



Plant-Based Nano-Formulations for the Treatment of Wounds in Animals

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ABSTRACT

The term wound describes any disturbance in the integrity of the soft tissues caused by the influence of different chemical, physical, biological, or pathological agents. They concurrently diminish the structural and functional capacity of a body. They are known to be categorized according to their depth, healing process, and etiology into classes like superficial, partial thickness, full thickness, acute, chronic, surgical, traumatic, infectious, chemical, or thermal type wounds. Despite all other chemical injuries, acid wounds are more severe as they happen due to exposure to corrosive materials, making the affected victims include hydrochloric or sulfuric acid; the severity is dependent on the concentration, time of exposure, and the area of injury. The occurrence of acid injuries is not common in developed countries where regulations are strict, but these injuries are still common in India, Bangladesh, and Pakistan. Systemic conditions such as diabetes mellitus or immune disorders may also cause chronic wounds. The proper treatment requires proper classification of the wounds and knowledge of the wound healing process, that involves hemostasis, inflammation, proliferation, and tissue remodeling. The new developments in nanotechnology have made a considerable contribution to the management of wounds. Nano-formulations, including nano-emulsions, nano-gels, silver nanoparticles and nanofibers, provide controlled, targeted, and sustained therapeutic delivery. Nano-emulsions improve the solubility and penetration of the drug, nano gels allow keeping a moist environment and permitting the sustained drug release; silver nanoparticles have a strong antimicrobial effect, and nanofibers form the extracellular matrix that supports cell development and tissue repair. Collectively, these nanotechnologies speed up healing and combine antimicrobial activity, effective drug release, and tissue regeneration in a controlled and effective way.

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INTRODUCTION

A wound is defined as a breakdown in the continuity of soft tissue, which may be due to chemical, physical, and biological factors (Forrest 1983; Barakat-Johnson et al. 2022). Pathological conditions, trauma, and surgery may also play a key role in the pathogenesis of wounds. It can disturb the function and structure of the body. Wounds are classified in many ways depending on the depth, healing process, and causes (Abazari et al. 2022).

Based on depth, wounds are classified as full thickness, partial thickness, and superficial wounds (Tan et al. 2022). Based on the healing process, there can be acute and chronic wounds. Based on causes, it can be surgical, chemical, traumatic, infectious, or thermal wounds.

An injury caused by exposure to corrosive acids, like hydrochloric acid or sulfuric acid, is recognized as an acid wound (Acehan et al. 2021). Its severity depends upon the concentration of acid, type, interval of exposure, and body

part involved. Mild, moderate, and severe acid wounds are the levels of severity of acid wounds (Brumberg et al. 2021). Globally, the incidence of acid burns in developing countries is very rare due to the strict regulations and sale of acid. A significant number of cases have been reported in India, Bangladesh, and Pakistan (Björnsson and Björnsson 2022), or it can develop due to slowly progressing diseases such as diabetes mellitus or immunological disease. The techniques for treating wounds depend upon the classification of the wound, whether it is classified as open or closed wounds, so it is important to identify the type of wound for its proper management (Abazari et al. 2022). Whenever an organism comes into contact with a wound, there occurs a series of steps that collectively are known as wound healing, which is a normal physiological response of the body towards wounds. These steps include hemostasis, inflammation, proliferation, and followed by tissue healing (Soliman and Barreda 2022). The rate of wound healing can depend upon the age, tissue oxygenation, and certain medications, and can be affected (Haller et al. 2021)

Types of Wounds

Wounds can be broadly classified into closed and open wounds (Milne and Penn-Barwell 2023). An open wound can be identified, which involves the rupture of the protective body surface, i.e., mucous membrane or skin, and allows the entry of foreign substances or particles into the tissue (King et al. 2022). Open wounds further include various types, which include avulsions, abrasions, incisions, and puncture wounds (Falanga et al. 2022). Avulsion is a type of open wound in which all the layers of skin are torn off due to trauma (Falanga et al. 2022). Abrasion is a type of open wound that involves the scraping of the superficial layer of skin, or in other words, the epidermis is affected in such a type of open wound (Stürmer et al. 2024). Incision is another type of open wound which involves a clean and straight surgical cut made on the surface of the skin, while a puncture wound is typically caused by the invasion of pointed objects into the skin, like a nail or needle (Groenen et al. 2024). A closed wound is a broader wound which do not break the skin but affects the internal structure by a direct blow onto the tissues (Sullivan and Myers 2022). In simple words, it does not involve the exposure of damaged tissue to the external environment. Close wounds can be in the form of crush injuries, contusions, or hematomas (Long et al. 2023). Crush injuries are the compression of two body parts by two objects; they mostly affect the skin, muscles, and nerves. On the other hand, contusion is a type of closed wound in which the capillaries are ruptured due to blunt forces (Sorroza-Martínez et al. 2025).

