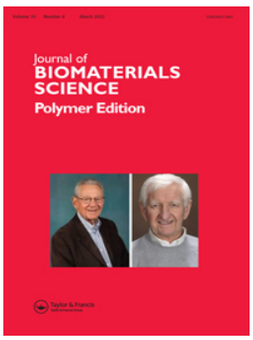

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Preparation, characterization and biological evaluation of silver nanoparticles and drug loaded composites for wound dressings formed from *Lallemantia royleana* seeds' mucilage

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ABSTRACT

After an injury, the wounds need to be covered with a dressing. Lack of absorptive potential and sticking of dressing with the wound causes pain and slows the healing process. The aim of this study was to develop wound dressings having more absorptive potential and less sticking with the wound. The hemicelluloses from *Lallemantia royleana* seeds possess desirable properties for a wound dressing. The hemicellulose was blended with chitosan/chitin and glutaraldehyde to enhance the absorptive properties of the hemicellulose through cross-linking. Two types of formulations incorporating silver nanoparticles and ciprofloxacin were prepared. The composites were characterized by elemental analysis, Fourier-transform infrared spectroscopy and scanning electron microscopy, and evaluated for their antibacterial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The dressings were subjected to *in vivo* studies on Albino rats. The dressings were found to be porous and the silver nanoparticles and drug particles were found to be uniformly distributed in the polymeric matrix. The composite containing ciprofloxacin released the drug in a sustained manner for 14–16 days. From extrapolation of the data, it was discovered that the formulation would release around 80% of ciprofloxacin in about two weeks. Silver-ciprofloxacin nano-composites exhibited comparable activity (zone of inhibition 19–30 mm) against *E. coli* to that of ciprofloxacin (standard, 21–35 mm) and relatively lower activity in case of *S. aureus* (zone of inhabitation 11–17 mm). The dressings did not stick to the wound site and the site remained wet during the healing process. Thus the use of hemicellulose from *L. royleana* seeds proved to be beneficial for preparing wound dressings with improved properties because of having high swelling index, porosity and spongy texture.

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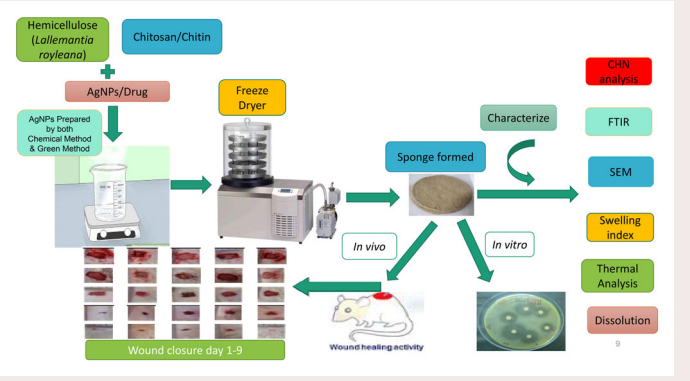
GRAPHICAL ABSTRACT



1. Introduction

Skin is the largest tissue covering the human body. It protects the body from external environment and helps maintaining homeostasis. Skin is made of three layers, i.e. epidermis – the upper layer, dermis – the middle layer containing blood supply and nerve network, and subcutaneous layer – lower layer containing connective tissues and fat [1]. Injury of the skin epithelium causes the wound and disruption of the function and structure of skin. It is estimated that 1–2% of the population in developed countries suffer from a chronic wound at some point and that global expenditure in treating these conditions is $13–15 billion annually [2].

Depending upon the cause of injury, the types of wounds vary greatly. Some wounds result in release of large amount of sticky exudate while some wounds are internals without any exudate. Wound healing process starts immediately after injury. Fibroblast, activated platelets, keratinocytes and white blood cells come into play and release a different set of anti-inflammatory cytokines including TNF alpha, PDGF, TGFβ and matrix metalloproteases leading to tissue regeneration [3]. Prevention of wound from microbial contamination is crucial for efficient and quick healing of a wound. From ancient times wounds are treated and protected from microbial contaminations by different types of dressings. Some of the dressings are incorporated with antimicrobial agents to safeguard the wound from bacterial infections. Some other types of wound dressings are occlusive, semi-occlusive and gauze dressings [4]. They are further divided into hydrocolloids, semipermeable films, hydrogels and foam dressings. Depending upon the nature of wound a particular dressing is required. Conventional dressings were cost-effective and highly absorbent, but not effective enough to promote hemostasis, adherence and in holding a moist wound bed [5]. Sometimes, these bandages cause skin irritation. Moreover, changing dressing on exuding wound caused a lot of pain and discomfort to the patient and sometimes bleeding starts. So, there is need of an ideal wound dressing which should be non-allergic, non-toxic, be able to maintain moist environment, possess thermal stability, permeable to gases,

prevent microbial growth, cost effective and, last but not the least, must not be adherent to the wound [6].

Many synthetic and natural polymers and their composites are used to develop wound dressings. These include hydrocolloids, alginates, hydrogels, polyurethane, collagen, chitosan, pectin, gelatin, hyaluronic acid and cellulose [6, 7]. Among the natural polymers chitosan, alginate and pectin have shown promise for this purpose [8–15]. But due to certain disadvantages such as less mechanical strength, stability, poor exudate-absorbing capacity, and adherence with wound site, there is a need for better natural polymers such as hemicelluloses [16]. Hemicelluloses are low cost, easily removable, non-toxic, easily available, biocompatible, biodegradable and possess some therapeutic activities such as anti-inflammatory, anti-oxidant, anti-tumor and antibacterial effects etc. These properties make them desirable candidates in drug delivery [17, 18]. Hemicelluloses can be plant gums or seed mucilages. The mucilages have diverse practical uses. However, they all have beneficial effects on burns, wounds, ulcers, inflammations and irritations, diarrhea and dysentery [19, 20]. One such mucilage is from *Lallemantia royleana* (*LR*) seeds, which possess several pharmacological effects including antimicrobial, antioxidant, antidepressant, anxiolytic, sedative, antiemetic, hypolipidemic and many other pharmacological effects. A review [21] discussed the *LR* as a beneficial medicinal plant. The mucilage from *LR* seeds has been traditionally used as a gelling agent, and natural matrix for sustained release of drugs. However, it seems that these compounds are not only good additives rather have several undiscovered pharmacological properties. The gel prepared from the mucilage causes a good analgesia with unknown mechanism [22].

