Biomedical Sciences and Clinical Medicine 2024;63(2):108-113.
doi: 10.12982/BSCM.2024.14
https://heo1.tci-thaijo.org/index.php/CMMJ-MedCMJ/index

BSCM
Biomedical Sciences and Clinical Medicine

Original Article

Open Access

Exploring Synergies of Lotus Seed Extract-Hyaluronic Acid Gel for Enhanced Local Drug Delivery

Kaviyaselvi Gurumurthy, Nidhita Suresh and Saranya K

Department of Periodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS) Saveetha University, Chennai, Tamil Nadu, India

Correspondence:
Nidhita Suresh, MDS,
Department of Periodontics,
Saveetha Dental College,
Saveetha Institute of Medical and Technical Sciences (SIMATS)
Saveetha University, Chennai,
Tamil Nadu, India.
Email: nidhitas.sdc@saveetha.com

Received: January 23, 2024;
Revised: March 15, 2024;
Accepted: March 20, 2024

ABSTRACT

OBJECTIVE The plant species *Nelumbo nucifera* (lotus) is widely used in traditional medicine and is known to contain flavonoids, alkaloids, and other polyphenols which contribute to its potent antioxidant, anti-inflammatory properties in addition to its being a remedy for cardiac diseases. Hyaluronic acid, present in skin and connective tissue, is well known for its tissue regeneration and wound healing properties. The present study aimed to determine the antioxidant and anti-inflammatory effect of the hyaluronic acid gel combined with lotus seed extract and how this combination could be used as an effective local drug delivery system for the treatment of periodontitis.

METHODS A 2% solution of hyaluronic acid gel was combined with increasing concentrations of prepared lotus seed extract. A DPPH test was conducted to determine the antioxidant activity of the resultant mixture at increasing concentrations. Additionally, anti-inflammatory activity was assessed using a UV spectrometer, and the spreadability of the gel was measured using the sliding glass slide method. The values obtained were plotted on graphs.

RESULTS The DPPH scavenging assay revealed that lotus seed extract with 2% hyaluronic acid gel exhibited increased antioxidant activity in a dose-dependent manner with IC_{50} at 76 µL. The lotus seed extract with 2% hyaluronic acid gel also showed increased anti-inflammatory properties in a dose-dependent manner with IC_{50} at 271 µL. Moreover, the spreadability of the lotus seed extract with 2% hyaluronic acid gel was found to be 42 mm.

CONCLUSIONS Lotus seed extract shows potent antioxidant and anti-inflammatory activities which vary with the concentration of the extract, and can serve as an effective local drug delivery system.

KEYWORDS flavonoids, lotus seed, hyaluronic acid, local drug delivery, quality of life, periodontitis

INTRODUCTION

Periodontitis is one of the most commonly encountered inflammatory diseases affecting teeth and supporting structures. A mechanical therapy involving root surface debridement is an efficient way of treating periodontitis. However, that method does not reduce the presence of microbes in tissues or other areas inaccessible to instrumentation. It is essential to incorporate antimicrobial therapy to enhance the efficacy of traditional treatment methods. Antimicrobial therapy can be in the form of either systemic or local delivery modes.

Local drug delivery aims for specific site delivery without systemic side effects, as seen in systemic antimicrobial therapy. In this study, we synthesized an HA-lotus seed extract gel for local drug delivery.

Lotus seed extract, derived from the seeds of the sacred lotus plant (*Nelumbo nucifera*), is renowned for its traditional medicinal uses in various cultures. Flourishing in aquatic environments, this perennial plant is recognized for its exquisite flowers and distinctive pad-like leaves (1). Lotus seed extract is rich in phytochemicals, offering numerous health benefits (2). *Nelumbo nucifera* contains a variety of phytochemicals, including saponins, alkaloids, polyphenols and carbohydrates. Its potent antioxidant activity, derived from its composition which includes flavonoids and phenols, gallic acid and chlorogenic acid, plays a vital role in preventing oxidative stress. The total phenolic content of the lotus plant studied by Leong et al. was found to be the highest in the seeds, with a range of 20.6 to 38.3 mg TAE/g of extract. These compounds neutralize free radicals (act as scavengers), reducing the risk of chronic diseases (3). The leaves and stems of the lotus plant are well known for exhibiting anti-inflammatory properties by boosting the natural defense cells in the body, thereby reducing the inflammatory disease affecting the body (4). However, the anti-inflammatory and antioxidant property in the lotus seeds was found to be the most effective as analyzed by Rai et al. The radical scavenging activity, assessed by the IC_{50} values, was found to be 6.12 ± 0.41 µg/mL (2), hence the present study focused on the antioxidant activity and anti-inflammatory activity and their combination with hyaluronic acid (HA) in the treatment of periodontitis. These bioactive components are also believed to enhance cognitive function and promote mental well-being (5).

