RESEARCH PAPER

Herbal nano-ointment containing encapsulated polysaccharide in repairing of superficial ulcers

Soraya Sajadimajd [1]*, Faranak Aghaz [2], Elham Arkan [2], Gholamreza Bahrami [3, 4]

[1]Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran
[2]Nano Drug Delivery Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
[3]Medical Biology Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4]School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran

ABSTRACT

Objective(s): Skin wounds are appraised as a rapidly growing threat to the economy and public health. Wound management is the main goal to promote rapid repair, with functional and esthetic outcomes. Among several wound healers, ointments are the most cost-effective and highly functional.

Materials and Methods: Here, polysaccharide was isolated from *Rosa canina* and structural analysis was performed by NMR, LC-MS/MS, and FTIR. Then, the polysaccharide was encapsulated in SLN through the dialysis process. Structural analysis was committed to a survey on the physical and chemical properties of polysaccharide-SLN (PS-SLN) complex by Uv-Vis spectrophotometry, dynamic light scattering (DLS), and scanning electron microscopy (SEM) technologies. The ointment was prepared by adding PS-SLN to *R. canina* oil and beeswax.

Results: The prepared PS-SLN nanoparticles had monodispersity with a size of about 217 nanometers. The nano-ointment showed high stability with a pH of about 6 and a high density near 3256 centipoises. The skin absorption of the compounds was determined by the Franz cells. The *in vitro* skin absorption indicated that during the first 12 hr 36% of our nano-ointment's skin permeation and then continued up to 12 hr to 51%. The higher healing rate of nano-ointment than positive control with no allergic effects confirmed its efficiency in wound management.

Conclusion: The results indicated that the nano-ointment could be applied for the healing of scars in pre-clinical and clinical trials. Owing to effectual scar healing, nano-ointment may be effective in the treatment of other wounds including burn, diabetic and chronic ones.

Keywords: *Encapsulation, Nano-ointment, Polysaccharide, SLN, Wound healing*

How to cite this article
Sajadimajd S, Aghaz F, Arkan E, Bahrami Gh. Herbal nano-ointment containing encapsulated polysaccharide in repairing of superficial ulcers. Nanomed J. 2024; 11(1): 72-79. DOI: 10.22038/NMJ.2023.73485.1793

INTRODUCTION

The wound is a disruption of tissue continuity that is breached by several injuries ultimately leading to structural interruption. Wounds can be open where occurred on the body surface in which the skin permits penetration of foreign components. Conversely, a closed wound like a hematoma, contusion, or seroma is developed in internal tissues and is usually created by blunt trauma that harms internal organs, muscles, and even skin [1]. A cutaneous wound is an open and/or outer injury in the skin originating from physical, chemical, and biological invaders and can be divided into acute and chronic insults. Acute skin lesions including surgical, traumatic, abrasions, and superficial burns heal with time, while infection can delay their recovery. Thus, management of healing and infection should be considered to prevent scar formation. On the other hand, in light of the non-healing character, chronic skin wounds are the most pressing medical problem that can affect the quality of life with organ failure and/or death so as to garner almost all of the attention [2]. In consonance with Medicare

* *Corresponding author: Email: s.sajadi@razi.ac.ir; sajadi.s62@gmail.com*
Note. This manuscript was submitted on July 1, 2023; approved on October 31, 2023

data, about 8.2 million patients were allocated to chronic non-healing wounds (CNHW) in the United States [3]. Moreover, a 5-year cohort study has demonstrated that the number of patients with wounds was raised by 71% from 2012/2013 to 3.8 million in 2017/2018 [4]. It is conceivable that wound healing in patients suffering from obesity, aging, diabetes, vascular abnormalities, other comorbidities, and even recent COVID-19 might cause chronic wounds leading to more vulnerability to infection and other environmental issues [5]. In this regard, research is in progress to assess molecular mechanisms of wound healing and develop new strategies for rapid healing of both acute and chronic wounds.

Wound healing is a normal and well-orchestrated process involving several cellular and molecular interactions to proceed with several overlapping stages such as inflammatory response, proliferation, regeneration, vascularization, and remodeling of tissue [6-8]. In this line, a major technological breakthrough in wound science is to strive for wound healers to shorten the healing time and mitigate infection and scarring [9]. Recent researches support the usage of herbal medicine, phytochemicals, and biologically active products as a traditional strategy to manage wound care and optimize the healing process [10, 11]. Among bioactive agents, compounds driven from medicinal plants like polysaccharides are of great interest. As an illustration, cellulose as a biodegradable glucose-driven polysaccharide can be extracted from bacteria, probiotics, and plants providing compatible wound dressings. Cellulose can also act as a convenient drug carrier of other wound healer molecules to accelerate the healing of wounds [12]. In addition, other polysaccharide polymers such as chitooligosaccharides and homopolysacchrides are used for repairing numerous wounds [13, 14].

