ISSN: 2690-8662

Journal of Clinical Images

Open Access | Research Article

Regenerating Effect of Combined Snail Mucus Extract and Herbs on Non-Healing and Necrotic Wounds

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Received: July 07, 2026

Accepted: Aug 04, 2026

Published Online: Aug 11 2026

Journal: Journal of Clinical Images

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Garden snail *Cornu aspersum*; Difficult-to-heal wounds; Marigold (*Calendula officinalis*); Plantain (*Plantago major*).

Abstract

Detailed information on the composition of the mucus of various snail species, as well as some healing properties in dermatological conditions, burns, acute and difficult-to-heal wounds, and tumour, has been published. However, currently applied protocols for the treatment of severe wounds and necrosis often lead to amputation of limbs, which requires a general rethinking of treatment.

The aim of the presented in vivo study is to establish the effect of a combined extract from the mucus of the garden snail *Cornu aspersum,* containing protein fraction with a Molecular Weight (MW) above 20 kDa with a native mucus extract in a 1:1 ratio, enriched with extracts of marigold (*Calendula officinalis*) and plantain (*Plantago major*), on acute, chronic, necrotic, and difficult-to-heal large-area wounds. A case of treatment 3 times a week with the combined extract for 6 months of a large-area diabetic wound on the sole of the right foot of a 64-year-old man with insulin-dependent diabetes is presented. During the treatment, the levels of tissue regeneration, the local inflammatory process, local pain on a Visual Analogue Scale (VAS), pH values, and microbiological control of wound secretion samples were monitored.

The results demonstrate complete tissue regeneration from the necrotic area of the large wound, resulting in the formation of functional skin and subcutaneous tissue, complete epithelialization, and a fine scar. It has been proven that the snail mucus extract *C. aspersum*, studied by us, in combination with herbal extracts, is a natural, safe, and effective alternative remedy for the treatment of difficult-to-heal wounds. A new protocol for the treatment of difficult-to-heal, chronic, and necrotic wounds of large areas has also been developed.

Cite this article: Kermedchiev M, Todorova M, Dolashki A, Velkova L, Krumova E, et al. Regenerating Effect of Combined Snail Mucus Extract and Herbs on Non-Healing and Necrotic Wounds. J Clin Images. 2026; 9(2): 1172.

Introduction

Chronic and non-healing wounds are a serious problem affecting millions of patients worldwide. The statistics are becoming increasingly alarming, with the mortality rate from chronic diabetic foot ulcers reaching 30.5%, which is comparable to the mortality rate from cancer (31%) [1]. This increased the scientific interest in researching non-toxic and effective natural substances that accelerate wound healing [2,3]. Human skin can be damaged to the point of extensive skin loss, deep burns, chronic wounds, non-healing ulcers, diabetes, etc. Diabetic foot affects about 15% of patients and is one of the main causes of limb amputation [4,5].

Most wound care products are based on synthetic and mixed chemicals, often with weak and toxic effects on wound surfaces. Science is increasingly focusing on investigating the therapeutic potential of natural molecules, such as herbal extracts, snail slime, and hydrogels loaded with various biologically active molecules and herbal extracts [6-8]. The natural compounds, calendula (*Calendula officinalis*) and plantain (*Plantago major*), are well-known and widely used in medicine and cosmetics. *Calendula officinalis* is mainly used as a wound healing agent and anti-inflammatory agent [9,10]. Plantago major is an antioxidant and antimicrobial agent that has been traditionally applied to purulent wounds [11-13].

The most recent studies have demonstrated the therapeutic potential of 100% *Aloe vera* extract in a clinical study conducted on 60 patients with diabetic foot ulcers [6]. Another clinical study used an extract from the plant *Inula viscosa*, which has been used for centuries in Eastern traditional medicine to treat skin injuries and diabetes. The effects of 2 types of hydrogels registered in the USA, one containing an extract from the plant *I. viscosa* and the other a synthetic product were studied in 65 patients [7]. A greater reduction in wound area and pain was found in patients treated with the hydrogel with *I. viscosa* extract.