HA is a naturally occurring anionic glycosaminoglycan found in connective tissues, joints, and skin. The most striking feature of this acid is its water retention capacity. In gel form, it acts as a lubricating agent and provides exceptional hydration to the cells and tissues (6). For that reason, HA is incorporated in cosmetic products, the demand for which has increased rapidly. It also exhibits viscoelastic behavior, allowing it to absorb any disturbances and shocks, and providing a cushioning effect to the joints (7). In gel form, it creates a conducive environment for the migration of cells and regeneration of tissues. This gel is utilized in various medical applications, including wound dressings and post-surgery treatments (8). HA gel in combination with lotus seed extract is known to display anti-edematous, anti-bacterial and pro-angiogenic properties. Previous studies have shown that decreased levels of gingival inflammation and increased periodontal attachment are found when antioxidant status is improved with HA gel. As reported by Asieh et. al, the IC_{50} values of HA gel were found to be around 55 ± 0.7 mM and demonstrating its substantially high radical scavenging properties (9). Because the antioxidant activity of the lotus seed extract and HA gel was found to be significant, the synergistic effect of these compounds was chosen for use in local drug delivery systems for periodontitis.

Unlike conventional systemic drug delivery systems, which circulate medications throughout the entire body, local drug delivery is able to focus on application and injection of drugs into targeted sites. This effectively reduces the potential damage to surrounding tissues while at the same time diminishing any side effects (10). These systems employ advanced technologies for the meticulous administration of the drugs directly to the intended area through microneedles, implants, or patches (11). This precise targeting is advantageous for treating chronic pain and inflammation, as well as localized bacterial and viral infections. The single most desirable significant advantage of local drug delivery is its ability to achieve desired concentrations at the target site, thus enabling the maximum beneficial effect of the drug and, at the same time, minimizing exposure to the drug (12).

Lotus seed extract can be encapsulated within carriers like nanoparticles, liposomes, or hydrogels. This encapsulation facilitates controlled release, ensuring a sustained localized delivery of therapeutic agents. The present study assessed the antioxidant and anti-inflammatory effect of lotus seed extract in combination with HA and its potential for further enhancement as a local drug delivery agent in the treatment of periodontitis.

METHODS

Preparation of the lotus seed HA

Organic lotus seed was purchased from a local market in T. Nagar, Chennai, India. 50 g of the seeds was soaked in 500 ml of 95% ethanol. The solution was left undisturbed at room temperature for two days, after which a fine filtrate was

obtained by filtration through a Whatmann filter paper. A 2% HA gel was combined with increasing concentrations of the plant extract. Encapsulated lotus seed extract with HA gel was prepared as per the method described by Mohammad et al. (13).

Test for antioxidant activity

To 100 mL of ethanol, 4 mg of DPPH was added to obtain a concentration of 0.1 mM. The lotus seed extract was then added at increasing concentrations (25, 50, and 100 mg/mL) to the prepared DPPH solution. The radical scavenging activity was observed at 517 nanometers by measuring the intake of the different solutions. As a reference, pure DPPH solution (20 mg/mL) was tested for antioxidant activity at the same wavelength (14). The percentage of radical scavenging activity of the samples was calculated using the following formula:

$$\% \text{ RSA} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where RSA is the radical scavenging activity, Abs control is the absorbance of DPPH radical + ethanol and Abs sample is the absorbance of sample.