Among the course of wound dressings, ointments are most commonly used as a commercial drug in the market. The ointment is a semisolid, viscous, and oily pharmaceutical formulation with a base containing mineral oil, petrolatum, and fatty alcohol compound that is used for external application on the skin [15]. The healer agents such as biologically active components should be added to the ointment base with an assured and controlled amount. To achieve a conceivable availability of compounds, novel drug delivery strategies seem to be used. Owing to the major technological breakthrough of nanotechnology in biomedical science, nanomaterial interventions may hold great attention for wound healing. To such an extent, solid lipid nanoparticles (SLNs) are a novel colloidal delivery system that remains solid in the body and at room temperature. SLNs are prepared from a mixture of several types of lipids and surfactants in a water medium with a size of nearly 40-1000 nm. They are a cost-effective and biocompatible group of nanocarriers that can be applied for the encapsulation of hydrophobic and hydrophilic drugs [16].

Taken together, it is worth considering that nanoencapsulation of drugs such as polysaccharides in ointment accommodates the impetus to get a rapid wound closure effect than previous traditional ointments.

MATERIAL AND METHODS

Isolation and characterization of polysaccharide from Rosa canina

Fruits from *R. canina* were collected from Kermanshah province (West of Iran) and were authenticated by a botanist. The hydro-ethanolic extract was prepared, and the polysaccharide was isolated by using several chromatographic procedures and characterized using LC-MS/MS, NMR, and FTIR techniques according to a report by Rahimi *et al* [17]. The isolated polysaccharide was named RCP (Polysaccharide isolated from *R. canina*).

Preparation of RCP-SLN

To obtain the RCP-SLN formulations with a particle size of 200 nm or smaller, the modified previous self-assembly method was used in this study [18]. Briefly, polysaccharide was solved (CH_2Cl_2 and DMSO, respectively) and added to one milliliter of lipid at room temperature (25 °C), which was then sonicated for 120 s at 80 WT. Then, the mixture was freeze-dried and stored at -20 °C. Also, the blank-SLN (B-SLN) was synthesized with a similar method. We examined the physical aesthetics, convenience of re-constitution, and quality characteristics of the nano-capsules when added to cell culture media.

Determination of EE and DL %

Following the centrifugation at 18,200×g for ten minutes at room temperature to produce the pellet (loaded drug) and supernatant (un-entrapped drug), the supernatant of the stock formulations was analyzed with a UV-VIS spectrophotometer, at wavelength of 263nm for polysaccharide and determination of entrapment efficiency (EE%) and drug loading (DL%). Calibration curves were

created with a straight line and R^2 of 0.9988 for polysaccharide. The DL% and EE% of RCP-SLN were calculated by the formula given below:

EE%: [Total drug −Free drug / Total drug] ×100.
DL%: [Total drug −Free drug / total weight nanoparticles] ×100.

All measurements were done in triplicate, and the data were presented as mean ± S.D.

In vitro drug release

We assessed polysaccharide released from RCP-SLN using a dialysis bag method (Sigma Aldrich, molecular weight cut off-20 kD) [19], in phosphate buffer (pH = 7.4). Briefly, 4 ml of the RCP-SLN was placed in a dialysis bag which was immersed in 80 ml of phosphate buffer solution (pH = 7.4) and put in a shaker incubator (200 rpm at 37 °C, for 55 h. In predetermined time points (0, 1, 2, 3, 4, 5, 6, 24, 48, and 55 hr), 1 ml of the release medium was removed and replaced with fresh PBS of the same volume. Then, polysaccharide was quantified by a UV-vis spectrophotometer set (Philips PU 8620) at absorption maxima (λ max), corresponding to 263 nm for polysaccharide. Drug quantification for the *in vitro* drug release profile was calculated as a percentage of the drug release quantity at each time interval relating to the amount of drug trapped within the SLN. The experiment was identically repeated in triplicate.

Fourier transform infrared spectroscopy (FT-IR)

By the potassium bromide disk method, FT-IR spectroscopy of synthesized compounds was used to assess the chemical interaction of different functional groups. About 2 mg of the samples was mixed and triturated with potassium bromide (100 mg). An FT-IR spectrophotometer set (IR prestige-21, Shimadzu Co., Japan) was used to measure the spectra of free isolated polysaccharide, B-SLN, and polysaccharide-SLN over 200–40000 cm^{-1} with a resolution of 4 cm^{-1} to determine any shift in individual peaks of the drugs.