Another extract used in medicine since ancient times is the mucus of land snails, which accelerates basic cellular processes; it is used in wound healing and tissue regeneration [14]. The mucus contains compounds such as hyaluronic acid and glycoproteins, which have a moisturizing and smoothing effect on the skin [15]. Mucopolysaccharide, hyaluronic acid, polyphenols and other bioactive molecules and minerals have also been found in the mucus of *Helix aspersa* Muller [16,17], which are associated with antimicrobial activity [18,19] and tissue regeneration [20,21]. Deng et al. [22] showed that sulfated glycosaminoglycans in the mucus of *Achatina fulica* and *Helix lucorum* effectively promoted the healing of chronic wounds in diabetic rat models by accelerating granulation tissue regeneration, angiogenesis, and collagen deposition [23,24]. Recently, the use of *C. aspersum* extract in the treatment of open burns has been reported [25].

Current conventional wound healing approaches involve bioengineered skin substitutes, which include three components: biomaterials, cells, and growth factors, which mimic normal skin and provide enhanced regeneration. Autografting is the gold standard for skin repair, where healthy skin is taken from the patient and transplanted into the damaged area [26].

Emerging therapeutic options in recent years, such as stem cell therapy, Platelet-Rich Plasma (PRP), various types of nanotherapeutics, phototherapy, microbiome therapy, and Reactive Oxygen Species (ROS), have shown promising results in the treatment of difficult-to-heal and chronic wounds [27,28]. Clinical trials with stem cell therapy have shown significant results in wound healing [29]. Another promising therapy for diabetic wounds is the topical application of Platelet-Rich Plasma (PRP), which has resulted in complete healing of leg wounds in 150 patients [30].

Chronic wounds are considered to be colonized by diverse microbial communities consisting of both bacteria and fungi [31]. It should be noted that, despite the most common Candida species, other unconventional fungi such as *Curvularia*, *Malassezia*, *Aureobasidium*, *Cladosporium*, *Ulocladium*, *Engodontium* and *Trichophyton* are also important in chronic wound infection [32]. The application of natural extracts aims not only to regenerate wound tissue but also to prevent infections, including fungal ones [33]. Recent trends are directed towards the development of combined treatments that include the use of traditional medicine and modern practices/products.

In this study, we report the outcome of the application of a novel protocol and a hydrogel mixture of snail extract (*Cornu aspersum*), calendula (*C. officinalis*) and plantain (*P. major*) extracts for the treatment of diabetic wounds.

Materials and methods

Development of a wound treatmentgel

Preparation of C. aspersum mucus extract

Mucus from garden snails *C. aspersum*, grown in Bulgarian organic farms, was collected by a patented technology using a device that does not damage the snails [34]. After several purifications, the mucus extract was concentrated on a polyethersulfone membrane with a pore size of 20 kDa (Microdyn Nadir™ from STER-LITECH Corporation, Goleta, CA, USA, respectively) using an Amicon ® Stirred Cell 200 mL (UFSC20001, MerckMillipore, Merck Group, Darmstadt, Germany). The concentrated protein fraction with a molecular weight above 20 kDa was mixed with a native mucus extract in a 1:1 ratio, and an extract with a concentration of 1.2 mg/ml protein was obtained. The concentrated fraction with a MW above 20 kDa was mixed with native mucus extract in a 1:1 ratio, resulting in a mucus extract with a protein concentration of 1.2 mg/ml (Bradford method [35]).

Preparation of calendula officinalis flower extract

After crushing, the dried flower baskets of marigold *C. officinalis* (purchased from an herbal pharmacy) were macerated with 40% v/v ethanol in a closed container for 48 hours in the dark at a temperature of 35°C and continuous shaking (150 rpm). The extraction process was completed with ultrasonic extraction at room temperature for 5 min (with a frequency of 40 kHz). After a series of operations, such as pressing, centrifugation, etc., the obtained extract was lyophilized. The used solution with distilled water contains 0.03 g/ml dry extract of marigold *C. officinalis* flowers.

