Journal of Vascular Nursing 40 (2022) 2–10

Contents lists available at ScienceDirect

Journal of Vascular Nursing

journal homepage: www.sciencedirect.com/journal/journal-of-vascular-nursing

Evaluation of a novel melatonin-loaded gelatin sponge as a wound dressing

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Background: For Melatonin, the pineal gland hormone, also known as the neuroendocrine hormone, influences angiogenesis by exerting a positive effect on fibroblast, monocyte, and cytokine proliferation. Formulation and study of characteristics of gelation-based Melatonin sponge crosslinked with fructose using the surfactant foaming and freeze-drying method for wound healing application was the objective of our study. The structure of the formulated gelatin sponge was observed using a scanning electron microscope and showed to have abundant and uniform pores. Characteristics were studied using digestibility test, water uptake test, porosity measurement test, moisture uptake test, tensile strength test, folding endurance test, scanning electron microscopy, Fourier transform infrared spectroscopy, and drug entrapment efficiency analysis.

Results: The obtained data showed that the formulated sponge had good mechanical properties, water uptake, and water retention capacities. In animal experiments, histological and macroscopic observations showed that wounds covered by gelatin loaded with a Melatonin sponge healed quickly.

Conclusion: The formulated sponge was a potential wound healing material having suitable physical, mechanical properties and biocompatibility. The graphical abstract is shown in Figure 1.

Introduction

Melatonin, an indoleamine discovered by Aaron Lerner, is chemically known as 5 methoxy-N-acetyltryptamine (Figure 1) [11]. Secretion of Melatonin occurs at night through the pineal gland, and hypothalamic nuclei regulate the secretion. Other than the pineal gland, Melatonin was found to be synthesized in lymphocytes, skin, ovaries, and gastrointestinal tract, producing an autocrine effect causing superimposition on the neuroendocrine response[21]. Various studies have suggested that enzymes like Acetylserotonin O-methyltransferase and Serotonin N-acetyltransferase, Melatonin synthesis primarily occur at mitochondria[16]. Melatonin at different body areas shows multiple biological activities by interacting with other molecules apart from binding with the receptors after crossing through the cell membranes, resulting from its amphipathic nature[1]. Biologically, wound healing occurs in three phases: the inflammatory phase, the proliferative phase, and the remodelling phase, which ultimately re-establishes the tissues' integrity and functions[19]. By inhibiting nuclear factor kappa B binding to deoxyribonucleic acid and its translocation to the nucleus, Melatonin as an anti-inflammatory agent decreases various factors which support inflammation such as interleukin-1β, tumour necrosis factor α, and interleukin- 6, [22,17]. By reducing oxidative stress, the production of adhesion molecules is inhibited by Melatonin[5]. Different studies have proved that Melatonin exerts an antifibrotic effect[23,4]. A different study observed that the accumulation of collagen is controlled by the pineal gland under the intact skin, and Melatonin[4] decreases this accumulation of collagen. With such encouraging characteristics, Melatonin gives off an impression of being an ideal candidate to be used in the healing of wounds. For fast and rapid recovery of the injury, wound dressing acts as a contributing component that soaks the exudates coming out of the wound and inhibits any bacterial infection, thus keeping the injured area clean. Ideally, a wound dressing should be inexpensive and able to provide support mechanically, biologically, and chemically[20].

According to researchers, generally used wound-dressing materials includes films, sponges, nanofibers and hydrogels which were

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https://doi.org/10.1016/j.jvn.2021.09.004
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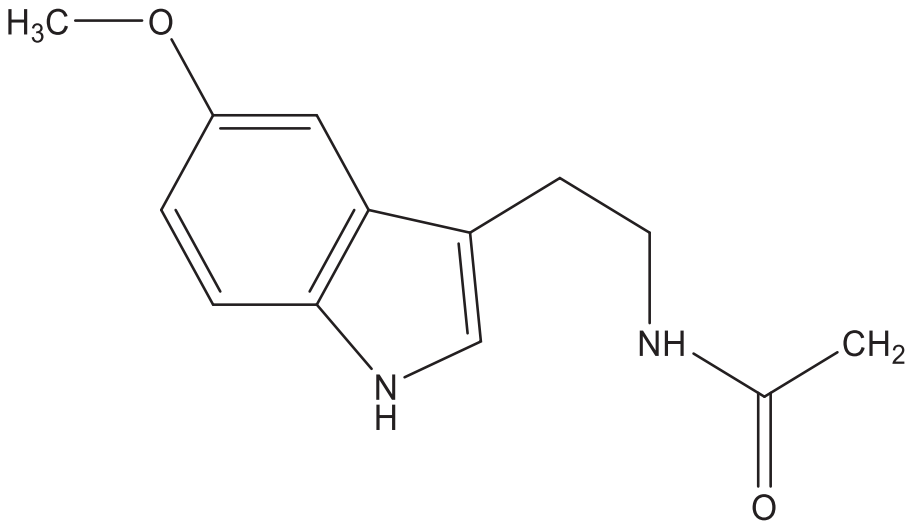

Fig. 1. Chemical structure of Melatonin.