Preparation of nano-ointment

Beeswax-based nano-ointment was prepared by melting at 65 °C. Then, salicylic acid (2%) and vitamin E (2.5%) was added to the 8-12% beeswax (13-15) with continuous stirring in a water bath. Then, *R. canina* oil (30%) was added and mixed with paraben (0.2%). After cooling, nano-encapsulated polysaccharide was added to the base and mixed with a homogenizer to produce a uniform ointment.

Physical and thermal stability of nano-ointment

Three prepared nano-ointment samples (15 g) were evaluated visually at 4 °C, 25 °C, and 45 °C for their color, appearance, and phase separation [20]. The uniformity of the product was also checked by a light microscope after 24 hr, 48 hr, 7 days, and 30 days.

pH determination of nano-ointment

The pH of gel formulations was determined using a digital pH meter (Techno Scientific products). 1 g of gel was dissolved in 100 ml of distilled water and stored for 2 hr. The measurement of the pH of each formulation was done in triplicate and average values were calculated [21].

Ex vivo skin permeability determination

The permeability potential of nano-ointment was measured through mouse gut skin samples placed on a Franz diffusion cell and left for 48 hr. The receptor compartment was filled with 50 mL PBS (pH=7.4) that was previously degassed with ultrasound for 5 min and freshly prepared nano-ointment (4 g) was poured into the acceptor chamber. To homogenization of nano-ointment in the receptor phase, inert Teflon-coated magnets were used to mix the solution with a continuous speed of 700 rpm. Sampling (1 mL) was performed at several time points (15, 30, 45, 60, 120, 180, 240, and 360 min) from the receptor phase and was immediately replaced with an equal volume of fresh buffer solution [22]. Collected samples were stored at 25 °C and used to analyze the amount of polysaccharide out from test nano-ointments by measuring the absorbance at a wavelength of 263 nm.

Sensitivity test

Skin sensitivity or irritancy test was carried out to assess each sign of adverse reactions to the nano-ointment formulation on the skin of Wistar rats. The nano-ointment (0.5 g) was applied on the shaved dorsal skin of the rats daily for 7 continuous days. The appearance of any reaction like edema and erythema was checked daily [23].

In vivo wound healing effect of nano-ointment

Four groups of Wistar rats of 5 each (negative control, positive control, ointment, and nano-ointment) were considered to evaluate the healing effect of nano-ointment. The full-thickness wounds (2 cm×2 cm) were created on the shaved dorsal skin of Wistar rats and treated with sterile

normal saline solution, Alpha® ointment, based ointment, and nano-ointment for 21 days. The process of healing was evaluated every 7 days. The wound diameter was measured every three days and visualized by ImageJ software.

All procedures were performed in accordance with the ethical guidelines for the care and use of laboratory animals (principles of Helsinki) and approved by the animal care committee of Kermanshah University of Medical Sciences, Kermanshah, Iran.

RESULTS

Isolated polysaccharide from Rosa canina

As reported by Rahimi *et al*, the characterization of the final extraction from *R. canina* through NMR, FTIR, and LC-MS/MS implied that the ultimate fraction (Fig. 1) was a derivative of pectin polysaccharides with methylated, acetylated, and carboxylated galacturonic acid monomers interlinked with 1-4 glycosidic bonds [17]. The isolated polysaccharide was named RCP (polysaccharide isolated from *R. canina*).

Properties of RCP-SLN

As shown in Fig. 2, empty SLN indicated main peaks at 2918 (CH stretch), 1701 (Carbonyl group), 1583 (C=C), 1463 (Carboxyl group), 1298 (CH stretch, 1109 (C-O). In polysaccharide fraction, prominent peaks include 3417 (NH stretch), 2924 (CH stretch or vibration), 2854 (asymmetric CH2), 1743 (C=O), 1643 (C=C), 1625 (C=C), 1516 (C=C-C), 1454 (C-O-H or asymmetric CH3), 1244 (C-O vibration), 1070 (C-O), 823 (CH). The premier peaks in polysaccharide-loaded SLN nano-encapsulate were at 3404 (NH brad stretch), 2918 (CH), 2848 (CH2), 1703 (carboxylic acid), 1467 (C=C-C), 1298 (C-H), 1105 (C-O), and 943 (C-H).