Severity of Wound

The severity of the wound depends on some factors like depth, size, cause, etc (Falanga et al. 2022). Wounds are generally classified as mild, moderate, and severe (Bassetti et al. 2024). A mild wound means small punctures, scrapes, and superficial cuts (LeSueur et al. 2025). They mostly affect the outermost layer of the skin. It causes less damage and less blood loss. It can be treated at home by applying simple ointments, antiseptics, and bandages. Moderate wounds are supposed to be more

serious than mild wounds; they can cause deeper lacerations, larger abrasions, second-degree burns, or bites (Wang et al. 2023). These wounds can expand to the dermis and muscle layers and can bleed moderately and need medical attention, antibiotics to avoid infection, and stitches in rare cases. Severe wounds are most critical because they can cause severe bleeding that can cause shock, sepsis, muscle, and bone damage (Eriksson et al. 2022). It needs immediate medical assistance. Incidence of wounds can be defined as the frequency or the rate of occurrence of new wounds in a certain population over a given period. The term is widely applied in a medical/healthcare context to keep track and monitor the frequency of occurrence of injuries (cuts, ulcers, lacerations, or surgical wounds) development in patients. As an illustration, a hospital can record the rate of post-operative wounds to understand the efficiency of surgical procedures or hygiene standards (Sinha 2019). In public health, knowing the rates of wound occurrence may enable the identification of a group at risk, e.g., people with diabetes, who have higher risks of getting chronic wounds because of poor circulation and delayed wound healing. Likewise, on the battlefield or in disaster areas, monitoring the occurrence of injuries in the civilian population or military personnel can help in the planning of medical assistance and preventive interventions. An incidence study will allow healthcare professionals to undertake specific measures to help decrease the occurrence of wounds and enhance patient outcomes.

Nano Formulation for Wound Treatment

Nano formulation in wound healing encompasses a range of nanotechnology-based delivery systems, such as nano-emulsion, nano-gel, silver nanoparticles, nano-fibers, and nano-emulsions increase the solubility and penetration of therapeutic substances, making sure that the wound site is efficiently delivered with the required substance (Ullah et al. 2025). The nano-gels offer a moist environment, favor a long release of the drug, and can be loaded with antibodies or growth factors and encourage healing. It is very common to know that silver nanoparticles have potent antimicrobial activity that prevents the infection of the wound and lowers inflammation (Wang et al. 2017). Nanofibers, many produced using electrospinning, recreate the extracellular context and can act as scaffolds on which cells attach, reproduce, capacity restore tissue capacity. Taken together, these nano-formulations have a large impact on the accelerated healing of the wound through the combination effects of antimicrobial performance, drug delivery, and tissue regeneration in a specific controlled manner.

Different kinds of formulations were made from different plants and tested on animals to check their effects. Some of them were described there (Kumar et al. 2021).

Nano-emulsions

A nano-emulsion is defined as a biphasic dispersion of two immiscible liquids that are thermodynamically stable and characterized by a surfactant (Ahire et al. 2021). Generally, it consists of an oily system dispersed in an aqueous system, forming droplets of nanometer

sizes. Some nano-emulsions used in wound healing are described below Table 1.

Table 1: Analysis of physicochemical parameters at the start of trial period

Sr. No	Parameters	Group A	Group B	Group C
1	pH	7.8	7.8	7.8
2	Temperature (°C)	29	29.1	28.8
3	EC (mS/cm)	0.77	0.74	0.73
4	Salinity (ppt)	0.3	0.4	0.3
5	TDS (ppt)	0.41	0.	0.38

Nano-emulsion, which is made by the plant *Licorice*, which is commonly called *Glycyrrhiza glabra* (Al Zahraa and Balata 2024). Its active ingredient is glycyrrhizic acid (Zhao et al. 2024). The method used in the preparation of nano-emulsions is spontaneous emulsification (Algahtani et al. 2022). The animal on which this emulsion is tested is a male Wistar rat, which shows the results that nano-emulsion with lavender essential oil is good for wound healing on 0, 4, 8, 12, and 16 days of wound production that including closure, epithelialization, and type I and type III collagen genes up-regulation. Nano-emulsion made from the *clove* plant, an aromatic flowering plant, has also been proven effective (Sun et al. 2022). Its active ingredient is eugenol, which is responsible for wound healing (Zari et al. 2021). The method used for its preparation is spontaneous emulsification. It is tested on female albino Wistar rats, which gives the result in wound healing and also for making some pharmaceutical products.