The objective of the present work was to prepare composite dressings, which are easily removable, and help in rapid wound healing. *LR* wound dressings have been prepared with chitosan/chitin using glutaraldehyde as a cross-linker, incorporating ciprofloxacin as a model drug and silver nanoparticles to prevent the microbial invasion of the wound site. The prepared dressings were characterized by various analytical techniques and tested, *in vitro* and *in vivo*, for their effectiveness as wound dressings. These dressings possess novel characteristics, including high water-holding capacity, porosity and non-sticking behavior at the wound site. These dressings will keep the wound site wet to fulfill the requirement of rapid healing. In addition to that the *LR* seeds mucilage has been reported to have analgesic properties [22].

2. Experimental

2.1. Materials

The *LR* seeds were purchased from a local market in Lahore, Pakistan. Chitin (Sigma-Aldrich, CAS No. 1398-61-4), chitosan (Sigma-Aldrich, CAS No. 9012-76-4), acetic acid (DAEJUNG, CAS No. 64-19-7), sodium hydroxide (Sigma-Aldrich, CAS No. 1310-73-2), glutaraldehyde (Sigma-Aldrich, CAS No. 111-30-8), sodium phosphate dibasic (DAEJUNG, CAS No. 7558-79-4), sodium phosphate monobasic (Riedel-deHaën, CAS No. 231-449-2) were used as received. All these chemicals were of analytical grade. Ciprofloxacin was gift from the Department of Pharmacy, Forman

Christian College (A Chartered University) Lahore. Distilled water was used throughout this research work.

2.2. Isolation of mucilage of LR

LR seed mucilage was extracted and purified as reported earlier [18]. The *LR* seeds (100 g) were weighed and cleaned to remove dirt and waste particles. The seeds were soaked in distilled water (1500 mL) overnight and the mucilage was separated from the swollen seeds by slightly blending in a kitchen blender. For the removal of impurities and obtaining a clear gel, the mucilage was filtered under vacuum for 3–4 times by using muslin cloth. The clear gel was spread on polythene sheets and air-dried at room temperature. The dried sheets were ground into powder and stored into airtight vials.

2.3. Preparation of silver nanoparticles

Silver nanoparticles were prepared by two different methods i.e. a chemical and a green method, which are described as follows:

2.3.1. Chemical method

A very famous method called Turkevich method [23] was followed for the preparation of silver nanoparticles. Briefly, $AgNO_3$ (50 mL; 0.001 M) was taken in a beaker and heated to boiling. Then 1% trisodium citrate (5 mL) solution in water was added dropwise and the mixture was heated under vigorous stirring until the color changed from colorless to pale yellow. After the color change, the mixture was cooled and UV spectrum was recorded after filtration.

2.3.2. Green method

For green synthesis of silver nanoparticles, a reported method was followed [24]. According to that, a diluted solution of *LR*, whose pH was adjusted to 8.5, was taken in a beaker. It was stirred and heated. After that, 1 mM $AgNO_3$ (20 mL) was added slowly at regular time intervals. The color changed from colorless to brownish yellow which indicated the formation of silver nanoparticles. The solution was cooled and UV spectrum was recorded.

Concentration of silver nanoparticles was determined by already reported method [25].

2.4. Preparation of wound dressings

Different protocols were followed for preparing composite dressings and after optimization the best one was selected. Two different formulations of composite dressings were prepared, i.e. *LR* with chitosan and *LR* with chitin. For that purpose chitosan (1%) solution was prepared in acetic acid (1%) and taken in a beaker. It was stirred for 6–7 h at room temperature. After that, a weighed amount of *LR* was added slowly to it and the contents were continuously stirred for complete mixing to form a

Table 1. Composition of the composite dressings.

Codes	*LR* (g)	Chitosan (%)	Chitin (%)	Green AgNPs (0.005%) (mL)	Synthetic AgNPs (0.005%) (mL)	Drug (0.005%) (mL)
*LRO*1	1	1	–	–	3	–
*LRO*2	1	1	–	3	–	–
*LRO*3	1	1	–	–	3	3
*LRO*4	1	1	–	3	–	3
*LRI*5	1	–	1	–	3	–
*LRI*6	1	–	1	3	–	–
*LRI*7	1	–	1	–	3	3
*LRI*8	1	–	1	3	–	3

homogenized blend. Silver nanoparticles were added to the reaction mixture under continued stirring. After that, glutaraldehyde solution (1%) was added dropwise as a crosslinking agent under constant and vigorous stirring using a homogenizer. Finally, the mixture was poured into petri dishes, frozen overnight and then lyophilized at −70 °C for 48 h. Similarly, *LR*-chitin composites were prepared by the same procedure.

For the preparation of drug loaded composite dressings, solution of ciprofloxacin (0.005%) was added to the above reaction mixture and lyophilized. The composition of the composite dressings thus prepared is given in Table 1.

2.5. Characterization

2.5.1. Chemical composition

Elemental analysis (carbon, hydrogen and nitrogen) was carried out by CHNS Elemental analyzer (630-200 LECO, USA), whereas, for the identification of functional groups in the composite dressings, the samples were subjected to Fourier-transform infrared (FTIR) spectroscopic analysis using CARY630 FTIR spectrophotometer (Agilent Technologies, USA) in the range of 4000–650 cm^{-1}.

2.5.2. Swelling index

For determining the percentage swellability of the composites, following equation was used:

$$S = \frac{W_W - W_D}{W_D} \times 100$$

where W_w is the mass of the wet sample at time t, W_D is the mass of the dry sample. A weighed amount of the dry sample was immersed in a buffer solution of different pH, i.e. 5.8, 7.4 and 8. These pH values were selected to simulate the swelling in skin environment (pH 5.8), blood (pH 7.4) and an alkaline environment (pH 8). After time t, the swollen sample was taken out from the buffer solution, wiped by a filter paper and weighed to determine the mass difference. The effect of time on swellability was checked at different time intervals i.e. 5, 10, 15, 20, 25, 35, 45 min and then after 24 h. A graph was constructed to show percentage swellability.

