, DOXYCYCLINE AND TRIPHALA ON COLLAGEN MEMBRANE DEGRADATION: AN IN VITRO STUDY**Sukanya Ampili¹, Naveen Vital Kumar Gidijala^{2*}, Tejaswin Polepalle³, Shiva Shankar Gummaluri⁴, Trinath Kishore Damera⁵, Bhargavi Devi Vedula⁶**

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ABSTRACT

Background: Collagen membranes are widely used in periodontal regeneration. However, strategies to enhance membrane stability include the incorporation of antimicrobial agents or natural inhibitors of collagenase activity. Hence, present study aimed to evaluate and compare the efficacy of tetracycline hydrochloride, doxycycline hyclate, and Triphala in delaying collagen membrane degradation at varying concentrations in an in vitro model.

Methods: Present invitro analysis used Healiguide collagen membranes. They were treated with tetracycline (TTC), doxycycline (DOX), and Triphala (TRI) at concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml. Collagenase enzyme (Clostridial collagenase, HiMedia Laboratories, India) was used to induce degradation. A total of 48 specimens were used, with collagen degradation assessed at 4-time intervals (Days 2, 7, 14, and 21) using spectrophotometric analysis. Statistical analysis was performed using ANOVA with post hoc Tukey's tests to compare among the groups and $p < 0.05$ considered significant.

Results: All tested agents significantly reduced collagen degradation compared to the control group ($p < 0.05$). Doxycycline at 25 mg/ml and 75 mg/ml exhibited the most pronounced inhibition of collagen breakdown, showing statistically significant differences from the control. Tetracycline was also effective, particularly at 75 mg/ml, while Triphala at 50 mg/ml and 75 mg/ml demonstrated moderate inhibition but did not surpass the efficacy of doxycycline.

Conclusion: Present study concludes doxycycline as the most effective in delaying collagen membrane degradation, followed by tetracycline and Triphala. The given growing concern over antibiotic resistance, Triphala may serve as a promising natural alternative for membrane stabilization in periodontal applications.

Keywords: Collagen Membrane, Doxycycline, Matrix Metalloproteinases, Periodontal Regeneration, Tetracycline, Triphala

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INTRODUCTION

Achieving periodontal regeneration involves restoring lost structures in the periodontium using specialized materials like grafts, growth factors, and barrier membranes. In periodontal therapy, both non-resorbable and resorbable membranes have been employed to aid in healing. Expanded polytetrafluoroethylene (e-PTFE), a widely used non-resorbable membrane, requires surgical removal, which poses drawbacks such as the need for a secondary surgery. To address this, resorbable membranes made from poly-lactic acid, polyglycolic acid, polyurethane, and collagen are designed to naturally break down in the body over time, enhancing convenience and reducing surgical intervention.^{1, 2} Collagen-based membranes are widely preferred because they are biocompatible, have low immunogenicity, and support the migration and differentiation of cells needed for healing.³

Collagen membranes can degrade prematurely due to enzymes like matrix metalloproteinases (MMPs), which are released at the surgical site during healing. To increase membrane longevity and improve outcomes, various methods like stabilizing collagen structure through cross linking or adding antimicrobial agents to membranes have been used. These techniques aim to control MMP activity and maintain membrane integrity.^{4, 5} For example, tetracycline hydrochloride (TTC) has shown effectiveness in slowing down membrane degradation, extending the healing process and supporting regeneration.⁶ Doxycycline hyclate, a derivative of tetracycline, has shown significant antimicrobial effects against periodontal pathogens and inhibits collagenase, a factor that otherwise contributes to rapid degradation of collagen membranes.⁷

However, concerns about antibiotic resistance due to long-term antibiotic use have led researchers to explore safer alternatives like herbal medicine. Triphala, a combination of *Emblica officinalis*, *Terminalia chebula*, and *Terminalia bellerica*, is a traditional Ayurvedic formulation known for its collagenase-inhibitory properties.^{8, 9} Each component of Triphala has shown promising results in reducing collagenase activity, with *Terminalia chebula* identified as a potent collagenase inhibitor. Thus, present study aimed to evaluate the impact of tetracycline hydrochloride, doxycycline hyclate, and Triphala at various concentrations on the degradation of collagen membranes by collagenase enzyme in a laboratory setting.