For the study, gallic acid was chosen as the positive control due to its high antioxidant capacity. The antioxidant activity of the extract was compared with gallic acid, as it is known to have high free radical scavenging activity. The DPPH solution mixed with ethanol was used as a negative control to eliminate errors and miscalculations during incubation, as shown in Figure 1.

Test for anti-inflammatory activity

Different extract concentrations (25, 50, and 100 mg/mL) were added to 1.5 ml of bovine serum albumin (2% solution produced with 0.05 M Tris HCl). Tris HCl was used to alter the pH of the final solution. The samples were incubated for 30 minutes, then the prepared samples were submerged in water which was then heated to 75°C for ten minutes, after which the samples were brought to room temperature. The turbidity of the samples was measured using an ultraviolet spectrophotometer at 660 nanometers, and the percentage inhibition of albumin denaturation was calculated.

$$\text{Anti-inflammatory (\%)} = \frac{\text{Optical density}_{\text{control}} - \text{Optical density}_{\text{sample}}}{\text{Optical density}_{\text{control}}} \times 100$$

Test to assess spreadability

Two grams of the prepared gel was placed on a glass slide measuring 75 x 25 mm using a pipette. Then, a second glass slide, approximately 1 mm thick, was used to spread the gel which was then placed over the first glass slide. The time taken for the solution to spread evenly over the slide was calculated, and the spread diameter was measured after 1 minute.

RESULTS

Antioxidant activities of lotus seed extract with 2% hyaluronic acid gel

The values obtained from each test were tabulated and graphs were plotted. The antioxidant properties of lotus seed extract with 2% HA gel were determined by using the DPPH scavenging assay. The 1,000 µg/mL of gallic acid was used as a positive control and the antioxidant activity of the positive control was set as 100%. The results indicated that the lotus seed extract with 2%

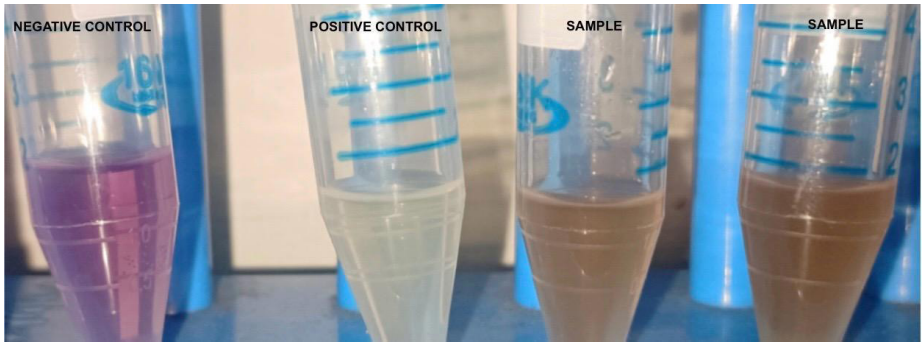

Figure 1. Samples containing ethanolic extract of lotus seed in combination with 2% HA gel. Positive control – Gallic acid (1,000 µg/mL); negative control – ethanol solution.

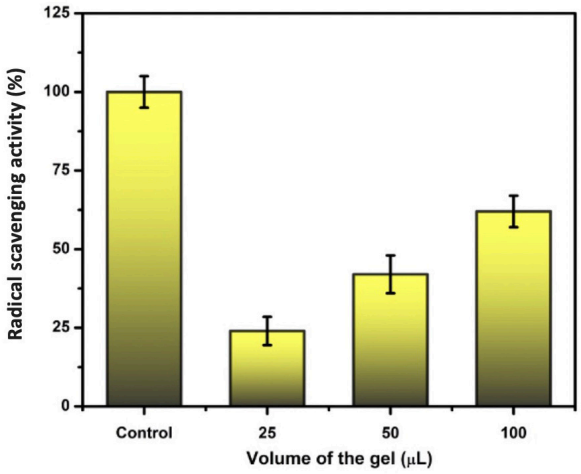

Figure 2. Graph depicting the antioxidant activity of ethanolic extract of lotus seed-HA at 25, 50, and 100 µL compared with the control (1,000 µg/mL of gallic acid).