2.5.3. Surface morphology

Surface morphology was studied by recording images on scanning electron microscope (SEM, Hitachi S-3400N and Jeol JSM-6060 LV).

2.6. Thermal stability

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were performed in the range ambient to 600 °C on SDT (Q600) thermal analyzer (TA Instruments, USA) under nitrogen (100 $cm^3 min^{-1}$ flow) at the heating rate of 20 °C min^{-1}. The samples were subjected twice to thermal analysis. One immediately after preparation and second time after 1 year.

2.7. Drug release study

The *in vitro* drug release study for ciprofloxacin loaded composite dressings was carried out in phosphate buffer saline (PBS) at pH 5.8. Drug loaded composite dressings were cut into equal size and having equal weight (0.25 g). The dissolution study was carried out at 50 RPM using USP apparatus. The quantity of the drug released was determined by measuring absorbance at 271 nm wavelength. The ε values were determined experimentally, by using standard solutions of standard solutions of ciprofloxacin (0.01%, 0.005%, 0.0025%, 0.00125%, 0.000625% and 0.0003125%). After each withdrawal an equal volume of the dissolution medium was replaced immediately. The cumulative release was plotted against time.

2.8. In vitro *antibacterial studies*

For determining antibacterial activity of the prepared composite dressings, disc diffusion method was used [26]. The antibacterial activity was checked against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) bacterial strains in Biological Safety Cabinet (Class II, Techno Scientific, Pakistan). These two strains were selected because *E. coli* was Gram-negative and *S. aureus* Gram-positive. Mueller-Hinton agar broth was prepared and autoclaved at 121 °C temperature and 15 psi pressure for 1 h. After that, the agar broth was poured equally into petri dishes and left to solidify. Then the bacterial strains were spread onto the agar medium by using glass rod. Small discs (5 mm dia) of filter paper loaded with standard solutions of ciprofloxacin (10 μL of 0.005%) and silver nanoparticles (10 μL of 0.005% Ag) were placed on the agar medium. Similarly, small discs of the composite dressings loaded with ciprofloxacin and silver nanoparticles were placed on the agar medium in each plate along with the blank discs. The petri dishes were incubated for 24 h at 37 °C. After that, the antibacterial activity of each sample was determined in terms of the zone of inhibition around all the samples.

2.9. In vivo *wound healing evaluation*

In order to evaluate the wound healing potential [27] of the prepared composite wound dressings, Wistar rats weighing 180–250 g were used. Animals were

Table 2. Elemental analysis of the samples.

Sample	Nitrogen (%)	Carbon (%)	Hydrogen (%)
LR	Not detected	33.079	5.198
Chitosan	0.122	38.161	6.177
Chitin	1.185	40.683	6.068
LRO1	0.120	38.259	6.080
LRO2	0.115	37.107	5.965
LRO3	0.121	36.766	6.066
LRO4	0.116	37.346	6.496
LRI5	1.129	40.980	5.950
LRI6	1.136	41.931	5.990
LRI7	0.150	41.269	6.263
LRI8	0.198	41.368	5.960

anesthetized by using ketamine (50 mg/kg) and diazepam (5 mg/kg) *via* subcutaneous injections. The hair on the dorsal side of the animals was removed with the help of a shaver. After that, the skin was sterilized by using 75% ethanol. Around $1 \times 1\,cm^2$ a full thickness wound was created with the help of a surgical blade under anesthesia. The animals were divided into 5 groups; each having 3 animals. Wounds in control group were covered with commercially available non- medicated gauze dressing (Soft Surgi Gauze, Karim Industries, Lahore, Pakistan), while in the test groups (*LRO*2, *LRO*4, *LRI*5 and *LRI*7) the wounds were covered with the composite wound dressings. After dressing, the animals were placed in the cages individually. The dressings were changed at 3, 5, 7 and 9 days and photographs of the wounds were also taken.

3. Results and discussion

The isolated *LR* seed mucilage was obtained in 12% yield (on dry substance basis). The molecular mass of the hemicellulose, consisting of xylose as major constitute and glucose as minor component was 2.3×10^6 Da. More information about this is reported in our previous paper [18]. The nanoparticles prepared by the chemical method, showed maximum absorption at wavelength 427 nm while nanoparticles from green method showed maximum absorption at 422 nm. The prepared composite dressings were porous, spongy, flexible and light brown in color.

3.1. Characterization

The CHN results (Table 2) indicated that *LR* seed mucilage and the composite materials contained carbon and hydrogen only as expected [18].

To confirm the polymeric structures and to confirm the purity, the blank samples as well as the drug and silver nanoparticle loaded samples were subjected to FTIR analysis. Each sample exhibited its characteristic peaks in the FTIR spectra at specific regions, which are summarized in Table 3 and depicted in Figure 1.

The FTIR spectrum of ciprofloxacin drug exhibited characteristic peaks at $3527\,cm^{-1}$ (O–H stretching), $2926\,cm^{-1}$ (C–H stretching), $1703\,cm^{-1}$ (C = O stretching), $1620\,cm^{-1}$ (quinolones) and $1025\,cm^{-1}$ (C–F stretching). The FTIR spectrum of pure *LR* exhibited characteristic peaks at $3308\,cm^{-1}$ (O–H stretching), $2924\,cm^{-1}$ (C–H stretching) and $1015\,cm^{-1}$ (C–O–C glycosidic linkage). The FTIR spectrum of

Table 3. Characteristic FTIR absorptions of the samples and their assignments.