MATERIALS AND METHODS

Sample Size Estimation

As there are no previous studies guiding for calculating sample size, we have taken a sample of 12 per group

with total sample size of 48.¹⁰ This sample size ensured adequate statistical power to detect significant differences in collagen membrane degradation among the study groups across multiple time intervals. THIS study was conducted at GITAM Dental College and Hospital, Visakhapatnam, following approval from the Institutional review board.

Preparation of Collagen Membranes:

5 mm diameter discs were created from bioabsorbable Healiguide collagen membrane for testing.

Preparation of Phosphate Buffer Solution (PBS) and Drug Solutions:

Phosphate buffer solution was prepared by mixing sodium chloride, potassium chloride, disodium phosphate, and monopotassium phosphate in distilled water, then adjusting the pH to 7.4. Tetracycline hydrochloride, doxycycline hyclate, and Triphala powder were dissolved in PBS to create solutions at 25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml concentrations.



Figure 1. Depict the armamentarium of the current study that shows the Healiguide membrane, doxycycline (Yellow tablets), Tetracycline (pink tablets), Triphala Churna box, collagenase enzyme, photo-colorimeter, micropipette (green colour), cuvettes etc.

Collagenase Enzyme Preparation:

Collagenase enzyme from Hi-Media laboratories was used. A solution with 1 mg of collagenase (containing 125 collagen digestion units) in PBS was prepared, and 120 µl of this solution was applied to each test sample.

Study Groups:

- Group 1: Control group
- Group 2 (Test Group 1): Tetracycline-treated group
- Group 3 (Test Group 2): Doxycycline-treated group
- Group 4 (Test Group 3): Triphala-treated group

Each test group received collagenase enzyme, and discs were incubated with PBS, tetracycline, doxycycline, or Triphala at four concentrations (25 mg/ml, 50 mg/ml, 75 mg/ml, 100 mg/ml) at 4°C for 24 hours.



Figure 2. Depict the 5mm collagen membrane discs that were used in the study

Incubation of Collagen Membrane in Collagenase

After initial incubation, collagenase enzyme solution (15 collagen digestion units per 1 ml PBS) was added to samples. Degradation of the collagen membrane was assessed at four time points: days 2, 7, 14, and 21. Using spectrophotometric analysis, protein release at each interval was measured, reflecting the degree of collagen degradation over time.

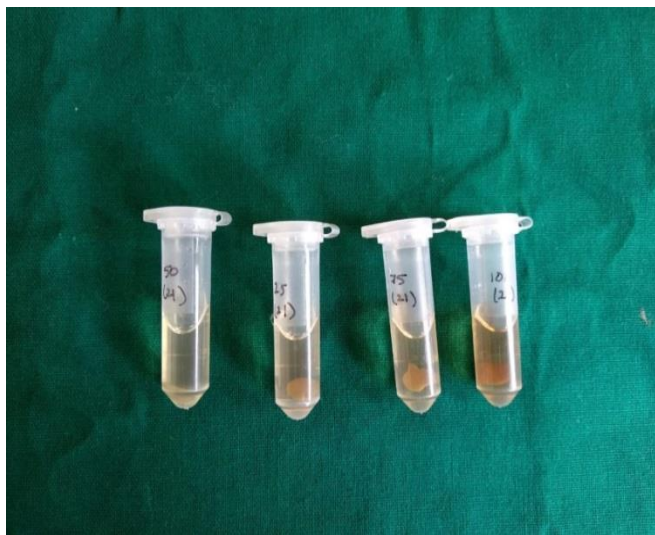


Figure 3. Depict the incubation of collagen membranes in the drugs

Spectrophotometric Analysis

A protein solution containing 10 to 100 µg protein in a volume of up to 1 ml was pipetted into a series of test tubes. The volume in the test tube was adjusted to 1 ml with acetate buffer. 5 ml of diluted reagent was added

to the test tube and the contents were mixed by vortexing. The absorbance at 595 nm was measured against a reagent blank prepared from 1 ml of buffer and 5 ml of protein reagent. A calibration curve was constructed with a concentration of protein (µg) against the corresponding absorbance and the amount of protein in the unknown sample was determined.