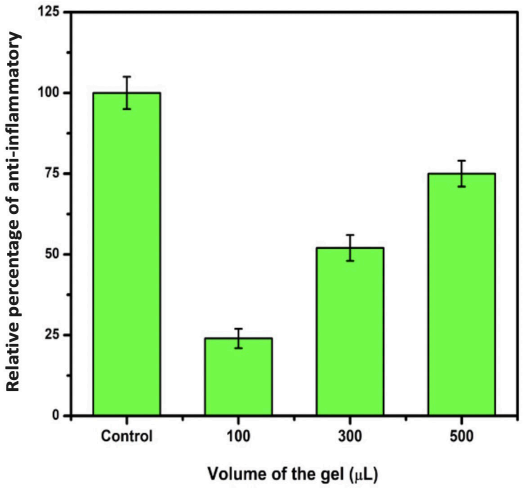

Figure 3. Graph depicting the anti-inflammatory activity of ethanolic extract of lotus seed-HA at 100, 300, and 500 µL compared with the control (1,000 µg/mL of gallic acid).

HA gel increased antioxidant activity in a dose-dependent manner. At 25, 50, and 100 µL of the lotus seed extract with 2% HA gel, the radical scavenging activity was found to be 25%, 37%, and 62%, respectively, as shown in Figure 2. The volume of the extract that was required to scavenge 50% of the initial DPPH radicals (IC_{50}) was shown to be 76 µL.

Anti-inflammatory activities of the lotus seed extract with 2% hyaluronic acid gel

The values obtained from each test were tabulated and graphs were plotted. The anti-inflammatory properties of lotus seed extract with 2% HA gel were determined using the BSA denaturation inhibition assay. The 1,000 µg/mL of gallic acid was used as a positive control and the antioxidant activity of the positive control was set as 100%. The results revealed that lotus seed extract combined with 2% HA gel exhibited increased anti-inflammatory activity in a dose-dependent manner. At 100, 300, and 500 µL of the lotus seed extract with 2% HA gel, the anti-inflammatory activity was found to be 25%, 61%, and 75%, respectively, as shown in Figure 3. Moreover, the volume of the extract that was required to inhibit 50% of the BSA denaturation (IC_{50}) was shown to be 271 µL.

The spreadability of the lotus seed extract with 2% hyaluronic acid gel

The spreadability of gel preparations refers to their ability to spread evenly across the skin's surface. The gel diameter was calculated by measuring the diameter of the gel on several sides. From this study, the spreadability of the lotus seed extract with 2% HA gel was found to be 42 mm, as shown in Figure 4.

DISCUSSION

The destruction of periodontal supporting tissues is due to the host immune inflammatory response triggered by red-complex bacteria (15). The present study evaluated the effectiveness of lotus seed- HA as a local drug delivery agent for the scavenging of free radicals as estimated by its antioxidant and anti-inflammatory ability (16). Ironically, in the present cosmetic era, use of natural remedies and herbal treatments has become increasingly popular for treating ailments such as cancer, skin diseases, poisoning, and periodontitis

Figure 4. Figure depicting the spreadability of the prepared extract (Lotus seed-HA)

(16, 17). The ethanolic extract of *Nelumbo nucifera* showed potent antioxidant and anti-inflammatory properties. Lotus seeds are the major reservoir and host to biologically active compounds such as flavonoids, tannins, and lignins (18). The hydroxyl groups in flavonoids alters their properties by chelating metal ions and breaking the antioxidant chain (19). Ascorbic acid, glutathione, and unsaturated fatty acids further enhance the antioxidant activity, making it a potent free radical scavenger (20). This activity is substantially amplified by the increased superoxide dismutase enzyme activity.

HA is widely used in periodontal therapy as adjunct to scaling and root planing and has resulted in greater reduction in probing depth and relative attachment level (21). It has also been used widely as nonsurgical treatment of papillary recession in esthetic areas. However, the use of HA injections provides only short term results (22). The use of HA gel as a drug delivery vehicle relies on its excellent biocompatibility and moisture-retaining capacity. These properties make it an ideal candidate for delivering therapeutic agents locally (23). Since HA is naturally present in the human body, the number of adverse allergic reactions and side effects to this acid are minimal (24).

