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Effectiveness of Plant-Derived Exosomes in Leprosy Ulcers: A case series

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Abstract: Background: Leprosy is a chronic skin infection which is caused by Mycobacterium leprae and can cause peripheral nerve damage, so that it is often accompanied by ulcer manifestation. Leprosy ulcers are still a stigma in society. Currently, the use of stem cell derivatives is starting to develop in the dermatology field, such as exosomes. Exosomes can be derived from mesenchymal stem cells, keratinocytes, or plant-based. Case report: In this case series, there were ten leprosy ulcer patients who had completed leprosy treatment. The average age of patients was 53 years old with various locations, such as in the cruris region (three patients), anterior pedis (two patients), and posterior pedis (five patients). The intervention given was plant-derived exosomes, given two injections with interval two weeks and the dosage was 1 cc diluted exosomes. No side effects were found during monitoring until the 28th day. At the end of study, all patients experienced a reduction in ulcer wounds based on the length, width, and depth of the ulcers. There were seven patients who also had re-epithelization. **Discussion:** Generally, leprosy patients often have damage in the posterior tibial nerve, so that leprosy ulcers are often found in the lower legs. Etiology of leprosy ulcers are the loss of protective sensation and deformity in the extremities. The location of ulcers, occupation, daily activities, and age are affecting the wound healing process of leprosy ulcers. Identification of risk factors is very important in ulcer management. The use of exosomes in leprosy ulcers play a role in the inflammatory process, increasing fibroblast growth, regulating gene expression, and stimulating blood vessel formation, so it provides tissue regeneration in wound healing process. Moreover, exosomes can regenerate nerves by inhibiting Schwann cell apoptosis, modulating glial cells, and increasing the expression of nerve growth factors. **Conclusion:** Plant derived-exosomes can be used in leprosy ulcers because it provides favorable wound healing. It can be used as a promising alternative in the management of leprosy ulcers.
Keywords: exosomes, leprosy, ulcers, wound healing.

INTRODUCTION

Leprosy is a neglected chronic infectious disease caused by *Mycobacterium Leprae* and is still commonly found in developing countries. The stigma about this disease is still high.[1-3] Indonesia is the third country with the highest prevalence of leprosy cases in the world. Leprosy is a polymorphic disease with diverse neurocutaneous manifestations and has an incubation

time of about 3 – 7 years. Clinical skin manifestations of leprosy patients are usually hypopigmented hypoesthesia macules, papules, plaques, and/or diffuse infiltration accompanied by xerosis. Leprosy can cause involvement of other organs, such the eyes, nasal mucosa, joints, lymph nodes, liver, lien and testes. The diagnosis of leprosy consists of clinical symptoms and confirmed by laboratory examination of acid – fast bacteria, namely skin slit smear. In endemic areas with limited resources, most leprosy cases are diagnosed based solely on the patient's clinical manifestations. Delayed diagnosis and treatment of leprosy patients remains one of the problems to date.[1,2]

Leprosy ulcers are not a typical feature in leprosy patients, unless the patient has leprosy reactions, Lucio's phenomenon (LP), or secondary neuropathy. Globally, about 30-40% of new leprosy cases are accompanied by disability. According to the World Health Organization/WHO (2020), the prevalence of leprosy cases with second-degree disability in the world could reach more than 1 million cases. Leprosy ulcers may result from peripheral neuropathy and/or vascular disorders. Therefore, other comorbidities in leprosy patients, such as diabetes and hypertension, can cause neuropathy and vascular disorders, resulting in a higher risk of leprosy ulcers.[2-4] In cases of leprosy ulcers, there is an increase in inflammatory cells and protease enzyme activity, extracellular matrix (ECM) damage, and failure of the epithelialization process which will prevent the wound healing process.[5] Therefore, leprosy ulcer management takes a long time. Daily wound care is required by cleaning the wound with cleaned water and changing the dressing. Ulcer management involves multiple factors, such as debridement of necrotic tissue, infection control, reduction of pressure areas, optimization of blood flow, and nerve decompression.[6]