Preparation of plantago major leaf extract

The extraction of active substances from 100 g of dried leaves of broad-leaved plantain (*P. major*) was carried out by maceration in the dark, with a water-alcoholic solution containing 60% ethanol for 36 hours at room temperature. The extraction process was completed in an ultrasonic bath at room temperature (25°C) for 5 min (with a frequency of 40 kHz), in order to more fully extract the active substances. After a series of operations, such as decantation, pressing, centrifugation, filtration, and concentration on a vacuum rotary evaporator, the obtained extract was lyophilized. An extract with concentration of 0.04 g/ml was used.

Gel preparation

The wound treatment gel was prepared by mixing the snail slime extract *C. aspersum*, marigold (*C. officinalis*) and plantain (*P. major*) extracts in a ratio of option 1 (15:1:1); option 2 (10:1:1) and option 3 (5:1:1). The gel option 3 (5:1:1) - or 50 ml of *C. aspersum* slime extract with a protein concentration of 1.2 mg/ml: 10 ml of aqueous solution of marigold *C. officinalis* flower extract with a concentration of 0.03 g/ml: 10 ml of aqueous solution of *P. major* leaf extract with a concentration of 0.04 g/ml) was used for wound treatment. The protein concentration was measured by the Bradford method [35].

Wound type and new treatment approach

The treated wound in this study is a dry and wet necrosis of the right foot of a 64-year-old man, obtained after primary inpatient surgical necrotectomy. The patient has comorbidities: insulin-dependent diabetes with diabetic polyneuropathy, cortical atrophy and multi-infarct encephalopathy.

A new approach and protocol were applied to treat the presented wound using a combined wound gel of snail mucus with extracts of calendula and plantain. The developed new wound treatment protocol includes the following steps:

1. Antiseptic and mechanical cleaning of the wound surface and surrounding skin with sterile 0.9% NaCl solution and Braunol.
2. Surgical removal of existing necrosis, crusts and biofilm covering the wound bottom.
3. Application of a thick layer of the previously prepared snail mucus gel with extracts of calendula and plantain directly onto the wound surface.
4. Covering with several layers of sterile 100% cotton gauze.
5. Periodic measurement of pain levels using VAS and pH of wound secretion.

Surgical treatment of the wound surface is 3 times a week, monitoring the phase and wound condition.

pH and microbiological test of wound secretion

The pH of the wound exudate was measured with each dressing using pH strips from ISOLAB Laborgeräte GmbH in the range of 0 to 14.

The Wound Extract (WE) collected from the wound gauze was evaluated for fungal viability by agar diffusion technique and Broth Microdilution Method (BMD). The antifungal activity was assayed through a diffusion technique on 3 growth media: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Sabouraud agar. Wound extract (200 µL) was spread onto the surface of the Petri dishes, which were cultivated at 28°C for 7 days. Using the BDM method with resazurin (an indicator of microbial growth), 96-well plates were prepared with the addition of 50 µL of Potato Dextrose Broth (PDB) and AN3 medium to each well, supplemented with 50 µL of the tested wound extract. Then, 30 µL of 0.02% resazurin was added, and the plates were incubated for 48 h at 28 °C. The following control samples were used for each variant: (i) medium + resazurin + chloramphenicol (control 1); (ii) medium + resazurin + chloramphenicol + GEL (mucus gel, control 2); (iii) medium + resazurin + chloramphenicol + collected extract; (iv) medium + resazurin + chloramphenicol + GEL with mixed extracts of mucus, calendula and plantain. The inhibitory effect was determined through the direct observation of growth and a change in the dye color from blue to pink to purple. All of the experiments were performed in triplicate.