The encapsulation of lotus seed extract within the HA gel ensures sustained and even release of the drug and prolongs the duration of the drug's interaction with tissues in the body. This property helps expand the duration of the effectiveness of the drug in addition to allowing concentration control (25). Additionally, combining lotus extract and HA can cause deeper drug penetration into the targeted tissues with immediate intake (26). As the antioxidant activity of the lotus seed extract and HA gel has been found to be significant, the synergistic effect of these compounds has resulted in their use in local drug delivery systems for periodontitis. Interestingly, the biological properties of lotus seed can also potentially promote wound healing and regeneration of tissues by amplifying the angiogenesis process. These properties make this substance an excellent option for local drug delivery (27, 28).

Previous research has shown that the physical properties of gels infused with HA can affect antioxidant activities more directly compared to those infused with HA (29). Additionally, the growth of *P. gingivalis* is known to be inhibited by high molecular weight hyaluronic acid. However, these properties fluctuate based on the acid concentration and the cell's metabolism (15). HA has a high binding affinity for CD44 cells which is expressed on the surface of leukocytes, found predominantly in individuals with periodontitis. The HA reduces leukocyte recruitment and there by decreases gingival inflammation. This research may pave the way for developing personalized drug delivery systems for inflammatory conditions. By altering the proportion of HA based on the type and purpose of the drug used, healthcare providers can potentially provide effective and efficient treatments to help resolve the specific ailments of each individual (16).

The synergy between lotus extract and HA provides a promising route for drug delivery by offering improved bioavailability, reduced side effects, and enhanced specific therapeutic outcomes. This approach could potentially revolutionize methods of delivering pharmaceuticals.

CONCLUSIONS

The present study effectively demonstrated the antioxidant and radical scavenging activity of *Nelumbo nucifera* (lotus seed) and how the addition of HA gel enhances these properties in a concentration-dependent manner. The present study provides scope for advanced research in this area, thus potentially enabling the formulation of other local drug delivery systems using lotus seed extract. Future studies should include both *in vivo* and *in vitro* studies to evaluate the efficacy of lotus seed extract-HA as a local drug delivery system in the treatment of chronic periodontitis.

ACKNOWLEDGEMENTS

We would like to thank Saveetha Dental College and Hospitals for supporting us in conducting the study.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