The test samples to be examined were added to a separate test tube followed by the addition of 5.0 mL of Coomassie blue to each tube and mixed. The total solution was transferred into a cuvette for analysis of protein. The spectrophotometer was adjusted to a wavelength of 595 nm and the amount of protein in the medium was analyzed as changes in the optical density at 595nm wavelength (OD595). Protein concentrations of the test samples were calculated on days 2, 7, 14, and 21. The data collected were statistically analyzed.



Figure 4. Depict the photo-colorimeter that was used in the current study

Statistical Analysis

Study data were entered in Excel and analysis was done using SPSS version 20, with ANOVA and post hoc Tukey's tests for pair-wise comparisons. A p-value < 0.05 was considered significant. All test groups, including those treated with tetracycline, doxycycline, and Triphala, showed reduced protein release compared to the control.

RESULTS

All concentrations of tetracycline with collagenase significantly reduced protein release, indicating inhibited collagen degradation. Among doxycycline-treated samples, all concentrations similarly showed a decrease in protein release, particularly at 25 mg/ml and 75 mg/ml, where differences from the control were statistically significant.

Table 1. Depict the mean collagen degradation/ protein release in Control and Tetracycline Groups at different time intervals and concentrations

Concentration (mg/ml)	Time Interval	Control Group (Mean Protein Release, SD)	Tetracycline Group (Mean Protein Release, SD)	p-value
25	Day 2	95.20 (±10.3)	38.75 (±7.27)	0.001*
	Day 7	102.30 (±9.7)	41.60 (±8.6)	0.001*
	Day 14	110.40 (±11.5)	42.30 (±7.8)	0.001*
	Day 21	118.10 (±10.2)	44.50 (±8.2)	0.001*
50	Day 2	95.20 (±10.3)	38.50 (±13.3)	0.044*
	Day 7	102.30 (±9.7)	40.20 (±12.4)	0.044*
	Day 14	110.40 (±11.5)	42.10 (±11.3)	0.044*
	Day 21	118.10 (±10.2)	43.70 (±10.2)	0.044*
75	Day 2	95.20 (±10.3)	47.50 (±9.1)	0.007*
	Day 7	102.30 (±9.7)	48.10 (±8.8)	0.007*
	Day 14	110.40 (±11.5)	49.00 (±9.0)	0.007*
	Day 21	118.10 (±10.2)	50.30 (±9.3)	0.007*
100	Day 2	95.20 (±10.3)	49.00 (±8.7)	0.024*
	Day 7	102.30 (±9.7)	50.20 (±8.4)	0.024*
	Day 14	110.40 (±11.5)	51.30 (±8.9)	0.024*
	Day 21	118.10 (±10.2)	52.00 (±8.7)	0.024*

mg/ml- milligrams/ millilitres, SD- standard deviation, * indicates significance, # indicates non-significant, p value <0.05 was considered statistically significant

For Triphala-treated samples, 50 mg/ml and 75 mg/ml concentrations significantly lowered protein release with collagenase. Comparisons between groups revealed that at 75 mg/ml, both tetracycline and Triphala were significantly different from the control (p = 0.046), with doxycycline at 75 mg/ml showing significant inhibition of degradation as well (p = 0.018).

Table 2. depict the significance regarding the protein release/ collagen degradation in Control and Doxycycline Groups at different time periods and concentration