The common peaks in the complex of polysaccharide-SLN were 2899 (CH bonds), and 1454 (C=C bonds). Given that polysaccharide was encapsulated in SLN, a number of interactions were changed in comparison with SLN and/or polysaccharide alone verifying the formation of a polysaccharide-SLN complex. In this line,

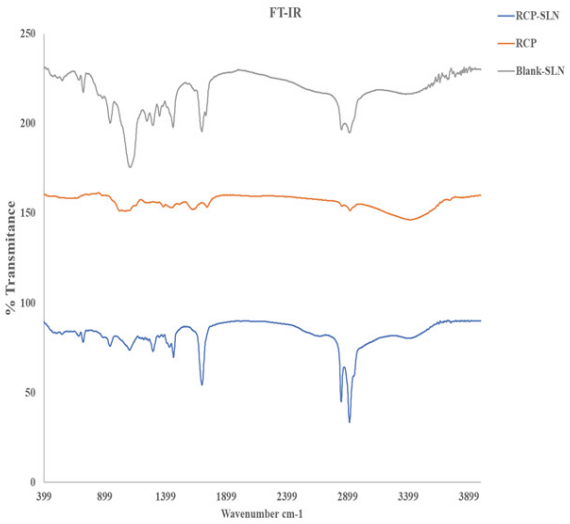

Fig. 2. FTIR diagram of Blank SLN, isolated polysaccharide (RCP) and nanoencapsulated RCP (RCP-SLN)

the alterations were 3381→3417, 2918→2924, 1734→1743, 1639→1643, 1463→1454, 1352→1336, and 1249→1294.

In vitro drug release of RCP-SLN at pH=7. 4 for 48 hr showed that the release of polysaccharide was about 25.97% after 48 hr incubation (Fig. 3). EE% and DL% were calculated as 86% and 20%, respectively.

The physicochemical properties of SLN and RCP-SLN were shown in Table 1 and Fig. 4. Data indicated that the approximate size of RCP-SLN was 217±2.5 compared to Blank-SLN with a size

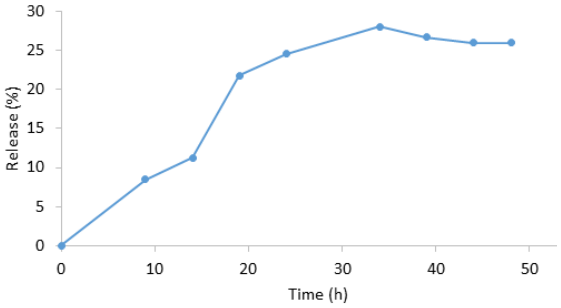

Fig. 3. *In vitro* drug release diagram of RCP-SLN during 48 hr

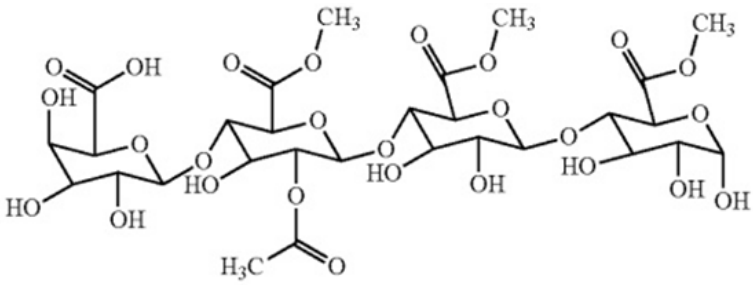

Fig. 1. Structure of isolated polysaccharide from *Rosa canina*

Table 1. Physicochemical properties of SLN and RCP-SLN

	Z-average diameter [nm]	Polydispersity (PDI)	Zeta potential [mV]	EE%	DL%
SLN	145±6.1	0.08±0.02	12.1	-	-
PS-SLN	217±2.5	0.245±0.08	-9.49	86	20

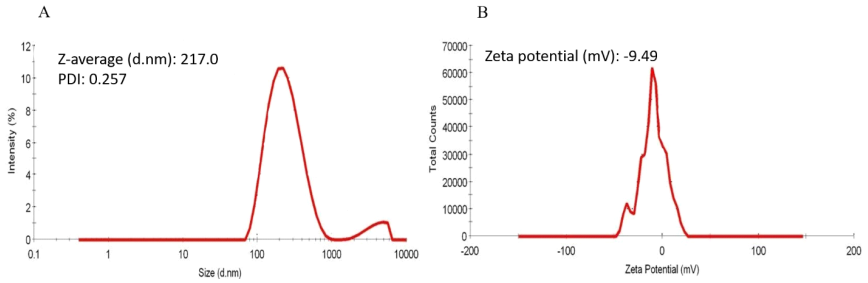

Fig. 4. The physicochemical properties of RCP-SLN. Polydispersity index (A), Zeta potential (B), and SEM micrograph (C)

of 145±6.1. PDI for polysaccharide-loaded SLN nanoparticle (RCP-SLN) was about 0.257 validating the low dispersity of size distribution.