Sample	O–H (str) (cm^{-1})	N–H (str) (cm^{-1})	C–H (str) (cm^{-1})	C = O (str) (cm^{-1})	N–H (ben) (cm^{-1})	C–O–C (cm^{-1})	C–F (str) (cm^{-1})
Ciprofloxacin	3527	–	2926	1703	–	–	1025
LR	3308	–	2924	1643	–	1015	–
Chitosan	3298	3280	2879	1649	1563	1025	–
Chitin	3436	3270	2873	1621	1554	1067	–
*LRO*1	3215	–	2880	1656	1561	1026	–
*LRO*2	3278	–	2951	1656	1561	1028	–
*LRO*3	3272	–	2949	1638	1561	1026	–
*LRO*4	3322	–	2946	1640	1552	1028	–
*LRI*5	3320	–	2932	1630	1561	1010	–
*LRI*6	3310	–	2950	1656	1561	1008	–
*LRI*7	3267	–	2931	1651	1548	1012	–
*LRI*8	3270	–	2939	1656	1559	1012	–

pure chitosan exhibited characteristic peaks at 3298 cm^{-1} (O–H stretching), 3280 cm^{-1} (N–H stretching), 2879 cm^{-1} (C–H stretching), 1649 cm^{-1} (C = O stretching), 1563 cm^{-1} (N–H bending) and 1025 cm^{-1} (C–O–C glycosidic linkage). The FTIR spectrum of pure chitin has shown peaks at 3436 cm^{-1} (O–H stretching), 3270 cm^{-1} (N–H stretching), 2873 cm^{-1} (C–H stretching), 1621 cm^{-1} (C = O stretching), 1554 cm^{-1} (N–H bending) and 1067 cm^{-1} (C–O–C glycosidic linkage). Similarly, all the composites have exhibited characteristic peaks of the pure polymers (*LR*, chitosan and chitin) as listed in Table 3 and depicted in Figure 1.

These results indicated that the characteristic peaks of the polymers have been retained in the composites and there was no new peak. This revealed that the polymers have not reacted with each other and retained their structural integrity. The peaks due to the drug in the composites could not be detected because the drug was used in minute quantity and the polymer peaks dominated the peaks of the drug. Based on these evidences, the structure of the composites can be proposed as shown in Figure 2.

The % swellability was checked on *LRO*4 and *LRI*7 by using phosphate buffer of pH 5.8, 7.4 and 8 (Figure 3). The results have shown that composites of both types swelled at all pH values under investigation. The swelling followed the order pH 5.8 > 8.0 > 7.4. Hence, they are suitable for wound dressings to be applied on skin (pH 5.5).

3.2. Scanning electron microscopy (SEM)

Figure 4 clearly revealed that the prepared composites have voids and layered structure. Moreover, the results have also revealed that the nanoparticles and the drug molecules have been successfully incorporated into the voids and layers of the porous composites. These results suggest that the prepared composites have the ability to encapsulate nanoparticles and drug molecules in them.

3.3. Thermal stability

Thermal analysis was performed immediately after composite preparation and after one year. TGA and DSC results of *LRO*2, *LRO*4, *LRI*7 and *LRI*8 composites are shown in Figure 5.

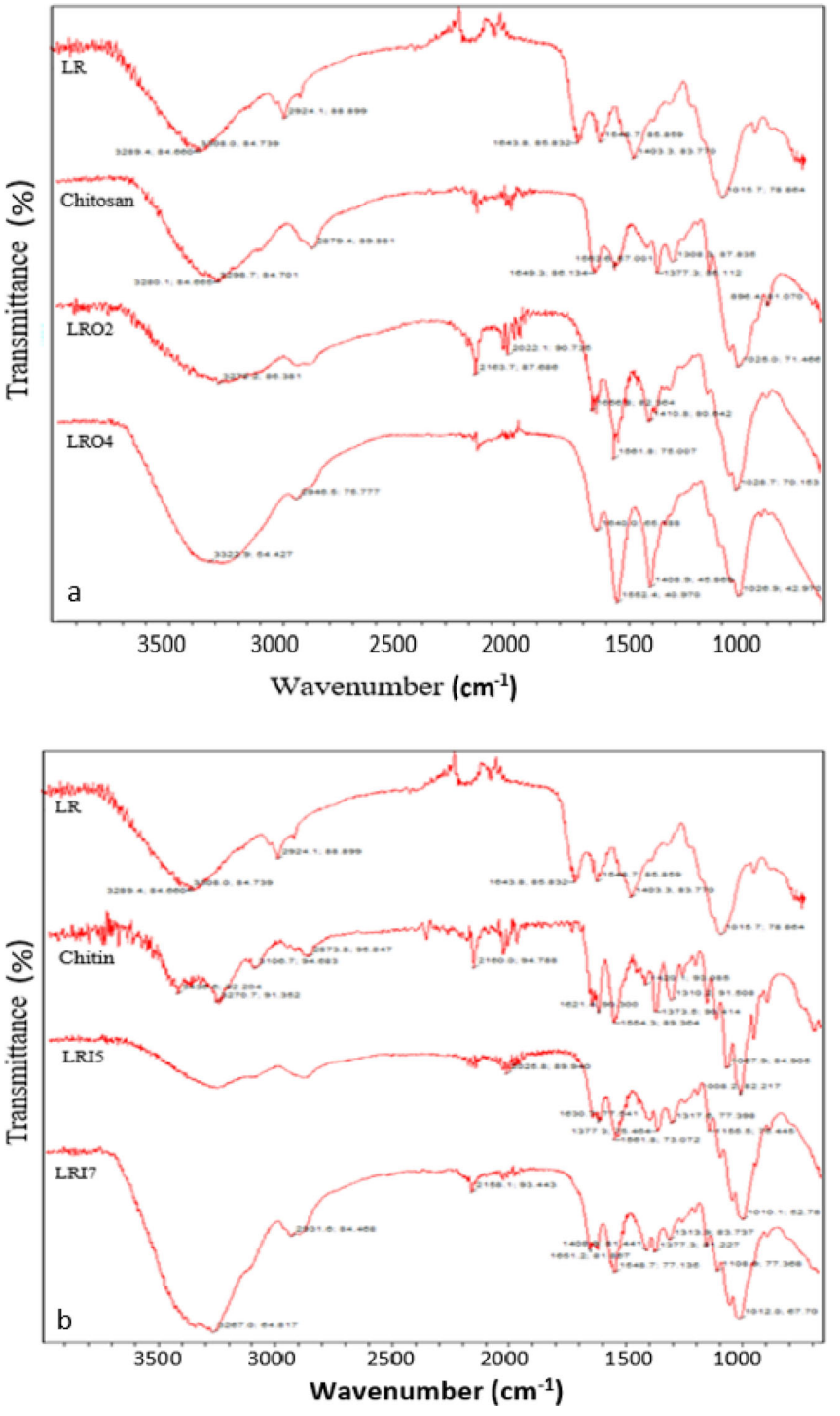


Figure 1. FTIR of (a) *LR*-chitosan composite and (b) *LR*-chitin composite.