Concentration (mg/ml)	Time Interval	Control Group (Mean Protein Release ±SD)	Doxycycline Group (Mean Protein Release ± SD)	p-value
25	Day 2	95.20 (±10.3)	52.00 (±16.7)	0.006*
	Day 7	102.30 (±9.7)	53.50 (±15.3)	0.006*
	Day 14	110.40 (±11.5)	54.20 (±14.7)	0.006*
	Day 21	118.10 (±10.2)	55.00 (±15.0)	0.006*
50	Day 2	95.20 (±10.3)	58.50 (±17.6)	0.008*
	Day 7	102.30 (±9.7)	59.30 (±16.4)	0.008*
	Day 14	110.40 (±11.5)	60.10 (±15.9)	0.008*
	Day 21	118.10 (±10.2)	61.00 (±16.2)	0.008*
75	Day 2	95.20 (±10.3)	63.00 (±14.1)	0.008*
	Day 7	102.30 (±9.7)	63.80 (±13.7)	0.008*
	Day 14	110.40 (±11.5)	64.60 (±13.9)	0.008*
	Day 21	118.10 (±10.2)	65.20 (±14.0)	0.008*
100	Day 2	95.20 (±10.3)	62.00 (±22.3)	0.003*
	Day 7	102.30 (±9.7)	63.50 (±21.1)	0.003*
	Day 14	110.40 (±11.5)	64.10 (±20.8)	0.003*
	Day 21	118.10 (±10.2)	65.30 (±20.4)	0.003*

mg/ml- milligrams/ millilitres, SD- standard deviation, * indicates significance, # indicates non-significant, p value <0.05 was considered statistically significant

Table 3. Depict the significant mean protein release/ collagen degradation in Control and Triphala Groups at different concentrations and time periods

Concentration (mg/ml)	Time Interval	Control Group (Mean Protein Release ±SD)	Triphala Group (Mean Protein Release±SD)	p-value
25	Day 2	95.20 (±10.3)	58.50 (±39.6)	0.138#
	Day 7	102.30 (±9.7)	60.10 (±37.2)	0.138#
	Day 14	110.40 (±11.5)	61.30 (±36.7)	0.138#
	Day 21	118.10 (±10.2)	62.50 (±37.8)	0.138#
50	Day 2	95.20 (±10.3)	58.50 (±31.5)	0.055
	Day 7	102.30 (±9.7)	59.70 (±30.1)	0.055
	Day 14	110.40 (±11.5)	60.80 (±30.8)	0.055
	Day 21	118.10 (±10.2)	61.70 (±31.4)	0.055
75	Day 2	95.20 (±10.3)	47.50 (±14.3)	0.003*
	Day 7	102.30 (±9.7)	48.70 (±13.7)	0.003*
	Day 14	110.40 (±11.5)	49.60 (±13.9)	0.003*
	Day 21	118.10 (±10.2)	50.50 (±14.0)	0.003*
100	Day 2	95.20 (±10.3)	65.50 (±29.4)	0.184#
	Day 7	102.30 (±9.7)	66.30 (±28.8)	0.184#
	Day 14	110.40 (±11.5)	67.00 (±28.2)	0.184#
	Day 21	118.10 (±10.2)	67.50 (±28.4)	0.184#

mg/ml- milligrams/ millilitres, SD- standard deviation, * indicates significance, # indicates non significant, p value <0.05 was considered statistically significant

Collagen Degradation over Time:

Day 2: Early collagen degradation was observed across all groups, with control showing the highest protein release, indicating active degradation. The test groups, particularly doxycycline and tetracycline, showed minimal protein release, indicating initial effectiveness in inhibiting collagenase activity. **Day 7:** By the seventh day, degradation in the control group continued to increase, while the test groups maintained relatively stable protein release levels, especially at higher concentrations (50 mg/ml and 75 mg/ml). Notably, Triphala at 50 mg/ml and 75 mg/ml showed strong inhibition by this stage.

Day 14: The control group experienced a steady increase in collagen breakdown, with a marked difference in protein release compared to all test groups. The tetracycline and doxycycline groups continued to show lower degradation, and Triphala’s effects were sustained at 50 mg/ml and 75 mg/ml, reflecting its mid-term stability.

Day 21: At this final time point, significant degradation was noted in the control group, whereas the doxycycline and tetracycline groups displayed the most sustained inhibition of degradation. Triphala continued to show effectiveness, particularly at 75 mg/ml, supporting its potential for longer-term collagenase inhibition. (Tables 1 to 4)

Table 4. Depict the significant inter group Comparison among Triphala, Doxycycline, and Tetracycline Groups at different concentrations