Results

Preparation of the wound gel

The developed wound healing gel contains three main ingredients: snail mucus extract *C. aspersum* and two plant extracts: marigold (*C. officinalis*) and plantain (*P. major*). The mucus was collected from garden snails *C. aspersum*, using a patented technology that does not harm the snails. After filtration, the active extracts were obtained. Calendula flower extract was extracted with 40% v/v ethanol for 48 hours in the dark, at T 35°C. The maceration of active substances from 100 g of dried leaves of the broad-leaved plantain *P. major* was carried out in the dark with 60% ethanol for 36 hours at room temperature. The three obtained extracts after filtration, pressing, centrifugation and concentration were lyophilized. Three different combinations of the three extracts were developed, with the best polymerized gel being obtained after mixing the extracts in a ratio of slime: plantain: calendula 5:1:1.

Wound treatment with the combination gel

Given the wound-specificity, a new protocol was applied for the treatment of difficult-to-heal and chronic wounds using a combined gel of snail mucus and calendula and plantain extract. It was applied in vivo to a patient with a large wound surface after extensive necrosis, and the local inflammatory process and tissue regeneration of the wound were observed at different times.

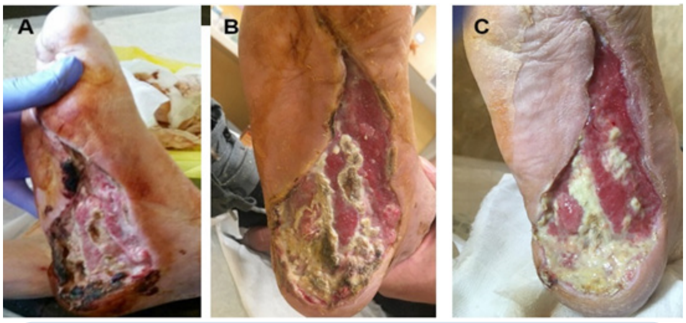

Figure 1: Monitoring the effect of snail mucus gel enriched with calendula and plantain extracts in the treatment of wet necrotic wounds on the patient's foot: **(A)** 10 days after primary hospital surgical necrotectomy; **(B)** after primary surgical debridement and application of the gel for the first time; **(C)** after one month and 12 times of treatment of the wound with the gel.

Figure 1A shows the condition of the wound 10 days after the primary removal of necrotic tissue. A significant improvement of the wound was observed one month after its primary cleaning and 5 treatments with the gel (Figure 1B). Figure 1C clearly shows the fresh and clean appearance of the wound surface, the presence of fresh and rapidly growing columns of granulations, and the absence of new necrosis. The medial edge of the wound has activated hypodermis and dermis, which grow and cover the granulations.

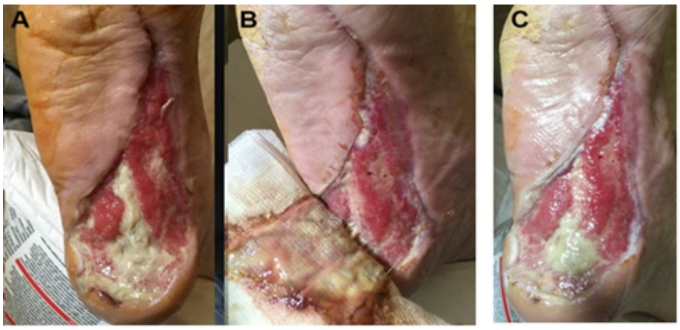

Figure 2: Monitoring of wound tissue changes after treatment with snail mucus gel enriched with calendula and plantain extracts: **(A)** after 5 weeks; **(B)** condition of the wound and wound secretion upon opening after 6 weeks, and **(C)** condition of the wound after 7 weeks.