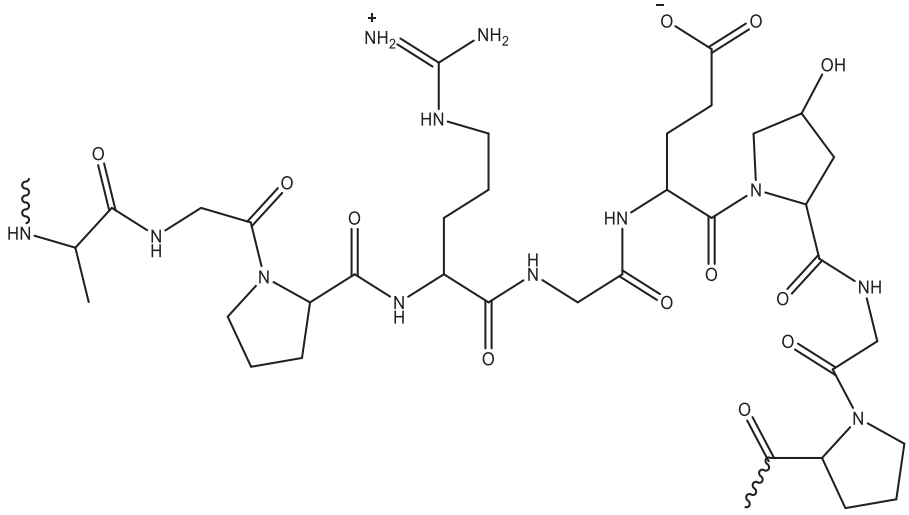

Fig. 2. Chemical structure of Gelatin.

based on natural or synthetic polymers and their combinations[14]. For wound healing management, natural polymers are mostly considered over synthetic polymers due to their advantages like being cost-effective, non-toxic to biological systems and environment friendly. Naturally occurred polymers such as cellulose, chitosan, pullulan, starch and β-glucan, as well as collagen, hyaluronic acid and alginate are among the most commonly used polymers as wound dressings[14].

Gelatin (Figure 2) consists of 4-hydroxyproline, glycine, and proline[18]. Absorbable gelatin sponges are used in various surgical procedures to control bleeding and to show a haemostatic effect. As the sponges are porous, blood plasma and platelets are caught in the sponges, and the coagulation factors are activated. Soluble fibrinogen is transformed into a net of insoluble fibrin, which stops the bleeding, which is helpful for wound dressing purposes. Thus, the sponge structure is believed to be of significant interest in developing a melatonin-loaded gelatin sponge for a wound dressing that provides dual benefits, i.e., medication and dressing material. Formulations of gelatin sponges were prepared by dissolving gelatin in water and mixing it vigorously to form firm foam. This foam is then dried and sterilized to use as a surgical haemostatic sponge and wound dressing material[9,8]. On doing a literature survey we did not find any similar work performed by any other researcher on Gelatin based melatonin sponge for wound healing and dressing purpose. Our study aimed to design, develop, and evaluate Melatonin loaded gelatin sponge for wound healing activity and investigate the effect of various parameters on the characteristics of the formulation.

Methods

Materials and reagents

Melatonin was received from Zhengzhou Lion Biological Technology Co. Ltd., USA. Gelatin, Fructose, Tween 80, analytical grade Sodium bicarbonate, n-octanol, and Potassium dihydrogen phosphate were procured from Himedia Laboratory Pvt. Ltd., Mumbai, India. Analytical grade Methanol, Ethanol, Chloroform, Acetone, Acetonitrile, and Glycerin were procured from Merck specialization Pvt. Limited, Mumbai, India. Analytical grade Sodium hydroxide, Hydrochloric acid, and Sodium chloride were obtained from Qualigens Fine Chemicals Ltd., Mumbai, India. Analytical grade Formaldehyde solution was procured from Rankem, RFCL Limited., New Delhi, India.

Table 1
Composition of Melatonin loaded gelatin sponge.

Formulation code	Melatonin (%)	Gelatin (%)	Tween 80 (%)	Fructose (%)
S9a	0.7	5	0.2	1.5
S9b	1.5	5	0.2	1.5
S9c	2	5	0.2	1.5
S9d	2.5	5	0.2	1.5

Instrumentation and operating conditions

Ultraviolet spectrophotometer UV 1800 procured from Shimadzu; Japan, Fourier transform infrared spectrophotometer procured from Bruker, Germany, Scanning electron microscope- JSM 6360 procured from JOEL, Japan, Differential scanning calorimeter procured from Perkin Elmer, Japan, Lyophilizer procured from IIC Industrial Corporation, India, Homogenizer procured from Ultra Turrax, Germany and Microscope with True Chrome IIS Camera procured from Primo Star, Karl-Zeiss, Germany were used while carrying out the research work.

Preparation of gelatin sponge

Melatonin-loaded gelatin sponge was made by surfactant foaming method where gelatin was accurately weighed and dissolved in warm distilled water. To the gelatin solution, tween 80 was added which acts as a foaming agent and the specified amount of the Melatonin was accurately weighed and added to the solution. Finally, as crosslinking agent fructose was added. The solution mixture was homogenized for 5 min by using a homogenizer at an optimum speed. The mixture was poured into a Petri dish and kept at a temperature of -4^{0}C for overnight and lyophilized the formulation using lyophilizer[6,12,7]. The detailed composition of the Melatonin loaded gelatin sponges is given in Table 1.

Characteristics of Melatonin loaded gelatin sponge

The Melatonin-loaded sponge was developed and evaluated for its characteristics, such as digestibility, water uptake, porosity, moisture uptake, tensile strength and folding endurance. SEM, FT-IR spectroscopy, drug content tests, drug loading tests, entrapment efficiency tests, and in vitro drug release studies were performed.

Digestibility

The unit weight of the dry sponge was taken and was placed in a beaker containing water. Between the finger, it was kneaded until it became thoroughly wet and all the air got removed. The sponge was removed from the beaker, and excess water was discarded with tissue paper. The wet sponge was then placed in 100 ml of 0.1N HCL at 37°C and was gently agitated continuously for 75 min[6].