Properties of the prepared nano-ointment

The measured pH and viscosity of nano-ointment were nearly 6 and 3256 cps, respectively. The stability test implied that nano-ointment showed no biphasic pattern as well as no physicochemical alterations such as color, smell, acidity, and density after 28 days of incubation. Skin absorption of nano-ointment on mice skin composed of dermis and epidermis showed an increased permeability potential in a time-dependent manner (Fig. 5A). The wound healing on the dermal wound revealed that nano-ointment

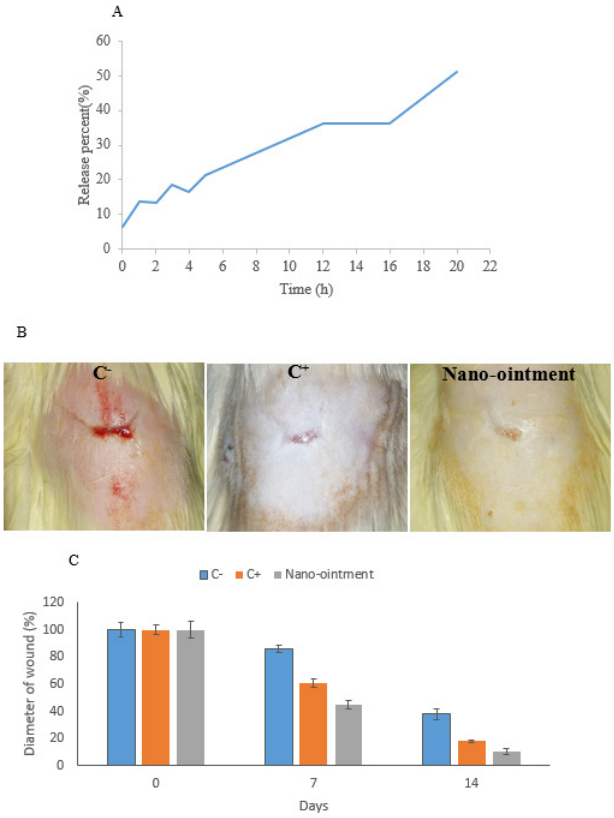

Fig. 5. *In vivo* properties of nano-ointment in rat skin wound model. Nano-ointment permeation profile through mouse skin within 20 hr of the Franz cell diffusion assay (A). Wound healing effect of nano-ointment in compared to negative and positive control after 14 days (B). Average wound diameters of wounds in rats treated with nano-ointment and Alpha® ointment in positive control (C)

causes the wound to heal faster than the positive control (Fig. 5B). The measurement of wound diameters confirmed the efficient wound healing effect of nano-ointment than controls (Fig. 5C).

DISCUSSION

One of the most challenging medical issues affecting the social and economic burden of health is the development of different wounds due to several environmental causes. From a therapeutic point of view, the quest for an efficient wound dressing is necessary to accelerate wound closure and restraint of tissue infection. In the present study, a novel nano-ointment based on polysaccharide-encapsulated SLN was designated to heal superficial wounds. Amidst several strategies being developed for wound healing, nano-ointment efficiency has been supported by a wealth of evidence from recent reports [24-26]. Given that the isolated polysaccharide from *R. canina* has actuated anti-inflammatory, blood glucose modulating, proliferative and regenerative effects in vitro and in vivo [27, 28], the results endowed us to focus on its accelerated wound closure impact in animal models. In this regard, to better characterize this aspect, we used nano-encapsulation to control the release of the isolated polysaccharide in the injury location.

Owing to the biocompatibility engagement, therapeutic pipeline of biomolecules such as polysaccharides in wound healing seems to provide impetus for their utility in clinical trials. For instance, cellulose acetate and gelatin scaffold containing berberine could accelerate wound closure in diabetic foot ulcers [29]. Nevertheless, the lack of cellulase enzymes in body limits its implication in wound healing. In this regard, research is in progress to obviate this issue by manufacturing novel cellulose derivatives such as carboxymethyl cellulose, nanocellulose, and cellulose-based nanocarriers [30-32]. The synthesized microbial cellulose as microfibrils and nanofibrils, bacterial cellulose loaded with papain used in wound healing [12, 33]. Applicability, cellulose nanocrystals from *Syzygium cumini* garnished with silver nanoparticles imply a convenient healer in diabetic wounds [34]. In addition, deep burns were ameliorated with carboxymethyl chitosan and cellulose nanocrystals in hydrogel structures [35]. SLN as a safe lipid nanocarrier was used to optimize the efficiency of extracted polysaccharide from *R. canina*. In the present study, polysaccharide was encapsulated in SLN nanocarrier including stearic acid by self-assembly method with a good encapsulation efficiency of 86% and a negative zeta potential indicating that weekly electrostatic and/or repulsive interactions between nanocarrier and polysaccharide which is in accordance with Kaliamurthi *et al* and Vijayan *et al* [26, 36]. Given the low PDI and Z-Average size of all SLN-polysaccharide (about 0.245), the uniformity of droplets is expected with well-disparities, increased stability, and no particle aggregation.