Currently, one of the new strategies in ulcer management is the use of exosomes. Exosomes are derived from adipose stem cells, bone marrow mesenchyme, or plant-based that can promote angiogenesis and tissue repair. Exosomes are extracellular substances measuring 30 - 150 nm, containing proteins, lipids, mRNA, and other metabolites. Exosomes can regulate macrophage activity, proliferation and migration of dermal fibroblasts and keratinocytes, and have the ability to convert myofibroblasts into ECM.[7,8] In addition, exosomes also have antibacterial properties such as antibiotics by performing regenerative activities through bioactive molecules (growth factors, nucleic acids, proteins) and non-bioactive substances. The use of exosomes has been done in cases of diabetic ulcers and pressure ulcers. However, it has not been done for leprosy ulcers.[8,9] Therefore, we present a case series report on the treatment of leprosy ulcers using exosomes.

METHOD

These serial cases consist of ten leprosy ulcer patients who have obtained consent from the patients for exosome injection with the inclusion criteria that the patient had completed leprosy treatment. Exclusion criteria were patients who were lost to follow-up and had comorbid of diabetes mellitus.

The exosome used is P198 Exo-HL™, which is the second generation of exosome skin booster taken from edelweiss flower stem cells and then cultured in the media that are already conditioned for absorption and bind the active ingredients maximally (Edelweiss Exo^{pro}). P198 Exo-HL™ consists of 1 vial of powder (50 grams) containing growth factors and 1 vial of solvent (5 mL) containing 22 amino acids, 13 vitamins, 7 peptides, 4 antioxidants, and hyaluronic acid. Exosomes can be dissolved with the solvent or with 2-5 mL normal saline as an alternative.

Preparations were made for patient intervention by dissolving 1 vial of exosome powder with the solvent. Approximately 1 cc of diluted exosomes was injected to the patient at the base and edges of the ulcer lesion which has been cleaned. Exosome injection is done at 2-week intervals with a total of 2 injections. The evaluation will be made at pre-injection (initial), 2nd week, and 4th week. Monitoring of side effects is done every 2 weeks. Ulcer size is measured based on the length, width, and depth of the wound. The final result of observation is the presence or absence of re-epithelialization and reduction of the lesion sizes.

ILLUSTRATION CASE AND RESULTS

This case series reported ten cases of leprosy ulcers, mostly found in males (70%) with a mean age of 53 years and ulcer locations on the lower limbs to the feet. The general characteristics of the patients are shown in Table 1 and the clinical features of each patient during monitoring are shown in Figure 1.

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Table 1. General characteristics of leprosy ulcer patients

Characteristics	**N=10**
Gender	
Male, n (%)	7 (70)
Woman, n (%)	3 (30)
Age, average ± SD	53 ± 9.7
Occupation	
Self-employed, n (%)	2 (20)
Mother household, n (%)	3 (30)
Unemployed, n (%)	5 (50)
Location of ulcer	
Cruris, n (%)	3 (30)
Anterior pedis, n (%)	2 (20)
Posterior pedis, n (%)	5 (50)
Fore foot , n (%)	4 (40)
***Hind foot* , n (%)**	**1 (10)**

Notes: n, number of patients; %, proportion; SD, standard deviation.

Table 2. Characteristics of each leprosy ulcer patients

Case	**Gender**	**Age (years)**	**MDT Status**	**Time the appearance of ulcers**	**Ulcer location**	**Re-epithelialization**
1	Man	45	RFT	6 months	*anterior pedis sinistra*	(+)
2	Man	75	RFT	More than 1 year	*anterior pedis sinistra*	(+)
3	Woman	55	RFT	More than 1 year	*fore foot dextra*	(+)
4	Woman	58	RFT	More than 1 year	*fore foot dextra*	(+)
5	Man	62	RFT	More than 1 year	*fore foot dextra*	(-)
6	Woman	53	RFT	More than 1 year	*hind foot dextra*	(-)
7	Man	50	RFT	More than 1 year	*fore foot dextra*	(-)
8	Man	41	RFT	1 year	*cruris sinistra*	(+)
9	Man	46	RFT	6 months	*cruris sinistra*	(+)
10	Man	44	RFT	6 months	*cruris dextra*	(+)

Notes: MDT, multi drug therapy; RFT, release from treatment.