Water uptake

A unit portion of the dry sponge was weighed and place on the wet surface of tissue paper. Individual sponge weight was measured at different time intervals. The water up-take percentage was calculated from the difference in the sponge's final weight and initial weight[12].

$$Water\,uptake\,(\%) = \{(Final\,wt. - Initial\ wt.)/Initial\ wt.\} \times 100$$

Porosity measurements

Solvent replacement method was used to measure the porosity. In an oven, the unit weight of the sample was first dried till constant weight (Wi) was obtained. For overnight, the dried test sample was immersed in absolute ethanol and excess ethanol present over the surface was removed by blotting on tissue paper and weighed (W_f) immediately[12,7]. Calculation of the % porosity was Calculated as follows:

$$Porosity\ (\%) = \{(W_f - W_i)/V\rho\} \times 100$$

Where ρ is the density of absolute ethanol and V is the volume of the sponge.

Moisture up-take

Unit areas of the sponge were weighed and placed in a closed container with a saturated sodium chloride solution of RH 75% ±5. Calculation of moisture uptake of the sponges was done by calculating the weight difference from initial to final weight, using the formula given below[6].

$$Moisture\ \ up-take\ \ (\%) = \{(Final\ wt. - Initial\ wt.)/Final\ wt.\} \times 100$$

Tensile strength

Tensile strength of sponge was performed manually; unit length of the sponge was fixed with clip-in its two ends. One end of the sponge was mobilized, and another end was attached to a bottle by a non-stretchable string. Water was added drop by drop into the bottle until it detached from the sample. Breaking strength was calculated with the help of water volume, which is required to break the sample[6].

$$Tensile\,strength = \{Load/width \times thickness \times length\}$$

Folding endurance

To determine the flexibility of the sponge, folding endurance was performed manually. Determination of folding endurance was done by repeatedly folding the film at the same place until it breaks. Folding endurance is the number of times, without breaking, the films could be folded at the same place[7;14;25].

Scanning electron microscopy (SEM)

For observation of the internal structure of the gelatin sponges, Scanning Electron Microscopy (SEM), was used. Measurement of an average diameter of wall thickness and pores was done by image analyzer using on-screen geometrical observation[14,25].

Fourier transform infrared spectroscopy

The excipients that are to be used in the formulation should be compatible with each other and with the drug also. Interaction between the excipient and drug may cause drug degradation and affect the rate of dissolution of the drug, uniformity of dose, efficacy, and stability of the drug. The FT-IR spectra of all excipients, their physical mixture, and gelatin sponge-loaded Melatonin were carried out using an IR spectrophotometer (Shimadzu 8400) 4000cm^{-1} to 400cm^{-1}. The IR spectrums revealed the functional groups of the sample from their distinguishing peaks and were interpreted for any potential interaction between the components.

Drug content, entrapment efficiency, and drug loading

100 mg of gelatin sponge was dispersed in 50 ml methanol, sonicated for 90 minutes in bath sonicator, and filtered. 1 ml of filtrate was withdrawn and diluted with methanol. The absorbance measurement of the resultant solution was done at 277nm against

a suitable blank using UV-spectrophotometer (UV1800, Shimadzu). A blank sponge (without drug) was treated similarly and was used as a reference control[5,25]. Calculation of drug content, drug entrapment efficiency, and drug loading was done using the following formulae:

$$\text{Drug Entrapment efficiency (EE) \%} = \frac{\text{Total drug added - amount of free drug}}{\text{Total drug added}} \times 100$$

$$\text{Drug loading \%} = \frac{\text{Amount of drug in formulation}}{\text{Weight of the formulation}} \times 100 \qquad (1)$$

In-vitro drug release study

In-vitro dissolution study was performed in modified USP apparatus II (paddle). 100mg sponge was kept in the dissolution medium using 100 ml of pH 7.4 phosphate buffer. It was stirred (50 rpm) using a magnetic stirrer while maintaining the temperature of 37±0.5°C. 2 ml solution was withdrawn from the system at a different time interval from the reservoir and replaced with an equal volume of fresh dissolution medium to maintain sink condition. The aliquots were diluted and analyzed by UV spectrophotometer at 277nm [25]; The Pharmaceutical codex 2000). The in vitro release study data were statistically plotted in various kinetic models' dependent approaches, including zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas order to determine drug release mechanism.

In vivo wound healing study on animal model

In vivo wound healing study of prepared Melatonin loaded gelatin sponge has been performed using Swiss albino rats as an animal model given through topical route. The in vivo pharmacodynamic study was approved and certified (vide approval No. IAEC/DU/117, Dated 18/02/2016) by the Institutional Animal and Ethics Committee, Department of Pharmaceutical Sciences, Dibrugarh University (Reg No. 1576/GO/E Re/S/11/CPCSEA, Dated 23/12/2014), Dibrugarh, Assam, India.

In vivo wound healing study of the prepared Melatonin loaded gelatin sponge has been performed on Swiss albino rats. Animals were randomly assigned to either control or experimental groups, divided into five groups: Positive control, Negative control, Standard Marketed formulation, Blank and Test formulations. At first, the wound has been induced to each group except the positive control group by excision methods. In brief, the rats were anaesthetized with an intraperitoneal injection of ketamine (60mg/Kg) and xylazine HCl (8mg/Kg). The rats were depilated on the back, the skin was excised at a predetermined area of 1cm square total thickness and 1cm length incised in the dorsal interscapular region, respectively. Wound dressing has been done every day with respective sponge formulation to each group until the wound was completely healed. The test group has been compared with other groups treated with marketed formulation and blank formulation. With the help of a scale, the length and diameter of the wound area have been measured from when the wounds were induced until completely healed [6,10]. The reduction in wound size was measured at different day intervals, and the wound contraction rate was expressed in terms of percentage. The wound contraction rate was calculated using the following formula:[24]

$$\text{\% wound contraction} = \frac{\text{wound diameter(day 0)} - \text{wound diameter (day n)}}{\text{wound diameter (day 0)}} \times 100$$

Where n= diameter of the wound on 3rd day, 8th day, and 12th day.