Concentration (mg/ml)	Group Comparison	Mean Difference	p-value
25 mg/ml	Tetracycline vs. Doxycycline	-13.25	0.922#
	Tetracycline vs. Triphala	-19.75	0.790#
	Doxycycline vs. Triphala	-6.50	0.990#
50 mg/ml	Tetracycline vs. Doxycycline	-20.00	0.752#
	Tetracycline vs. Triphala	-20.00	0.752#
	Doxycycline vs. Triphala	0.00	1.000#
75 mg/ml	Tetracycline vs. Doxycycline	-15.50	0.787#
	Tetracycline vs. Triphala	0.00	1.000#
	Doxycycline vs. Triphala	15.50	0.787#
100 mg/ml	Tetracycline vs. Doxycycline	-13.00	0.912#
	Tetracycline vs. Triphala	-16.50	0.839#
	Doxycycline vs. Triphala	-3.50	0.998#

mg/ml- milligrams/ millilitres, SD- standard deviation, * indicates significance, # indicates non-significant, p value <0.05 was considered statistically significant

DISCUSSION

An effective periodontal regeneration membrane should be durable, biocompatible, and facilitate healing with minimal complications. Collagen membranes, commonly used in this role, degrade under the influence of collagenase enzymes, potentially shortening their functional period. Studies by Hutmacher and Hyder demonstrate that these membranes perform best when degradation is controlled, making collagenase inhibition a key focus in improving regeneration.¹¹

In this study, the use of tetracycline in varying concentrations (especially at 75 mg/ml) effectively slowed membrane degradation, corroborating findings from previous studies. Moses et al. and Zohar et al. found that collagen membranes treated with tetracycline at 50 mg/ml resisted degradation, providing a protective effect through MMP inhibition.^{12, 13}

Doxycycline is another widely used antibiotic for periodontal treatment, where it is known to inhibit collagenase and MMPs. Studies by Kutan et al. highlight doxycycline's osteogenic and antimicrobial properties when applied to collagen membranes, supporting healing and protecting against infections.¹⁴

In the current study, doxycycline at 25 mg/ml and 75 mg/ml significantly delayed degradation, aligning with Chang et al.'s findings on its anti-collagenase efficacy.¹⁵ Our results indicate that 75 mg/ml doxycycline concentration is particularly effective in stabilizing collagen membranes.

Triphala, a natural compound used traditionally in Ayurveda, has shown promising anti-collagenase properties. Zaveri et al. and Nair et al. demonstrated Triphala's role in enhancing periodontal healing by inhibiting collagenase activity.^{16, 17} The tannins in Triphala, particularly from Terminalia chebula, possess strong anti-collagenolytic properties, which contribute to delayed membrane degradation.¹⁸ The presence of bioactive compounds, such as phenols and flavonoids, further supports its potential as a natural inhibitor of collagen breakdown.

In the current study, Triphala at 50 mg/ml and 75 mg/ml significantly reduced membrane degradation in the presence of collagenase. The findings align with Nair et al., emphasizing Triphala's potential in periodontal healing. Although Triphala was effective, it did not surpass the performance of tetracycline or doxycycline at any concentration, possibly due to differences in the mechanism of action and bio-availability of its active components.

Triphala offers an appealing alternative to antibiotics, particularly for patients concerned about resistance or side effects.

Future studies could explore lower concentrations of Triphala and its use in combination with other bioactive agents to optimize its effects on collagen membranes.

This study's limitations include its restriction to a single type of bioresorbable membrane and the testing of only four concentrations. Further research on various membrane types and Triphala's efficacy at different doses would provide broader insights. Additionally, examining collagen membrane structure under different treatments microscopically could add valuable details about degradation patterns.

CONCLUSION

Present study concludes that tetracycline, doxycycline, and Triphala in various concentrations effectively slow down collagen membrane degradation when collagenase is present. Doxycycline exhibited the highest efficacy, particularly at 25 mg/ml and 75 mg/ml concentrations, followed by tetracycline and Triphala. Triphala's results support its potential as a natural, antibiotic-free option in periodontal therapy, though additional studies are needed to maximize its clinical application.

DECLARATIONS

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Conflicts of interest and financial disclosures: The authors declare no conflict of interest and there was no external source of funding

Ethical approval

Approval for the conduction of the study was obtained from the Institutional Review Board GITAM Dental College and Hospital, Visakhapatnam, Andhra Pradesh, India.

Informed Consent

Not Applicable

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