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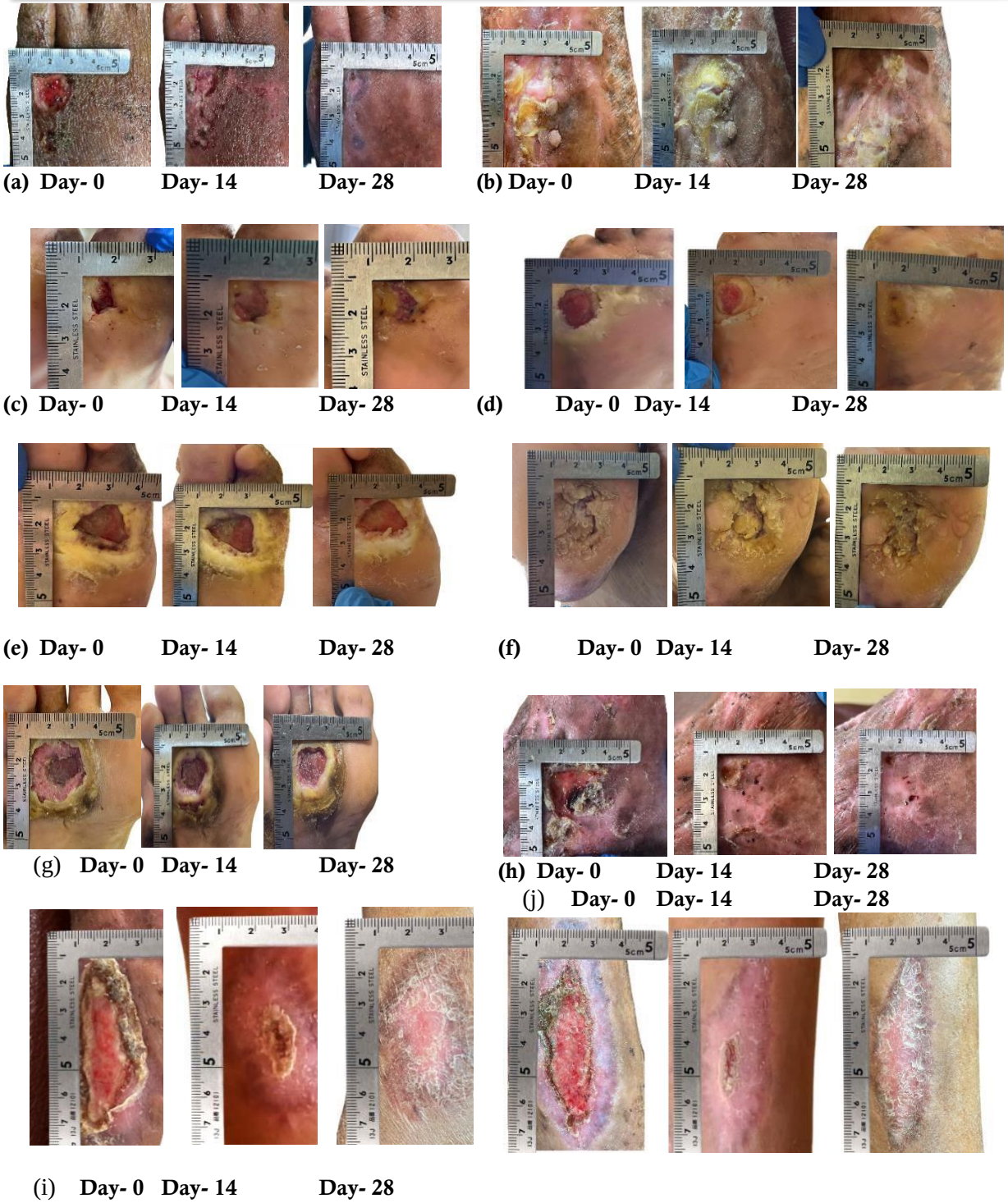

Figure 1. (a) case 1, *anterior pedis sinistra*; (b), case 2, *anterior pedis sinistra*; (c) case 3, *fore foot dextra*; (d) case 4, *fore foot dextra*; (e) case 5, *fore foot dextra*; (f) case 6, *hind foot dextra*; (g) case 7, *fore foot dextra*; (h) case 8, *cruris sinistra*; (i) case 9, *cruris sinistra*; (j) case 10, *cruris dextra*.