Histological evaluation

On the 8th day, the skin sample was collected from the wound area for incision wound and on the 15th day for excision. The skin sample was preserved in 10 % (v/v) formaldehyde and embedded in a block of paraffin. Tissues embedded in paraffin were sectioned (4mm thickness) and stained with Haematoxylin and Eosin (H&E) to check the infiltration of cells, epithelialization and collagen formation. The sections were further mounted under a microscope for examination.

Results

Characteristics of Melatonin loaded gelatin sponge

Melatonin gelatin sponge has been prepared and characterized the following parameters: water uptake, porosity, digestibility, tensile strength, adhesion, folding endurance, drug loading, and entrapment efficiency. The data obtained were reported in Table 2. All the formulations were wholly digested in the 0.1N HCl within 75 min, indicating that it might get digested entirely inside the biological system. Almost all the sponges had shown good water-holding capability with suitable porous structures. S9 exhibited minimum moisture uptake, i.e., 2.12%, which indicates that it might be more stable than other formulations. Formulation S9 had observed maximum tensile strength, adhesion properties, and more flexibility than other formulations. After evaluating all the parameters, S9 has been considered the suitable formulation to incorporate Melatonin in different per cent via 0.7%, 1.5%, 2%, and 2.5%.

Drug excipient compatibility studies

In the pre-formulation studies, investigation of the interaction between excipients and drug in the formulation is important as it gives the stability information of drug in the formulation, release pattern of the drug, and the lag time of the released drug from the formulation. Regarding the interaction between functional groups of drug and excipients, FT-IR spectroscopy provides a distinct idea among the different available methods. FT-IR spectra of all the individual compounds, physical mixtures of the excipients with Melatonin and Melatonin-loaded gelatin sponge, have been observed and interpreted in Figure 3. There was no significant shifting of the melatonin peak in the physical mixture as well as formulation. The slight shifting of the peak may indicate the physical interaction between the functional group of drug and excipients, either due to weak hydrogen bonds or van der wall force or dipole-dipole interaction.

Morphology of gelatin sponge

Morphology of Melatonin-loaded sponge had been observed through an optical microscope and scanning electron microscope. The average pore diameter has been measured by geometrical observation of SEM images. From Figure 4 it has been observed that under an optical microscope, the morphological structure of Melatonin loaded gelatin sponge was porous in structure. Using Scanning Electron Microscopy, the internal porous structure of the sponge and the surface of the sponges was observed with the continuity of pores size.

In vitro release of Melatonin from gelatin sponges

The in vitro release studies were performed for the formulation S9a, S9b, S9c, and S9d in phosphate buffer pH 7.4 as a dissolution medium. The cumulative percentage of drug release was calculated and shown in Table 3 and Figure 5.

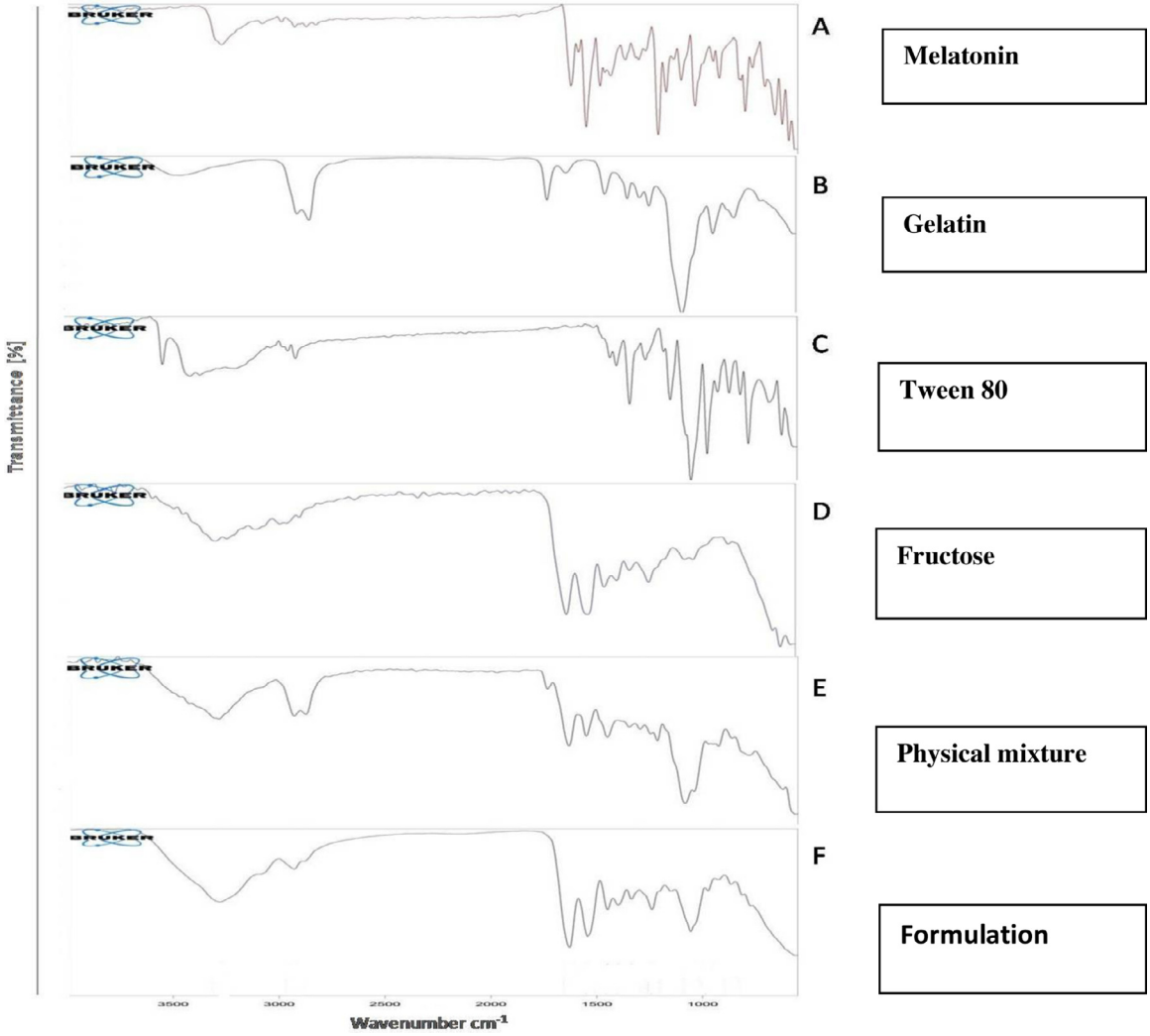

Fig. 3. FT-IR spectra of (a) Melatonin (b) Gelatin (c) Tween-80 (d) Fructose (e) Physical mixture (f) Melatonin loaded Gelatin sponge formulation.