The percentage of cumulative in vitro release of RCP-SLN was nearly 25.97% after 48 h. A burst release of polysaccharide was observed during the first 20 h while it slowed down implying the presence of unbounded polysaccharide on the outer surface of the nanocarrier. The sustained release of particles was likely correlated to the bounded polysaccharide in the inner layer of polysaccharide-SLN. The spherical shape of the nanoparticle with uniformity and non-aggregated particles was confirmed in the SEM imaging.

As discussed in previous reports, nano-ointments prepared from beeswax and Rosa oil illustrated the cytocompatibility and wound healing effects. In this line, Adibderm® ointment including *R. canina*, *Matricaria chamomilla*, Beeswax, ascorbic acid, oleic and linoleic acid ingredients. Adibderm® was used in a clinical trial to heal second-degree burns and indicated a faster healing in comparison to the silver sulfadiazine-treated group with no side effect [37]. Furthermore, as an Iranian herbal ointment that encompasses beeswax and *Lawsonia inermis* was also was examined in patients with second-degree burns indicating a powerful treatment for burn wounds [38]. The scar-improving efficacy of fresh fruits from *R. canina* in rats is corroborated with the use of this extract in Turkish folk medicine to treat gastric complaints [39]. The first clinical trial indicated that Rosehip oil from the fruits of *R. canina* was used to treat post-surgical scars implicating lower incidence of erythema, dyschromia, and atrophy compared to the control group [40]. Given the wide use of *R. canina* extracts, FDA recommended that maximum doses of *R. canina* vary in different manufactured products, namely, 0.2% in eye-area skin products and 1.5% in lipstick [41]. In this regard, the dose of isolated polysaccharide in the nano-ointment was chosen in accordance with FDA recommendation. Owing

to the healing efficacy of herbal polysaccharides in several reports [42, 43] and the regenerative activity of isolated polysaccharide from *R. canina*, this study indicated the wound-healing effect of the isolated polysaccharide was demonstrated in the form of nano-ointment in rats.

In the present study, Polysaccharide-SLN nano-ointment is more effective than other groups of skin wound rat models revealing the convenient wound closure effect of nano-ointment. The time to complete healing in groups with nano-ointment was about 14 days with the lowest wound diameter compared to positive (Alpha® ointment) and negative control groups. Despite the novelty and good effects, the mechanism of action of nano-ointment should be clarified to develop its efficiency in clinical trials and commercialization.

CONCLUSION

To draw a conclusion, Beeswax, polysaccharide and oil extracted from *R. canina* as well as SLN carrier were the main ingredients of nano-ointment. Given the cytocompatibility, stability, skin permeability potential, and fast closure of wounds, nano ointment could be chosen as an expedient option for wound healing in clinical trials.

ACKNOWLEDGEMENTS

The authors appreciate the financial support of this investigation by Tava Ebtekar Pakan knowledge-foundation company and Razi university.

FUNDING DECLARATION

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

CONFLICTS OF INTEREST

None.