A nano-emulsion made from the plant *Zataria multiflora* Boiss has also been proven effective (Osanloo et al. 2024). There are multiple methods, but among the most convenient methods is using the Ultra homogenizer method (Pereira et al. 2021). Excision was demonstrated in Wistar rats. The result is that *Zataria multiflora* Boiss has hostile to anti-inflammatory, antimicrobial properties, which makes this plant suitable for wound healing (Nosratabadi et al. 2023). Similarly, a nano-emulsion made of garlic and ginger has also been found effective (Ibrar et al. 2022). By using the Ultrasonic cavitation method (Song et al. 2024). Excision in vivo model in healthy albino rabbits. This study used an ultrasonic method to create nine neomycin sulfate nano-emulsions utilizing ginger and garlic oils (Ibrar et al. 2022). Nine nano-emulsions with sizes ranging from 145 to 304 nm were tested for stability over three months. The thermodynamic stability investigations using freeze-thaw, centrifuge, and heat-cool cycles confirmed the remarkable stability of these nano-emulsions. The nano-emulsions remained stable for three months with no noticeable changes in physical properties or phase separation. The nano-emulsions showed superior antibacterial efficacy against both gram-positive and gram-negative bacterial strains compared to neomycin sulfate, the positive control. Furthermore, NEs enhanced wound healing compared to Neomycin sulfate ointment.

Nano-emulsion made by the plant *Tinospora smilacina* has also been proven effective for wound management (Saki et al. 2023). There are many methods to make, but we use the high-pressure homogenization method (Kotta et al. 2015). This study created a stable nano-emulsion by combining

extracts from *T. smilacina* and *C. inophyllum* (Saki et al. 2023). The nano-emulsion contained bioactive compounds from both plants in a single formulation. Flavonoid compounds were identified in the *T. smilacina* extract, some of which have been previously studied for their medicinal properties. The nano-emulsion was optimized using statistical techniques and was found to be stable with a small particle size and low polydispersity index (PDI). The nano-emulsion showed antioxidant activity, improved cell viability, and enhanced wound healing properties compared to the individual plant extracts (Schlesier et al. 2002). The addition of a plant-based water component improved the overall effectiveness of nano-emulsion. This study demonstrates the potential for using nano-emulsions for various biomedical applications Table 2.

Table 2: shows clinical signs at day 7.

Clinical Signs	Treated Groups		
	G ₀	G ₁	G ₂
Gasping	+	+	++
Jerking	+	+	++
Convulsions	-	-	+
Faintness	-	-	+
Surface breathing	+	+	+
Surface Running	+	+	++
Bottom Running	+	+	++
Body unbalancing	-	-	+
Operculum movements	+	++	++
Tilting of Fin	+	+	++
Static position	+	+	+

Nano-emulgel

Nano-emulgel made from plant *Curcumin* has been observed to be effective in the wound healing process (Jeengar et al. 2016). There are multiple methods, but we use the high-energy ultrasonication method to make it (Li et al. 2018). Ex-vivo skin deposition Determination of nitric oxide (NOx) (µmol/g tissue), determination of tissue malondialdehyde (MDA) (nmol/g tissue) Determination of the skin content of proline, hydroxyl-proline (HPro) and glycine (mg/g tissue), Determination of myeloperoxidase, prostaglandin E2 (PGE2) and interleukin 1 beta (IL-1β). Myeloperoxidase (MPO), prostaglandin E2 (PGE2). The result is that Cur-NE was prepared from phenyl-propanol by ultrasonication and QbD software, the best result being recorded in Smix. The structure of the fine NE was smooth, and it had Cur in it, as REE analysis using SEM and TEM revealed. Skin permeation studies depicted the study's Cur release. Compared with the effects of clove oil and Cur-S, the wound healing effects of curcumin were comparable to those of fusidic acid. It was also noted that Cur-NE had no toxicity, and there were no inflammatory cells identified in wounds. In conclusion, the optimized Cur-NE exhibited good anti-inflammatory action in rat models (Gharat et al. 2024).