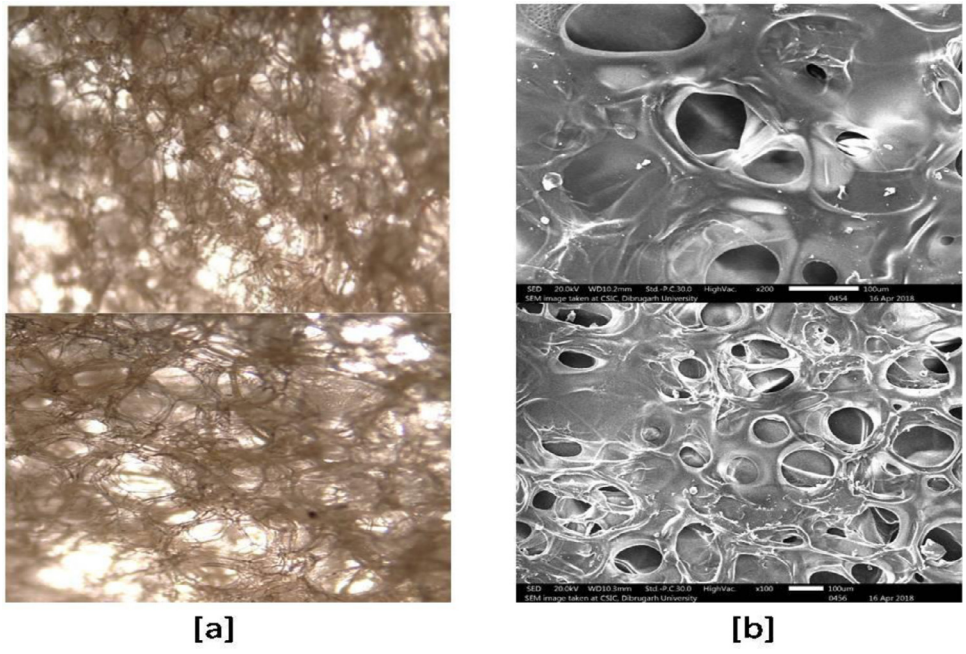

Fig. 4. (a) Optical Microscopic Structure of Sponge (b) SEM of Melatonin Loaded sponge.

Table 2
Result of evaluation parameter of Melatonin loaded sponge.

Formulation	S9a (0.7%)	S9b (1.5%)	S9c (2%)	S9d (2.5%)
Water uptake (%)*	77.96±0.601	76.45±0.169	77.02±0.077	78.46±0.183
Moisture uptake (%)*	2.01±0.077	2.03±0.007	2.00±0.0778	1.71±0.070
Digestibility (%)	Completely digested	Completely digested	Completely digested	Completely Digested
Porosity (%)*	74.05±0.106	73.51±0.120	73.80±0.134	74.90±0.777
Tensile strength (dyne/ cm^2)*	340.8±0.494	327.5±0.070	336.0±0.282	339.7±0.353
Adhesion (N) *	155.8±0.558	151.7±0.212	154.0±0.282	156.2±0.424
Folding Endurance*	297±0.707	289±0.707	294±0.707	301±1.414
Drug loading (%)*	0.6011±0.0402	1.2302±0.0913	1.6803±0.1755	2.2004±0.1409
Entrapment efficiency (%)*	82.704±0.2184	82.403±0.3521	84.102±0.7078	88.404±0.7665

*Each value represents mean with SD n=3

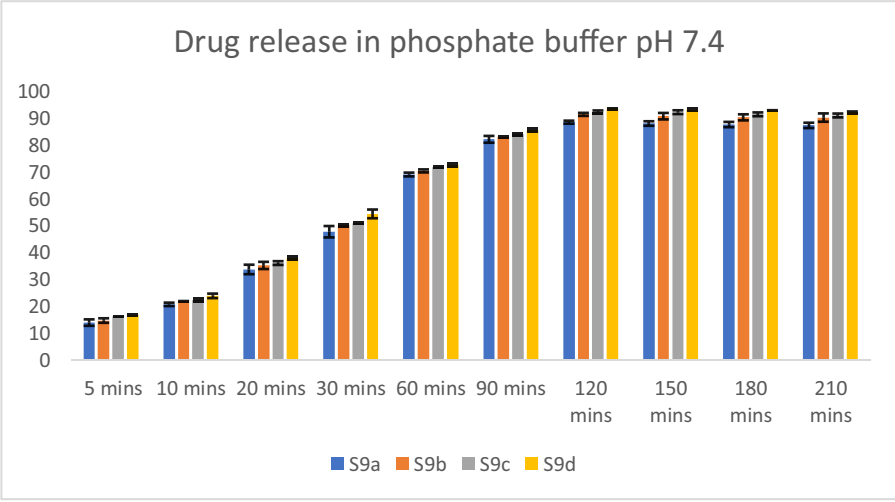

Fig. 5. Bar graph of cumulative drug release percent v/s time.