REFERENCE

1. Benirschke SK, Kramer PA. Wound healing complications in closed and open calcaneal fractures. J Orthop Trauma. 2004;18(1):1-6.
2. Sen CK. Human Wound and Its Burden: Updated 2020 Compendium of Estimates. Adv Wound Care (New Rochelle). 2021;10(5):281-292.
3. Nussbaum SR, Carter MJ, Fife CE, DaVanzo J, Haught R, Nusgart M, Cartwright D. An Economic Evaluation of the Impact, Cost, and Medicare Policy Implications of Chronic Nonhealing Wounds. Value Health. 2018;21(1):27-32.
4. Guest JF, Fuller GW, Vowden P. Cohort study evaluating the burden of wounds to the UK's National Health Service in 2017/2018: update from 2012/2013. BMJ Open. 2020 22;10(12):e045253.
5. Ennis W. Assessing the impact of coronavirus on wound care patients and practice. 2020.
6. Yang SW, Geng ZJ, Ma K, Sun XY, Fu XB. Comparison of the histological morphology between normal skin and scar tissue. J Huazhong Univ Sci Technolog Med Sci. 2016;36(2):265-269.
7. Profyris C, Tziotzios C, Do Vale I. Cutaneous scarring: Pathophysiology, molecular mechanisms, and scar reduction therapeutics Part I. The molecular basis of scar formation. J Am Acad Dermatol. 2012;66(1):1-10.
8. Chouhan D, Dey N, Bhardwaj N, Mandal BB. Emerging and innovative approaches for wound healing and skin regeneration: Current status and advances. Biomaterials. 2019;216:119267.
9. Maver T, Maver U, Stana Kleinschek K, Smrke DM, Kreft S. A review of herbal medicines in wound healing. Int J Dermatol. 2015;54(7):740-751.
10. Das U, Behera SS, Pramanik K. Ethno-Herbal-Medico in Wound Repair: An Incisive Review. Phytother Res. 2017;31(4):579-590.
11. Shedoeva A, Leavesley D, Upton Z, Fan C. Wound healing and the use of medicinal plants. J Evid Based Integr Med. 2019;2019:2684108.
12. Ahmed J, Gultekinoglu M, Edirisinghe M. Bacterial cellulose micro-nano fibres for wound healing applications. Biotechnol Adv. 2020;41:107549.
13. Jafari H, Bernaerts KV, Dodi G, Shavandi A. Chitooligosaccharides for wound healing biomaterials engineering. Mater Sci Eng C Mater Biol Appl. 2020;117:111266.
14. Naseri-Nosar M, Ziora ZM. Wound dressings from naturally-occurring polymers: A review on homopolysaccharide-based composites. Carbohydr Polym. 2018 Jun 1;189:379-398.
15. Bremecker KD, Strempel H, Klein G. Novel concept for a mucosal adhesive ointment. J Pharm Sci. 1984 Apr;73(4):548-552.
16. Firooz A, Namdar R, Nafisi S, Maibach HI. Nano-Sized Technologies for Miconazole Skin Delivery. Curr Pharm Biotechnol. 2016;17(6):524-531.
17. Rahimi M, Sajadimajd S, Mahdian Z, Hemmati M, Malekkhatabi P, Bahrami G, Mohammadi B, Miraghaee S, Hatami R, Mansouri K, Moahammadi Motlagh HR, Keshavarzi S, Derakhshankhah H. Characterization and anti-diabetic effects of the oligosaccharide fraction isolated from Rosa canina in STZ-Induced diabetic rats. Carbohydr Res. 2020;489:107927.
18. Motiei M, Kashanian S. Novel amphiphilic chitosan nanocarriers for sustained oral delivery of hydrophobic drugs. Eur J Pharm Sci. 2017;99:285-291.
19. Herndl GJ, Kaltenböck E, Müller-Niklas G. Dialysis bag incubation as a nonradiolabeling technique to estimate bacterioplankton production in situ. InHandbook of methods in aquatic microbial ecology 2018; 553-556. 20.Koliyote S, Misal B. Evaluation of anti-inflammatory activity of topical gel by carrageenan induced paw oedema method. Int J Pharm Stud Res. 2013;2:579-584.
21. Nour S, Shalaby S, Afify N, Abdel Aal S, Kamal M. Formulationand evaluation of econazole nitrate emulgels. J Drug Res. 2002;24(1-2):63-71.
22. Huh Y, Lee DH, Choi D, Lim KM. Effect of cosmetics use on the *in vitro* skin absorption of a biocide, 1, 2-Benzisothiazolin-3-one. Toxics. 2022;10(3):108.
23. Draize JH. Methods for the study of irritation and toxicity of substances applied topically to the skin and the mucous