Nanofibers

Nanofibers are fiber materials with at least one dimension less than 100 nanometers (Xue et al. 2017). They are generated from different polymers and have different properties and applications, such as wound healing. One of them is described below Table 3.

Table 3: Clinical signs at day 14

Clinical Signs	Treated Groups		
	G ₀	G ₁	G ₂
Gasping	+	+	-
Jerking	+	+	++
Convulsions	-	-	++
Faintness	-	-	+
Surface breathing	+	+	+
Surface Running	+	+	-
Bottom Running	+	+	++
Body unbalancing	-	-	+
Operculum movements	++	++	+++
Tilting of Fin	+	+	++
Static position	+	+	+

Nanofiber, made from the *Moringa* plant by using the method of electrospinning, was tested on rats (Fayemi et al. 2018). *Moringa* has antibacterial properties that show wound potential, and a study showed that *Moringa* incorporated into nanofibers increased wound healing.

Nanofiber, which is made from *Centella asiatica* it is commonly known as Gotu kola or Asian pennywort. It is used in traditional medicine (Bozkaya et al. 2022). The electrospinning method is used in making this nanofiber because it is a convenient method to make it. It was tested in SD rats; its result was that adding the *C. asiatica* extract to the electro-spun membranes improves the rate of wound healing in rats. It shields against bacteria, fungi, and virus invasion, and enhances cell proliferation.

Nanofibers made by the plant *Peppermint* showed good results in the wound healing process (Sünter Eroğlu and Canoğlu 2025). The crosslinking method was used for making this nanofiber because it has been proven to be an effective method for making it (Schiffman and Schauer 2007). It was tested on male Wistar rats. The study prepared hydrophilic polyurethane-based wound dressings containing peppermint extract using electrospinning. The addition of crosslinked extract-gelatin nanoparticles controlled the release of the extract, resulting in fast and effective wound healing. The dressings showed antibacterial properties, controlled release, and high absorbency. They promoted tissue granulation, collagen synthesis, and faster wound closure compared to conventional gauze bandages. The dressings were non-toxic and enhanced cell viability. Overall, the extract-containing wound dressings showed great potential for treating diabetic ulcers and bacterial infections.

Silver nanoparticles

Silver nanoparticles are extremely small-sized nanometre-sized particles, which possess very peculiar characteristics owing to their high surface area to volume ratio as well as size characteristics (Mishra et al. 2014).

Silver nanoparticles made by the *Melia azedarach* plant (Ma-AgNPS), commonly known as the chinaberry tree, were found effective in wound healing (Jebri et al. 2020). There are many other methods, but the solvent displacement method is commonly used to assemble them. Are results show that Ma-AgNPS possessed higher bioactivity than the Ma-extract and also demonstrated promising wound healing activity.

Silver nanoparticles made by the plant *Tridax procumbens*, known as coatbuttons or tridax daisy, are a species of flowering plant in the family *Asteraceae*

(Amutha et al. 2019). It was tested on rats, and its results show that it is very useful for wound healing. Silver nanoparticles, made by *Prosopis juliflora Neltuma juliflora* formerly *Prosopis juliflora*, are a shrub or small tree in the family *Fabaceae*, a kind of mesquite (Anwar et al. 2019). Nanoparticles showed antibacterial activity and have the potential in wounds faster than the standard group.

Silver nanoparticles, made by the plant *Azadirachta indica*, have wound healing properties and were made by using the Solvent displacement method (Ahmed et al. 2016). It was tested on a male, pale-skinned person. The result was that flavonoids and terpenoids were displayed in neem leaves and contributed to the formation of silver nanoparticles from neem extricate. Based on the information presented, these AI AgNPs were demonstrated to be secure for use and compelling within the treatment of wounds. The paper also suggests that neem silver Nanoparticles have an extraordinary scope for reasonable green solutions. Silver nanoparticles that are made by the plant *Caulerpa scalpelliformis* using the cold suspension method (Manikandan et al. 2019). It was demonstrated in albino male rats. Its result is that the *C. scalpelliformis* extract-intervened blend of Ag-NPs appears critical in enhancing wound mending.