REFERENCES

1. Ming R, VanBuren R, Liu Y, Yang M, Han Y, Li L, et al. Genome of the long-living sacred lotus (*Nelumbo nucifera* Gaertn). Genome Biol. 2013;14:1-11.
2. Rai S, Wahile A, Mukherjee K, Saha B, Mukherjee P. Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds. J Ethnopharmacol. 2006;104:322–7.
3. Leong ES, Tan S, Chang YP. Antioxidant properties and heavy metal content of lotus plant (Nelumbo nucifera Gaertn) grown in ex-tin mining pond near Kampar, Malaysia. Food Sci Technol Res. 2012;18:461-5.
4. Chen C, Li G, Zhu F. A novel starch from lotus (*Nelumbo nucifera*) seeds: Composition, structure, properties, and modifications. Food Hydrocoll. 2021;120:1-15.
5. Rajeshwaran N, Ramamurthy J, Rajeshkumar S. Evaluation of antioxidant and inflammatory activity of grape seed oil infused with silver nanoparticles an in vitro study. Int J Dent Oral Sci. 2021;8:3318-22.
6. Sudha P, Rose M. Beneficial effects of hyaluronic acid. Adv Food Nutr Res. 2014;72:137-76.
7. Burdick J, Prestwich G. Hyaluronic acid hydrogels for biomedical applications. Adv Mater. 2011;23:41-56.
8. Brown M, Jones S. Hyaluronic acid: a unique topical vehicle for the localized delivery of drugs to the skin. J Eur Acad Dermatol Venereol. 2005;19:308-18.
9. Soozanipour A, Taheri-Kafrani A, Razmjou A, Asadnia M. Efficient enzymatic degradation of hyaluronic acid by hyaluronidase-conjugated polyamidoamine dendrimer: A robust and stable nanobiocatalyst. Authorea. 2024.
10. Harvey RJ, Schlosser RJ. Local drug delivery. Otolaryngol Clin North Am. 2009;42:829-45.
11. Wolinsky J, Colson Y, Grinstaff M. Local drug delivery strategies for cancer treatment: gels, nanoparticles, polymeric films, rods, and wafers. J Control Release. 2012;159:14–26.
12. Greenstein G, Polson A. The role of local drug delivery in managing periodontal diseases: a comprehensive review. J Periodontol. 1998;69:507-20.
13. Mohammed M, Devnarain N, Elhassan E, Govender T. Exploring the applications of hyaluronic acid-based nanoparticles for diagnosis and treatment of bacterial infections. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2022;14:e1799. PubMed PMID: 35485247
14. Kim M, Shin H. Antioxidative effect of lotus seed and seedpod extracts. Food Sci Biotechnol. 2012;21:1761-6.
15. Meenakshi S, Sankari M. Effectiveness of chitosan nanohydrogel as a bone regenerative material in intrabony defects in patients with chronic periodontitis: a randomized clinical trial. J Adv Oral Res. 2021;12: 222-8.
16. Yen G, Duh P, Su H, Yeh C, Wu C. Scavenging effects of lotus seed extracts on reactive nitrogen species. Food Chem. 2006;94:596-602.
17. Ke C, Sun L, Qiao D, Wang D, Zeng X. Antioxidant acitivity of low molecular weight hyaluronic acid. Food Chem Toxicol. 2011;49:2670-5.
18. Kumar S, Pandey A. Chemistry and biological activities of flavonoids. Sci World J. 2013;2013:1-16.
19. Seyoum A, Asres K, El-Fiky F. Structure–radical scavenging activity relationships of flavonoids. Phytochemistry. 2006;67:2058-70.
20. Ramamurthy J, Jayakumar N. Anti-inflammatory, anti-oxidant effect and cytotoxicity of Ocimum sanctum intra oral gel for combating periodontal diseases. Bioinformation. 2020;16:1026-32.
21. Shah S, Vijayakar H, Rodrigues S, Mehta C, Mitra D, Shah R. To compare the effect of the local delivery of hyaluronan as an adjunct to scaling and root planing versus scaling and root planing alone in the treatment of chronic periodontitis. J Indian Soc Periodontol. 2016;20:549-56.
22. Alhabashneh R, Alomari S, Khaleel B, Qinawi H, Alzaubi M. Interdental papilla reconstruction using injectable hyaluronic acid: A 6 month prospective longitudinal clinical study. J Esthet Restor Dent. 2021;33:531-7.
23. Yadav AK, Mishra P, Agrawal GP. An insight on hyaluronic acid in drug targeting and drug delivery. J Drug Target. 2008;16:91-107.
24. Jin Y, Ubonvan T, Kim D. Hyaluronic acid in drug delivery systems. J Pharm Investig. 2010;40:33-43.
25. Vasvani S, Kulkarni P, Rawtani D. Hyaluronic acid: A review on its biology, aspects of drug delivery, route of administrations and a special emphasis on its approved marketed products and recent clinical studies. Int J Biol Macromol. 2020;151:1012–29.
26. Rajeshwari H, Dhamecha D, Jagwani S, Rao M, Jadhav K, Shaikh S, et al. Local drug delivery systems in the management of periodontitis: A scientific review. J Control Release. 2019;307:393-409.
27. Juncan A, Moisă D, Santini A, Morgovan C, Rus L, Vonica-Țincu A, Loghin F. Advantages of hyaluronic acid and its combination with other bioactive ingredients in cosmeceuticals. Molecules. 2021;26:4429. PubMed PMID: 34361586
28. Deepika B, Ramamurthy J, Girija S, Jayakumar N. Evaluation of the Antimicrobial Effect of Ocimum sanctum L. Oral Gel against Anaerobic Oral Microbes: An In Vitro Study. World J Dent. 2022;13(Supplement_1): S23-7
29. Xu Q, Torres J, Hakim M, Babiak P, Pal P, Battistoni C, et al. Collagen- and hyaluronic acid-based hydrogels and their biomedical applications. Mater Sci Eng R Rep. 2021;146:100641. PubMed PMID: 34483486