Prolonged treatment of the wound leads to significant changes in the tissue and formation of clean and fresh columns of granulation tissue (Figure 2A). There are no stigmata of inflammatory reaction at the edges and in the surrounding tissues. Necrotic connective tissue material is still visible at the lower pole. The medial edge of the wound demonstrates growth of the hypodermis over fresh granulations. When the dressing was removed after 6 weeks of wound treatment, abundant, mucoid wound secretion was observed, without an unpleasant odor Also, mucous wound secretion was observed, without an unpleasant odor (Figure 2B). The wound was clearly visible, almost filled with fresh granulation tissue up to the level of the skin edges. Active epithelialization (covering) of the granulations was noted from the upper-medial edge, with new hypodermis forming and maturing into dermis. A more advanced phase of tissue regeneration was noted after the 7th week of treatment, where the hypodermis from the lateral edge grew actively, covering the mature and healthy granulations (Figure 2C). The regeneration process is presented in Figure 3A with a creeping, petal-like hypodermis /fibroblasts and keratinocytes/, on fresh granulations with subsequently generated dermis and maturation.

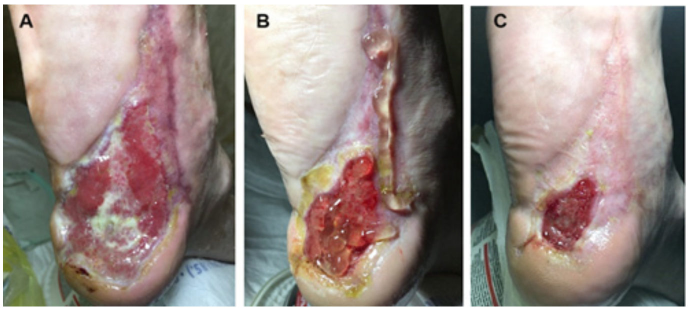

Figure 3: Elements of the wound regeneration process after treatment with snail mucus gel with calendula and plantain extracts: **(A)** after 10 weeks; **(B)** after 14 weeks; **(C)** after 18 weeks.

The visualization of several important processes is presented in Figure 3B, ten weeks after surgical debridement of the wound bed and removal of the existing biofilm, and application of a large amount of the gel. A freshly cleared wound bed of biofilm, fresh granulations in depth, actively growing hypodermis with hyperkeratosis, a less pronounced hyperkeratinized stem in the area of formation of the active dermis and its maturation on the hypodermis are observed.

A significant change in the wound condition occurred after 27 weeks of treatment with the gel. Figure 4A shows an almost completely healed wound bed with a scab formed in the area, clearly showing the dry, cracked and flaky skin of the entire foot and in the area of the still mature dermis, including the hyperkeratotic areas. Visualization of the complete closure of the wound bed by the hypodermis is presented in Figure 4B, through the formed fibrin, keratin and elastin threads at the edges, which actively cover the still immature dermis and hypodermis.

Figure 4: Visualization of complete wound healing after: **(A)** 27 weeks; **(B)** 28 weeks; **(C)** 29 weeks.

Traces of bruising and microhemorrhages are also observed in the scar area. After 29 weeks of wound treatment, a completely healed wound surface is observed in the upper part of the wound, with the formation of a fine scar that continues to smooth, mature, and acquire characteristics of fully healthy and functional skin (Figure 4C).

The ongoing processes of collagen, fibrin and keratin synthesis in the area are clearly visible. In-depth analysis of the presented wound surface revealed a lack of local and systemic immune-inflammatory response. An important indicator of a wound is the pH of the wound secretion, which is monitored with each dressing. After opening the dressing after 72 hours, a pH value of 7 was recorded in the wound secretion (Figure 5), which was followed until the end of wound healing.The analysis showed preservation of physiological pH values after 72 hours of wound treatment with gel in the neutral to slightly acidic environment of wound secretion, in the range of pH 6 - 7.

Figure 5: Monitoring the pH values of wound secretion after 72 hours of treatment with the gel.

Antifungal effect of combined mucus gel on wounds

The extract from gauze used for covering diabetic wounds was evaluated for fungal viability by the agar diffusion technique. As can be seen in Figure 6, even after 7 days of cultivation, no fungal development was observed on the Petri dish surface.