Table 3
Cumulative amount released percentage of S9a, S9b, S9c and S9d.

Time (min)	S9a*	S9b*	S9c*	S9d*
0	0	0	0	0
5	13.95±1.20	14.72±0.84	16.24±0.14	16.95±0.21
10	20.75±0.63	21.84±0.14	22.35±0.49	23.91±0.84
20	33.75±1.76	35.25±1.34	36.13±0.70	38.12±0.42
30	47.81±2.12	50.23±0.28	51.05±0.07	54.45±1.62
60	69.12±0.70	70.46±0.56	71.92±0.14	72.75±0.49
90	82.23±1.27	83.17±0.14	84.15±0.35	85.75±0.35
120	88.61±0.56	91.52±0.56	92.41±0.56	93.63±0.28
150	88.12±0.84	90.85±1.20	92.34±0.70	93.31±0.28
180	87.74±0.98	90.41±1.13	91.54±0.70	92.93±0.14
210	87.42±0.99	90.33±1.55	91.12±0.71	92.25±0.35

*Each value represents mean with SD, n=3

Kinetic study of in vitro drug release

In-vitro drug release kinetic studies of formulations S9a, S9b, S9c and S9d in different kinetics model is shown in Table 4. The different kinetic models of in vitro drug release profile of different formulations S9a, S9b, S9c, and S9d has been calculated. Based on the highest regression (R^2) values, all the formulations were best fitted in Korsemeyer-Peppas kinetics (R^2 = 0.99, 0.9913, 0.9917, and 0.9862 for S9a, S9b, S9c, and S9d, respectively). However, they tended to follow the Higuchi model and the values. The drug release exponent (n-value) in the Koresmeyer-Peppas plot for S9a, S9b, S9c, and S9d was 0.6295, 0.6374, 0.6524, and 0.6584, respectively. These values were within the range of 0.45 to 0.89, suggesting that the drug shown Fickian diffusion from the formulation.

In vivo wound healing activity in rat's model

Wound healing activity of Melatonin loaded gelatin sponge has been carried out using Swiss albino rats as an animal model. The wound has been induced to each animal group by excision method and incision method; the wound area has been treated and monitored from the initial day to the 15^{th-}day Figure 6. The rate of wound healing ability has been observed in the following order: Standard > Test> Blank> Negative control, respectively. It was observed that the healing rate of incision wounds was much faster than excision wounds for each group. The excision wound area of the standard group has been shown to completely healed within 12-13 days for the test group, whereas for both blank and negative control groups, complete healing was not observed on the 15^{th} day. However, it is worth mentioning here that healing was slightly better in the blank group than in the negative control group. The wound contraction rate was expressed in terms of percentage as reported in Figure 7.

Histological study

On the 15^{th} day, a skin sample from the wound treated area has been collected and evaluated histologically for any changes like collagen formation, epithelialization, and infiltration of cells. The histological cross-section of the rat's skins was shown in Figure 8. The histological analysis of Standard, Test, Blank, Negative Control, and Positive Control of rat's skin was examined. In positive control, i.e., normal skin, epidermis tissue had shown more continuous and complete collagen in the skin. Epidermis

Table 4
In vitro drug release kinetic study.

Formulation Codes	Kinetic model	Equation	R2
S9a	Zero order	23.821x +25.467; K_0 = 23.821	0.7707
	First order	-0.2947x +1.8715; K_1= 0.2947	0.8709
	Higuchi	52.084x +5.941; K_H = 52.084	0.9283
	Hixon-Crowell	-0.7747x +2.1705; K_{HC} = 0.7747	0.5073
	Korsemeyer-Peppas	0.6584x - 0.1383; n = 0.6584	0.99
S9b	Zero order	24.717x +25.752; K_0 = 24.717	0.7841
	First order	-0.3409x +1.8809; K_1 =0.3409	0.8946
	Higuchi	53.821x +5.7102; K_H = 53.821	0.8946
	Hixon-Crowell	-0.7857x +2.1556; K_{HC} = 0.7857	0.513
	Korsemeyer-Peppas	0.6524x - 0.1315; n = 0.6524	0.9913
S9c	Zero order	24.701x +26.518; K_0 = 24.701	0.7778
	First order	-0.3508x +1.8743; K_1 =0.3508	0.8838
	Higuchi	53.911x +6.3673; K_H = 53.911	0.9334
	Hixon-Crowell	-0.7804x +2.1296; K_{HC} = 0.7804	0.5061
	Korsemeyer-Peppas	0.6374x -0.1275; n = 0.6374	0.9917
S9d	Zero order	24.691x +27.378; K_0 = 24.691	0.7727
	First order	-0.3647x +1.8705; K_1 = 0.3647	0.8886
	Higuchi	54.005x + 7.1202; K_H =54.005	0.9314
	Hixon-Crowell	-0.7753x +2.1022; K_{HC} = 0.7753	0.4986
	Korsemeyer-Peppas	0.6295x - 0.1188; n = 0.6295	0.9862