membranes. J Pharmacol Exp Ther. 1944;82:377-390.
24. Yadav E, Yadav P, Verma A. Amelioration of full thickness dermal wounds by topical application of biofabricated zinc oxide and iron oxide nano-ointment in albino Wistar rats. J Drug Deliv Sci Technol. 2021 ;66:102833.
25. Kubendiran L, Theerthagiri S, Al-Dhabi NA, Palaninaicker S, Subramanian SM, Srinivasan V, Karuppiah P. *In vitro* preparation of biosurfactant based herbal-nano topical ointment from Tridax procumbens infused oil using gelatin stabilized silver nanoparticle and its efficacy on fibroblastic cell lines. Appl Nanosci. 2023;13(1):719-734.
26. Kaliamurthi S, Selvaraj G, Thirugnanasambandam R, Thangavel B. Topical delivery of nano-encapsulated rutoside medication for diabetic foot ulcer in rat model. Nanoscience & Nanotechnology-Asia. 2018;8(1):116-129.
27. Fattahi A, Niyazi F, Shahbazi B, Farzaei MH, Bahrami G. Antidiabetic mechanisms of Rosa canina fruits: an in vitro evaluation. J Evid Based Integr Med. 2017;22(1):127-133.
28. Bahrami G, Naseri R, Khosravifar M, Sajadimajd S, Mohammadi B, Heydarpour F, Cheraghialiakbari S. The Safety and Effectiveness of a Polysaccharide Extracted from Rosa Canina in Patients with NAFLD: A Randomized Trial. Clin Diabetol. 2022;11(4):239-244.
29. Samadian H, Zamiri S, Ehterami A, Farzamfar S, Vaez A, Khastar H, Alam M, Ai A, Derakhshankhah H, Allahyari Z, Goodarzi A. Electrospun cellulose acetate/gelatin nanofibrous wound dressing containing berberine for diabetic foot ulcer healing: *In vitro* and *in vivo* studies. Sci Rep. 2020 20;10(1):8312.
30. Sartika D, Syamsu K, Warsiki E, Fahma F, Arnata IW. Nanocrystalline cellulose from kapok fiber (Ceiba pentandra) and its reinforcement effect on alginate hydrogel bead. Starke. 2021;73(9-10):2100033.
31. Chang C, Duan B, Cai J, Zhang L. Superabsorbent hydrogels based on cellulose for smart swelling and controllable delivery. Eur Polym J. 2010;46(1):92-100.
32. Hu D, Zeng M, Sun Y, Yuan J, Wei Y. Cellulose-based hydrogels regulated by supramolecular chemistry. SusMat. 2021;1(2):266-284.
33. Asanarong O, Quan VM, Boonrungsiman S, Sukyai P. Bioactive wound dressing using bacterial cellulose loaded with papain composite: Morphology, loading/release and antibacterial properties. Eur Polym J. 2021;143:110224.
34. Singla R, Soni S, Patial V, Kulurkar PM, Kumari A, Padwad YS, Yadav SK. Cytocompatible anti-microbial dressings of s yzygium cumini cellulose nanocrystals decorated with silver nanoparticles accelerate acute and diabetic wound healing. Sci Rep. 2017;7(1):10457.
35. Huang W, Wang Y, Huang Z, Wang X, Chen L, Zhang Y, Zhang L. On-demand dissolvable self-healing hydrogel based on carboxymethyl chitosan and cellulose nanocrystal for deep partial thickness burn wound healing. ACS Appl Mater Interfaces. 2018;10(48):41076-41088.
36. Vijayan V, Rao DS, Jayachandran E, Anburaj J. Preparation and characterization of anti-diabetic drug-loaded solid lipid nanoparticles. JITPS. 2010;1(8):320-328.
37. Ala A, Ebrahimi Bakhtavar H, Shams Vahdati S, Rahmani F, Azargoun M, Ebrahimi Bakhtavar H. Effects of Silver Sulfadiazine and Adibderm® Herbal Ointments in Treatment of Patients with Second Degree Burns: A Randomized Clinical Trial. Trauma Mon. 2018;23(2):1-7.
38. Pool H, Quintanar D, de Dios Figueroa J, Mano CM, Bechara JE, Godínez LA, Mendoza S. Antioxidant effects of quercetin and catechin encapsulated into PLGA nanoparticles. J nanomater. 2012;2012:86.
39. Gürbüz I, Ustün O, Yesilada E, Sezik E, Kutsal O. Anti-ulcerogenic activity of some plants used as folk remedy in Turkey. J Ethnopharmacol. 2003;88(1):93-97.
40. Valerón-Almazán P, Gómez-Duaso AJ, Santana-Molina N, García-Bello MA, Carretero G. Evolution of post-surgical scars treated with pure rosehip seed oil. Journal of Cosmetics, Dermatol Sci Appl. 2015;5(02):161.
41. Johnson W, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, Marks JG, Shank RC, Slaga TJ, Snyder PW, Gill LJ, Heldreth B. Safety Assessment of *Rosa canina*-derived Ingredients as Used in Cosmetics. Int J Toxicol. 2022; 41(1_ suppl):44S-60S.
42. Zhao B, Zhang X, Han W, Cheng J, Qin Y. Wound healing effect of an Astragalus membranaceus polysaccharide and its mechanism. Mol Med Rep. 2017;15(6):4077-4083.
43. Trombetta D, Puglia C, Perri D, Licata A, Pergolizzi S, Lauriano ER, De Pasquale A, Saija A, Bonina FP. Effect of polysaccharides from Opuntia ficus-indica (L.) cladodes on the healing of dermal wounds in the rat. Phytomedicine. 2006;13(5):352-358.