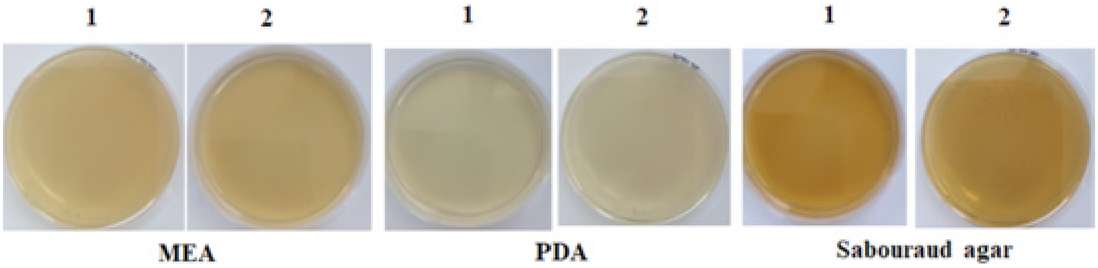

Figure 6: A lack of fungal growth after 7 days of cultivation of the extract from gauze covering diabetic wounds on MEA, PDA, and Sabouraud agar, 1 – upper surface; 2 - reverse surface.

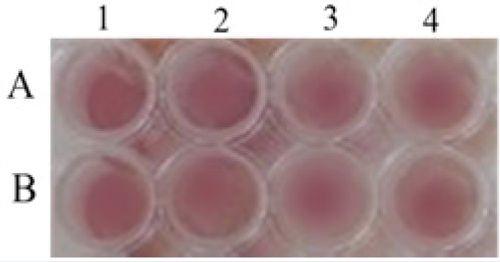

Figure 7: Resazurin dye used to identify fungal viability on the gauze after wound treatment by wound extract: A - medium 4/4; B – medium AN3; 1 – PDA + resazurin + chloramphenicol (control 1); 2 – PDA + resazurin + chloramphenicol + GEL (control 2); 3 – PDA + resazurin + chloramphenicol + calendula + plantain; 4 – PDA + resazurin + chloramphenicol + GEL + calendula + plantain.

The resazurin-based assay also confirmed the lack of fungal cell viability. It should be noted that, even at the beginning of the cultivation process, the sterile control wells exhibited a dark pink color instead of a blue color (see Figure 7, columns 1 and 2, lines A and B). Additionally, the test wells (containing growth medium and wound extract (WE) or WE+GEL) exhibited the same dark pink color at 0 hours and 24 hours after incubation (Figure 7, columns 3 and 4, lines A and B).

It is important to note that even at the beginning of the cultivation process, the sterile control wells have a dark pink color instead of blue (see Figure 7, columns 1 and 2, rows A and B). This color change, even in the absence of microbial growth, has been reported [36]. One possible reason is autoreduction of resazurin in most growth media [37].

Discussion

The use of various agents, such as mud, milk, and various types of plants, to treat severe wounds has been documented since ancient times [38]. There are two main types of wounds: acute and chronic wounds. Acute wounds, following the normal healing process, reach complete recovery after 4–12 weeks of therapy. Chronic wounds due to underlying pathology, such as diabetes, venous stasis, autoimmune diseases, or atherosclerosis, are much more difficult to treat. [39,40]. In turn, chronic wounds are represented by six types: burns, diabetic wounds, malignant ulcers, venous ulcers, decubitus ulcers, and pyoderma gangrenosum. Of these, Diabetic Foot Ulcers (DFU), Venous Foot Ulcers (VFU), and Decubitus Ulcers (DFU) are the most common, more complex, and most expensive to treat. Therefore, our study was conducted on a chronic, poorly healing wound, 10 days after primary inpatient surgical necrotization, in a patient with comorbidities such as insulin-dependent diabetes with diabetic polyneuropathy, MSD with cortical atrophy, and multi-infarct encephalopathy (Figure 1A).