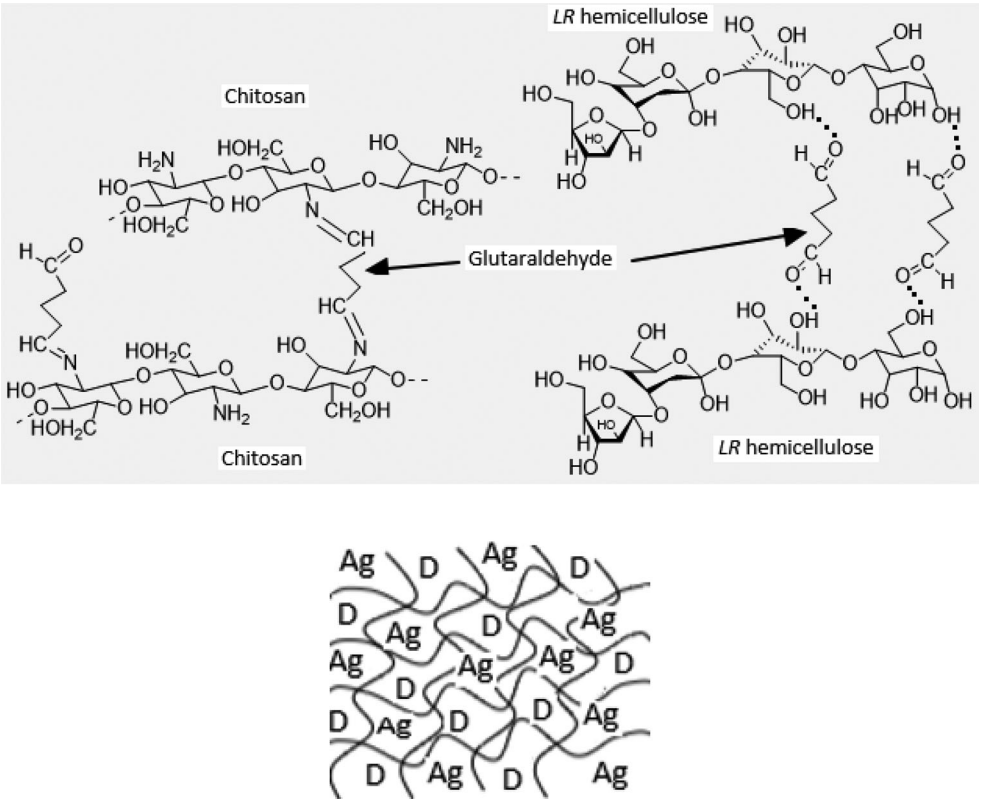


Figure 2. (a) Crosslinking between *LR* hemicellulose and chitosan with glutaraldehyde. (b) The drug (D) and the AgNPs in the *LR* composite matrix.

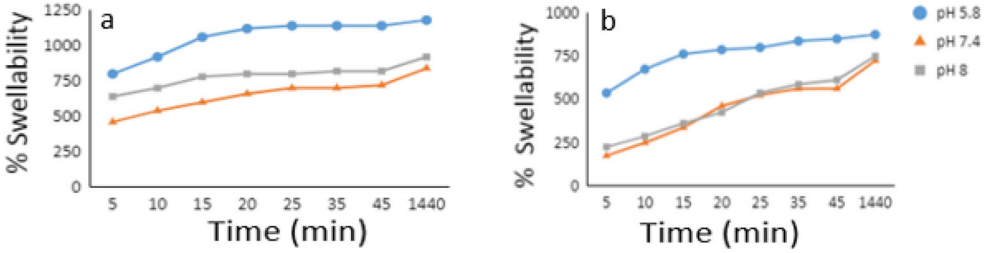


Figure 3. % Swellability of (a) *LR*O4 composite and (b) *LR*I7 composite.

The TGA and DSC results remained unchanged after one year, indicating that these composites are stable. Moreover, the TGA data revealed that all the composites have undergone three-step degradation. The first step is endothermic weight loss of 12% from ambient to 100 °C, which was due to the loss of trapped moisture. In the second step, the major weight loss (40%) in the temperature range 200–350 °C (Figure 5(a)), which was also endothermic due to detachment of side chains producing mono-/oligosaccharides [19]. The third step (400–500 °C, endothermic 30% weight loss) involved decomposition of the main polymeric chains producing monosaccharides followed by charring to produce CO_2 (exothermic peak in the DSC scan).

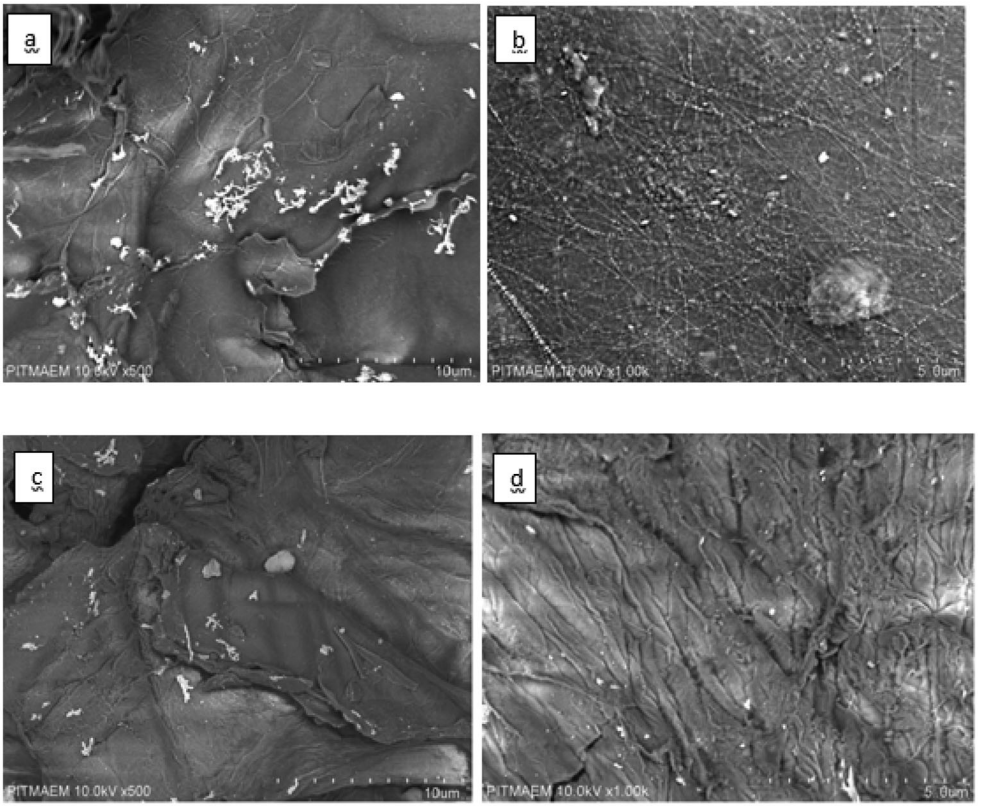


Figure 4. SEM images of (a) *LRO*2, (b) *LRO*4, (c) *LRI*5 and (d) *LRI*7.