Silver nanoparticles made by the plant *Scutellaria barbata* showed good results in the wound healing process (Veeraraghavan et al. 2021). It was tested on the wound scratch assay in fibroblasts. The result was that (Forrest 1983) the *Scutellaria barbata* extract, which possesses antimicrobial action, also possesses wound-healing and mending movement in fibroblast cells. Silver Nanoparticles made by the plant *Indigofera aspalathoides* have good wound healing properties (Arunachalam et al. 2013). The result was that *Indigofera aspalathoides* can be used to prepare green silver nanoparticles through a process that is quite straightforward and friendly to the environment (Krishnasamy et al. 2014). Silver nanoparticles are synthesized from plant extract because the extract contains phytochemicals that assist in the reduction of silver ions and the subsequent formation of nanoparticles. It is important to prove successful synthesis of cobalt ferrite nanoparticles; the nanoparticles were analyzed using UV-visible spectroscopy, SEM, EDAX, FTIR, and XRD techniques. This work further revealed that the formed nanoparticles of silver from *Indigofera aspalathoides* extract can potentially be used in wound healing treatments in the form of a hydrogel dressing. As this study reveals, medicinal plants that are sourced naturally might be used in the green synthesis of nanoparticles for application in the medical sector (Habeeb Rahuman et al. 2022).

Limitations

Although important advances have been achieved concerning wound pathophysiology and the formation of high-level treatment regimens such as nano-formulations, there are still certain limitations and issues that should be mentioned (Krishnaswami et al. 2022). Although nano-formulations, such as nano-emulsions, nano-gels, silver nanoparticles, and nanofibers, have promising potential, their transformation to the common clinical practice remains minimal (Sabarees et al. 2022). This is because

there are no long-term safety data, regulatory barriers, as well as limited larger-type clinical trials to confirm their efficacy and safety in varied populations.

The other limitation is the inconsistency of wound types (St-Supery et al. 2011). Wounds vary tremendously in terms of etiology (chemical, traumatic, surgical, or infectious), depth, and whether acute or chronic (Shakir et al. 2020). This heterogeneity creates an impediment to the standardization of treatment methods. As an example, nano-formulations that have good outcomes in treating acute wounds cannot be expected to work similarly in chronic acid-induced wounds, which are most often more complicated to treat because of their longstanding inflammation and lack of proper vascularization.

Although the situation with acid wounds is covered, there is no strong clinical evidence proving the application of nanotechnology-based interventions (Malik et al. 2023). The infrequency of such wounds in certain areas and their rather violent nature (particularly in developing countries such as India, Bangladesh, and Pakistan) does not allow the engineering of controlled experiments or the creation of specific treatments for this type of wound. Also, nano-formulations may need advanced production procedures, storage facilities, and administration routes, which are not always available in resource-restricted areas (Petrovic et al. 2024). In nations or regions that lack much of a medical infrastructure, it may not be possible to use nanotechnology-based treatment, especially where the traditional treatment of wounds is still the norm, because it is more cost-effective and simpler. There is also a big challenge of toxicological concern. Whereas silver nanoparticles, e.g., are characterized by the presence of antimicrobial effects, in the case of intensive concentration in the tissues, cytotoxicity or inflammatory reactions can take place. The effects of long-term exposure to these nanoparticles on human tissue are not yet fully comprehended, and there is a necessity for in-depth biocompatibility research (Ferdous and Nemmar 2020). Lastly, although the promise of nano-formulations is astronomical, there is minimal access to knowledge of and expertise on the same as perceived by the populace and those in the medical field (Petrovic et al. 2024). The doctors might need some educational messages about the effective methods of using nano-based therapy, its suitable dose, and anticipated results. In the absence of proper training, chances are high that such advanced materials will be misused or underutilized.

Conclusively, nano-formulations possess a good future in the healing of wound healing, but it will be essential to overcome their limitations by conducting more research, clinical validation, regulatory certainty, and accessibility of such nano-formulations so that they can be widely and successfully embraced (Lin et al. 2022).

Conclusion

Management of wounds has been a very complicated and sensitive issue in the healthcare field that requires understanding the classification of wounds, the cause of a wound, and how a wound is normally healed biologically. Acid-type wounds and chronic wounds are among the many categories under which a person can experience as far as wounds are concerned, because of their severity and cause. Nanotechnology has led to the

development of new nano-formulations, i.e., nano-emulsions, nanogels, silver nanoparticles, and nanofibers, which have transformed wound care. Such nanostructures lead to increased delivery of drugs, antimicrobial protection of wounds, tissue regeneration, and healing promotion. They have unique properties and can stimulate specific, sustained, effective therapeutic applications. Therefore, the incorporation of nanotechnology into wound therapy regimens is one of the new trends that may prolong the overall recovery time, eliminate the complications, and enhance the recovery of these patients both in acute and chronic wound conditions.

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