Wound treatment using a novel in vivo protocol demonstrated the synergistic effect of the developed combined gel of snail mucus extract *C. aspersum*, marigold and plantain on the state of local inflammation and tissue regeneration on a large wound surface after extensive necrosis.

Conventional practices for the treatment of poorly healing and chronic wounds are mainly focused on the application of surgical and non-surgical therapies. Surgical debridement of devitalized, inflamed, and necrotic tissues in the wound remains the “gold standard.” The advantages of surgical therapy are that good wound cleansing from devitalized and necrotic tissues is the basis and beginning of the healing process. Therefore, the conventional method applied in our protocol includes surgical debridement and local therapy with the hydrogel of snail mucus *C. aspersum* in combination with herbal extracts of calendula and plantain.

Monitoring the effect of the gel on the treated necrotic wound on the patient’s foot showed a significant change after the primary surgical cleansing (Figure 1B) and after 5-fold treatment of the wound with the gel for one month (Figure 1C). Surgical debridement of necrotic tissue allows the formation of fresh and rapidly growing columns of granulation tissue, which are observed much more clearly after the 5th week of treatment (Figure 2A). An important factor is the lack of inflammatory reaction at the edges and in the surrounding tissues. A thin 1-2 mm brighter red shaft is clearly visible between the granulations and the already growing hypodermis, probably a zone of active migration of fibroblasts and keratinocytes, as well as their rapid maturation.

Evidence of activation of tissue regeneration processes is the completely closed upper pole at the “cortex” level and the complete alignment of Langer’s lines, recorded after the 7th week of treatment (Figure 2C). Growth of the hypodermis from the lateral edge is observed, which actively covers the mature and healthy granulations.

An important element of the wound healing process is non-surgical therapy, which includes local therapies: antibiotics, proteolytic enzymes, growth factors, etc. Also, proteolytic enzymes are used for biochemical cleansing of necrosis, which is possible only in the necrotic stage and does not stimulate tissue growth and regeneration [41]. Also, in the era of antibiotics, they are applied systemically and locally in the treatment of chronic and difficult-to-heal wounds. Topical antibiotics in the form of creams and ointments are effective against a wide range of bacterial strains, but despite expectations, a number of shortcomings arise in antibiotic therapy. Low or suboptimal tissue penetration has been found with topical application of antibiotics, as well as the development of bacterial multidrug resistance with their prolonged use [42].

The presented study using snail mucus and extracts of calendula and plantain showed the rapid elimination of the local inflammatory process and drainage in and around the wound, as well as the prevention of the spread of infection through the lymphatic and blood routes in the body without the use of topical antibiotics (Figure 2). Evidence is the lack of any signs of local inflammation (Figure 2B) after opening the dressing 6 weeks after treatment, despite the abundant and thick wound secretion and the presence of pathogenic microorganisms in the biofilm.

The presented studies confirm that the combined gel of *C. aspersa* snail mucus with calendula and plantain extracts not only prevents wound infection, but also has a strong regenerative effect (Figure 3). Figure 3A clearly shows elements of the regeneration process, and at the edge - granulation tissue and hyperkeratosis, as evidence of stimulated keratinocytes. This regeneration process develops more clearly from the 14th (Figure 3B) to the 18th week (Figure 3C), with the maturation of the dermis layers and the process of neovascularization (formation of a new capillary and lymphatic vascular network) clearly outlined in the upper covered edge of the wound.

A significant change in the wound condition was achieved after 29 weeks of treatment with the gel (Figure 4), with an almost completely healed wound bed with a scab formed in the area, cracked and flaky skin throughout the foot, and still maturing dermis, including in the hyperkeratotic areas (Figure 4A). Visualization of the complete closure of the wound bed by the hypodermis is presented in Figure 4B, through the formed strands of fibrin, keratin, and elastin at the edges, which actively cover the wound. In recent years, wound healing growth factors, such as Epidermal Growth Factor (EGF) and Fibroblast Growth Factor (FGF-10), have been widely used in practice for topical application in various forms. Their application, encapsulated in nanoparticles, increases their stability and ability to stimulate the synthesis of collagen and new vessels [43].