A small amount (approx. 10%) of residue was left as ash at the end. So, on the basis of these results, it can be concluded that the prepared composites have thermal stability up to 210 °C even after one year.

3.4. Dissolution studies

Drug release through dissolution studies were carried out to determine the % release of the incorporated drug (ciprofloxacin) from the loaded composites at regular time intervals by measuring absorbance at 271 nm. The results revealed that the composites *LRO*2, *LRO*4, *LRI*7 and *LRI*8 show slow and steady release of the drug over a period of 10 days with gradual rise in % drug release. In 3 days only 12 to 15% of drug was released from the composites. Release in first 3 days was slow because the composites were swelling and as the polymer swelled, the release increased. Thus these dressings continue to release the drug and protect the wound from infections for an extended period of time, eliminating the need to change the dressings more frequently.

All the composites showed a sustained release with % release of 20%–30% respectively over 9 days period. The remaining drug will continue to be released for about 15 days (approx. 80% of the load) as revealed by extrapolation of the data. So on the basis of these results; *LRO*4 and *LRI*7 were selected for *in vivo* evaluation (Figure 6).

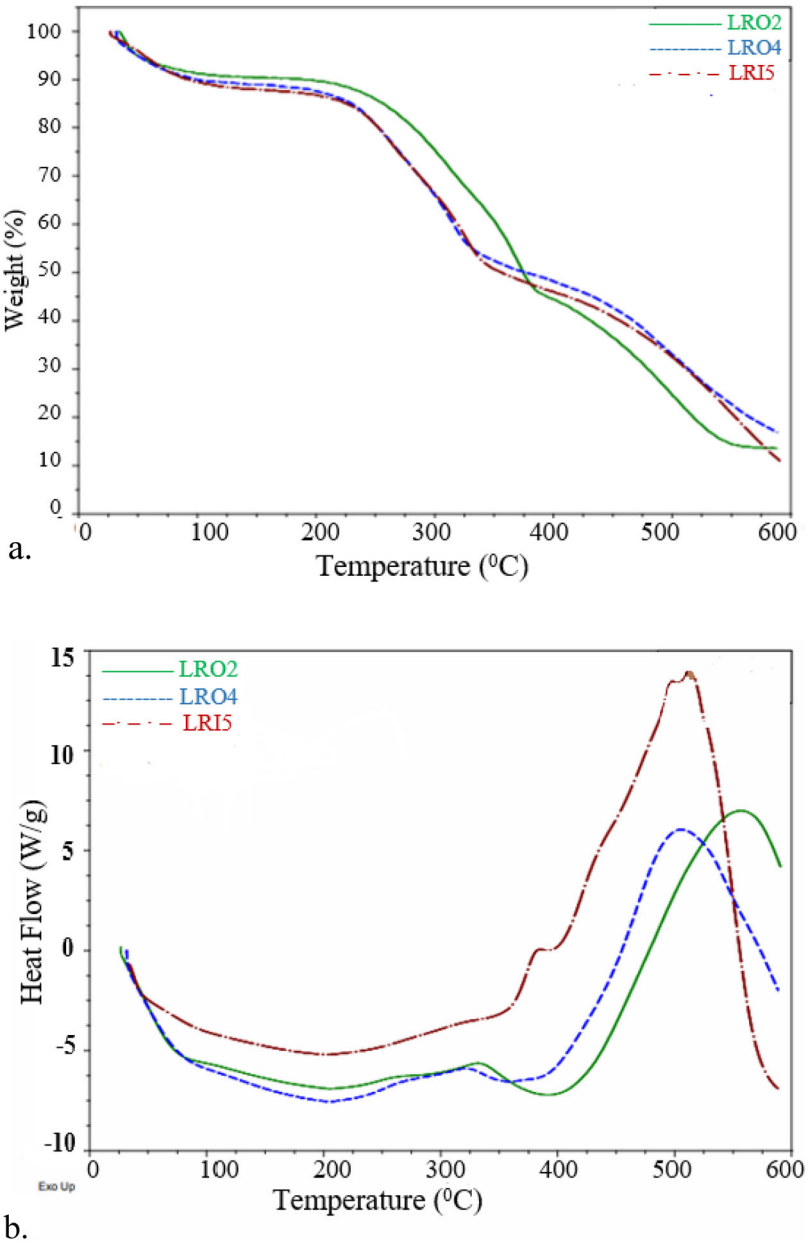
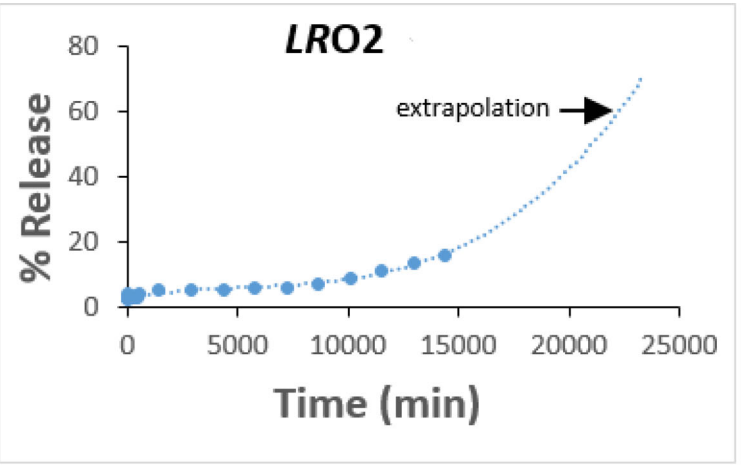


Figure 5. Thermal analysis (a) TGA and (b) DSC scans.