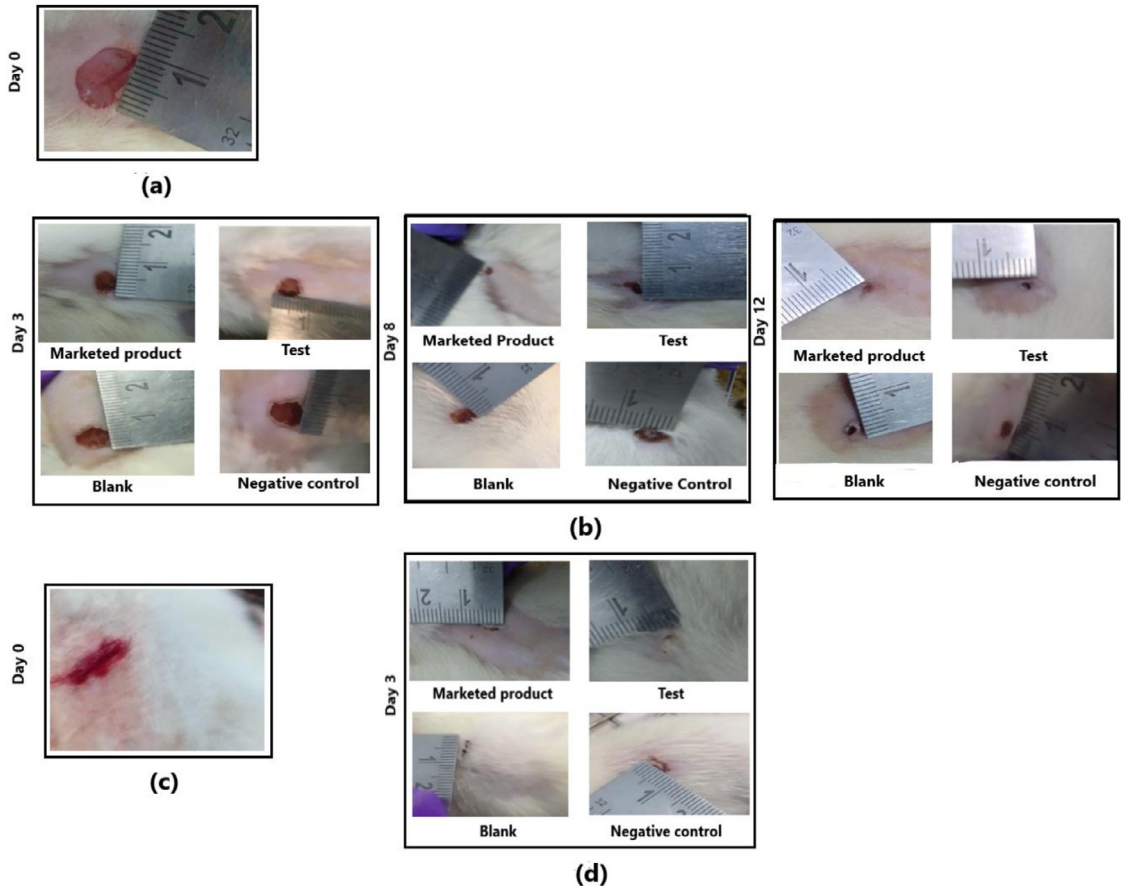

Fig. 6. (a) Excision wound at Day 0 (b) Excision wound contraction at Day 3, Day 8 and Day 12 (c) Incision wound at Day 0 (d) Incision wound contraction at Day 3.

tissue regeneration was significantly similar between the Standard and Test groups, but there was a slight difference in the collagen synthesis. Collagen-induced regeneration of tissue in the dermis layer was almost entirely synthesized in the Standard group followed by the Test group, which showed an almost similar structure as that of normal skin of the positive control group. In the Blank and Negative control group, the epidermis was not continuous like that of the Standard and Test group. Tissue regeneration was not wholly achieved in the Blank and Negative control, but better healing was observed in the Blank group than Negative Control due to gelatin in the blank formulation. In all the cases, no granular cells (inflammatory cell) were found, which indicates that the entire wound had crossed the inflammatory phase but were still in the regeneration phase. The histological evaluation conducted after 15 days of treatment proved that wound healing rate was faster in the order of Standard > Test> Blank> Negative control, respectively.

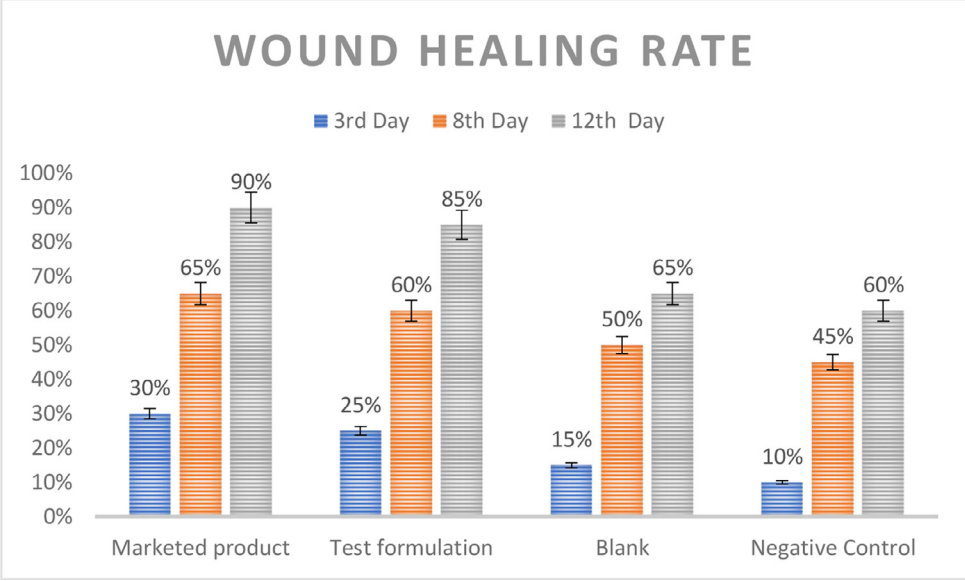

Fig. 7. Bar graph of wound healing rate in terms of percentage.