The results show that the developed combined gel also achieves a very good effect on the healing of severe, diabetic wounds. It not only accelerates tissue regeneration, but also the treatment with the gel protects the wounds from fungal infection. The absence of fungal infection in the wound and in the gauze extract shows that the gel protects the wound from fungal infection for a long time (Figures 6 & 7) Proof of this are the results of the antifungal experiments conducted, which show that the gel does not allow viability of fungi in the treated wounds. The established antifungal properties confirm that the gel can be applied topically to the wound to prevent the development of fungal infections. The use of new and effective agents for the treatment of diabetic wounds remains an area of intensive research. Fungal infection is a major complication of diabetic wounds, which causes serious harm to patients [44]. They are responsible for the non-healing nature of these wounds [45]. However, very few drugs exhibit both antibacterial and antifungal effects. Shevelev et al. (2020) reported that three polyphenols isolated from Norway spruce bark can induce the elimination of *Pseudomonas aeruginosa* and *Candida albicans* [46]. Similar positive results have been demonstrated by extracts of Combretum and Terminalia species against *Candida albicans*, *Cryptococcus neoformans*, *Microsporum canis*, and *Sporothrix schenckii* [47].

Another advantage of the developed mucus hydrogel and plant extracts is that it provides the necessary moisture to the wounds through high absorption and cooling effect. It has the same function as wet wound dressings based on hydrogels, which are applied for wound healing due to their high ability to absorb exudate while maintaining a moist wound environment, promote the migration and proliferation of keratinocytes and fibroblasts by mimicking the extracellular membrane of the skin, and accelerate the healing process.

In recent years, nanotechnologies have been developed that provide new routes for the delivery of drugs, antimicrobials, antibiotics, various biomacromolecules (proteins, peptides), growth factors, DNA/RNA, etc. They are applied to heal chronic wounds by balancing the control of microbial infection, inflammation and promotion of cell proliferation. The obtained extracts from snail mucus, calendula and plantain contain various active substances important for wound healing [19,25]. Therefore, they can be used as reducing agents for the synthesis of metal nanoparticles to be used in new therapeutic approaches. Nanoparticle-based treatments have shown efficacy in wound healing using different types of nanomaterials [48,49].

Conclusion

The results presented in this study prove that the successful synergistic combination of garden snail *C. aspersum* mucus extract enriched with marigold (*C. officinalis*) and plantain (*P. major*) extracts provides a moist environment, protects against fungal infections and accelerates the healing of acute, chronic, necrotic and difficult-to-heal wounds with a large area. Therefore, the developed protocol and gel fulfill the three main factors and problems that need to be overcome in the healing of difficult-to-heal and chronic wounds: 1. Control and prevention of local infection, which can lead to the 2nd factor - acute and chronic inflammatory process in the tissues, which compromises healing. The third factor is related to the stimulation of rapid growth and regeneration of tissues in the wound. Therefore, the developed strategy and gel presented in this study are promising in the treatment of acute, chronic, necrotic and difficult-to-heal wounds, which has high social and health importance.

Author declarations

Funding

This research was carried out with the support of the project KP-06 N 61-8/2022 funded by the Bulgarian National Science Fund.

Acknowledgements

Grant Project №BG16RFPR002-1.014-0015: “Clean Technologies for Sustainable Environment – Water, Waste, Energy for Circular Economy”, financed by the European Regional Development Fund through Bulgarian Programme “Research, Innovation and Digitalisation for Smart Transformation”.

Ethics statement

Ethics obtained from the Institutional Review Board (LAUM-CRH.KY2.25/Nov/2020).

Consent

Informed consent statements were obtained from the patients.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability statement

The authors have nothing to report.

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