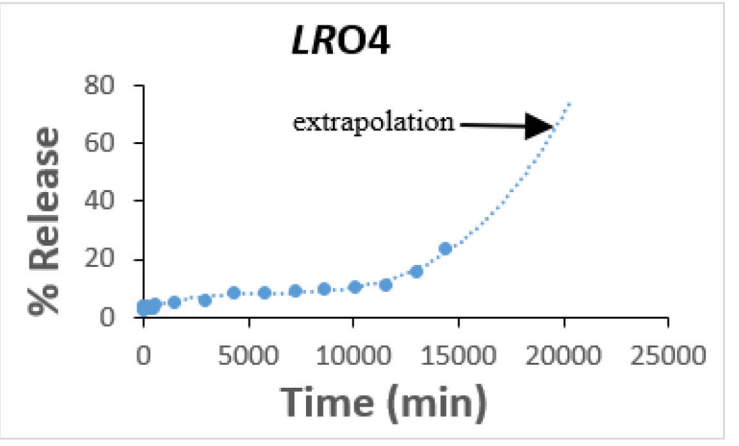
3.5. In vitro *antibacterial studies*

The use of silver in wound dressings has been wide spread, as it shows broad anti-microbial (against both Gram-negative and Gram-positive organisms) [28, 29] and anti-fungal activities [30]. Although there is a debate regarding the efficacy of silver [31, 32] and its potential toxicity [33].

The role and significance of microorganisms in wound healing has been debated for many years. While some experts consider the microbial density to be critical in predicting



a.



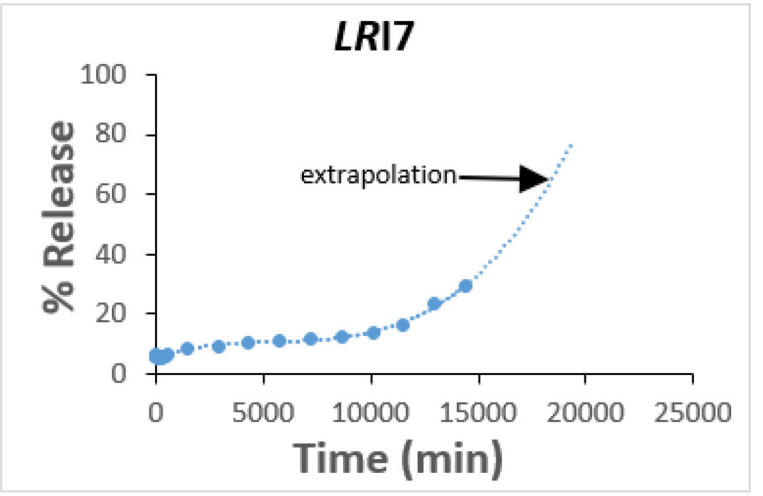
b.

Figure 6. *In vitro* drug release from the composites.

wound healing and infection, others consider the types of microorganisms to be of greater importance. However, these and other factors such as microbial synergy, the host immune response, and the type of tissue must be considered collectively in assessing the state of infection [34]. So, the prepared composites were loaded with anti-microbial agents (silver nanoparticles and ciprofloxacin), their antibacterial potential was checked against different bacterial strains. The antibacterial activity was performed on agar medium against *E. coli* and *S. aureus* bacterial strains. These strains were used because they are usually involved in infections and they represent the Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) strains. Silver-ciprofloxacin nano-composites showed zone of inhibition (19–30 mm) for *E. coli*, which was found to be close to the ciprofloxacin standard (21–35mm) but in case of *S. aureus* zone of inhabitation was less (11–17 mm) than the standard.

These results revealed that AgNPs loaded discs were effective against only Gram negative *E. coli* whereas the discs containing both AgNPs and drug were effective against both *E. coli* as well as *S. aureus*. The values of zones of inhibition around the loaded discs are shown in Table 4.

c.



d.

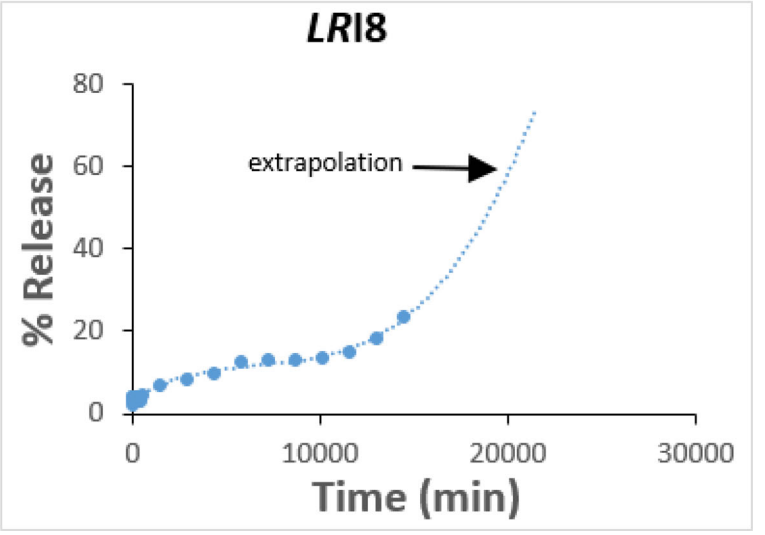


Figure 6. Continued.

Table 4. Zones of inhibition against different bacterial strains.