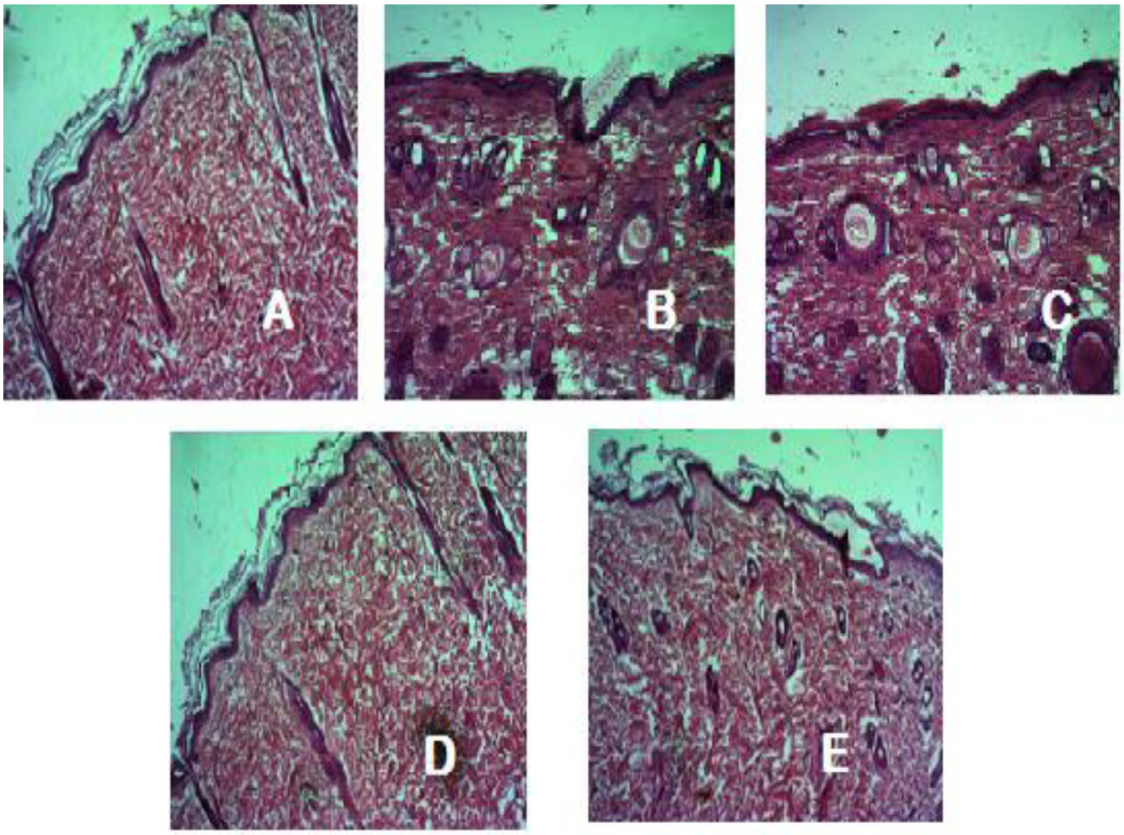

Fig. 8. Histological Cross-section of rat's skin of (a) Positive control, (b) Negative control, (c) Blank, (d) Standard and (e) Test.

Discussions

Medical gauze and other traditional disinfection and cleaning methods that are commonly used during wound healing cannot be degraded in vivo due to their poor absorption of moisture [13]. Our study aimed to explore the characteristics of Melatonin in the healing acceleration of wounds. Melatonin was introduced to the wound in a sponge using gelatin as a carrier and fructose as a crosslinking agent. The gelatin-based Melatonin sponge prepared using the freeze-drying method exerted different degrees of retention and absorption because of high porosity, allowing the sponge to absorb and transport the exudates of the wounds to encourage healing of the wound. Gelatin sponge can enhance blood coagulation, the release of factors responsible for platelet coagulation after absorbing a large amount of blood [3]. The presence of gelatin improvised the Melatonin-based wound healing sponge's stability

and performance, which might be highly beneficial in the clinical aspect.

As per previously conducted studies [15], Melatonin was found to improve the scarring quality significantly, both in terms of the orientation of collagen fibres and maturity, it generates proline which is essential for the synthesis of collagen by increasing the activity of arginase and the protein profiles of heme oxygenase isoforms-(HO-1) and HO-2. Our study has shown improved scar both in terms of collagen orientation and maturity upon treatment with gelatin-based Melatonin sponge.

Additionally, in in-vivo wound healing studies, the gelatin-based Melatonin sponge could rapidly retain wound exudates and keep a moderately moist climate to advance injury mending. The poriferous gelatin-based Melatonin sponge upgraded the development of epidermal cells and sped up the repairing of skin absconds without holding with the injury; in this manner, there was no auxiliary harm to the wounds. Conversely, it was drier attributable to its low water ingestion, and water retention limits and the injury recuperated gradually; in this way, the injury dressing all the more without any problem [2]. Consistent with this, the formation of new blood vessels occurred in the wounds treated with gelatin-based Melatonin wound sponge dressing. The excision wound was significantly repaired after 12 days, whereas the Incision wound was repaired entirely after three days. Based on the results obtained from the experiment, the data supported the application of gelatin-based Melatonin sponge dressing to accelerate the healing of the wound.

Conclusion

In this work, composite sponges of Melatonin and gelatin was made at various ratios. Sponge containing 2.5 % Melatonin and 5% gelatin was found to be most efficient. The morphology and structure characteristics were studied via FTIR and SEM analysis. The composite of Melatonin loaded gelatin improved water uptake ability, water retention ability and degradation rate and wound closure. Based on the results of Melatonin drug release, the higher content of gelatin in the composite sponge exerted a faster release behaviour. The collagen formation and wound closure in in vivo studies was enhanced using these composite sponges containing Melatonin and gelatin, which improved the wound healing activity.

Declaration of Competing Interest

None

Authors' contributions

The first and last author were responsible for the study design and experiments. The first, second, third and fourth author did the initial data analysis. The final data analysis was discussed and consented to by all authors. All authors were responsible for the drafting of the manuscript, revision and approval of the final manuscript.

Acknowledgements

The authors wish to thank Department of Pharmaceutical Sciences, Dibrugarh University for supporting this work.

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