	E. coli		*S. aureus*	
Sample	Standard (mm)	Loaded disc (mm)	Standard (mm)	Loaded disc (mm)
LRO1	15	11	–	–
LRO2	10	7	–	–
LRO3	21	19	21	12
LRO4	22	21	25	17
LRI5	11	8	–	–
LRI6	17	12	–	–
LRI7	35	30	25	15
LRI8	30	25	20	11

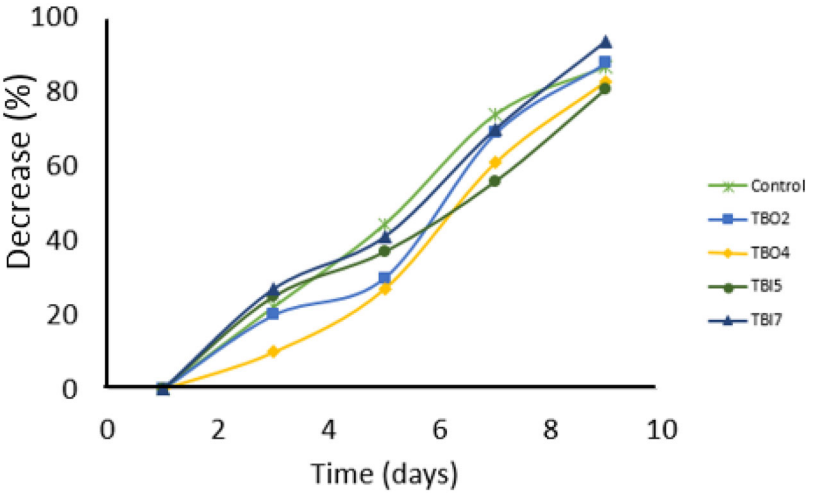


Figure 7. Decrease (%) in wound size with time.

3.6. In vivo *wound healing evaluation*

Wound healing experiment was performed on Wistar rats to determine the potential of the prepared composites as well as to confirm whether these dressings are easily removable or not. The results of wound recovery are shown graphically in Figure 7 and pictorially in Figure 8.

The results clearly revealed that the prepared composite dressings cured the wound effectively over a period of 9 days. None of the composites showed adverse effects on the wound site. In the control group, the dressing got stuck to the wound and when it was removed on 3rd day, it caused bleeding. Of the composites, only *LR*O4 remained stuck to the wound, which was removed by spraying the sterile water. Other composites showed consistent and comparable recovery of wound as shown in Figure 8. Therefore, it can be concluded from these results that the composites *LR*O2, *LR*I5 and *LR*I7 were better, non-sticky to the wounds, non-allergic, non-toxic, moistening, thermally stable, preventing microbial growth, absorbed more wound exudate as compared to the traditional gauze dressing and were economical over the commercially available sustained release dressing. Thus, they can provide better compliance and less pain to the patients during change of dressings. Therefore, these composite dressings have the potential to be used in the clinical trials.

4. Conclusion

The polysaccharide isolated from *LR* seeds was used to prepare composite wound dressings in combination with chitosan and chitin. The composites possessed high water retention capacity and showed high swelling indices. The composites were porous in nature thus able to support oxygen permeability. The drug loaded composites remained stable, under stored condition for one year and their efficacy was comparable to that commercially available dressings. The composites can be loaded with any drug. The slow release pattern of the drug from these composites is desirable for

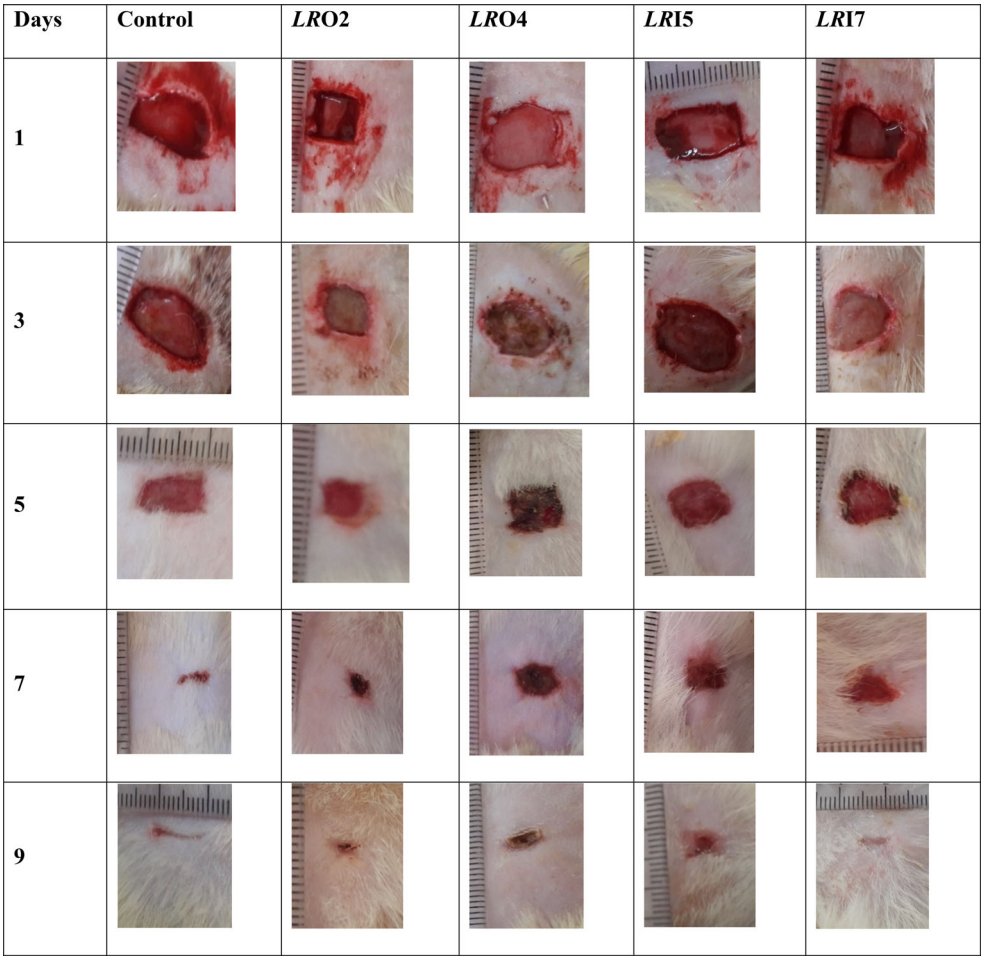


Figure 8. Wound closure after treatment.

protection against infections over an extended period of time. Based on this study, it can be concluded that the prepared composites have the potential to be used as wound dressings in clinical trials for treating different types of wounds.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethics statement

All experiments were carried out according to EU Directive 2010/63/EU for animal experiments and after approval of IRB of the Forman Christian College (A Chartered University) Lahore, Pakistan.

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