During the monitoring period, there were no adverse effects of exosome injection. Table 2 describes the characteristics of each leprosy ulcer patient. There were seven patients who had re-epithelialization in the ulcer lesions. Based on the ulcer location, we divided the case series into three groups, group 1 with ulcer location on the *cruris* or leg, group 2 with ulcer location on *anterior pedis*, and group 3 with ulcer location on the sole or *posterior pedis* (*plantar pedis*). The response of exosome therapy to ulcer lesions can be seen in Table 3. Based on Table 3 and Figure 2, all patients experienced reduction in ulcer lesions measured by the length,

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width, and depth of the ulcer.

Table 3. Response to exosome injection therapy

Time	Group 1 (n=3)	Group 2 (n=2)	Group 3 (n=5)
Reduction of length size ulcer (average ± SD) in unit cm			
Day – 0	4.70 ± 1.89	3.00 ± 1.41	2.08 ± 0.73
Day – 14	1.50 ± 0.50	2.65 ± 1.62	1.72 ± 0.66
Day – 28	0.10 ± 0.17	1.75 ± 0.00	1.14 ± 0.85
Reduction of width ulcer (average ± SD) in unit cm			
Day – 0	1.70 ± 0.28	2.25 ± 0.35	1.74 ± 0.51
Day – 14	0.83 ± 0.28	1.85 ± 0.49	1.56 ± 0.51
Day – 28	0.10 ± 0.17	1.00 ± 0.00	1.04 ± 0.75
Reduction of depth ulcer (average ± SD) in unit cm			
Day – 0	0.14 ± 0.05	0.20 ± 0.00	0.38 ± 0.15
Day – 14	0.03 ± 0.00	0.15 ± 0.07	0.28 ± 0.13
Day – 28	0.00 ± 0.00	0.05 ± 0.00	0.18 ± 0.08

Notes: n, number of patients; SD, standard deviation; cm, centimeters.

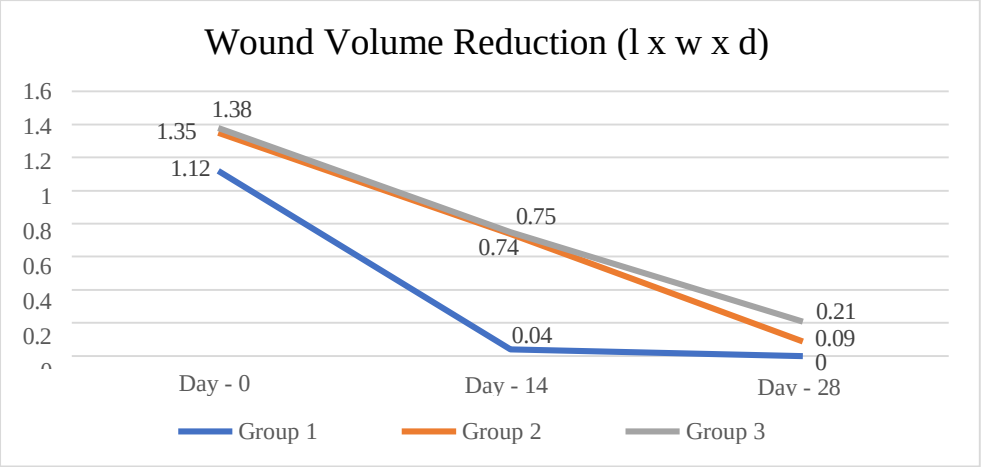

Figure 2. The chart of wound volume reduction (length x width x depth) cm^3 during the observation period.

DISCUSSION

Leprosy is a neglected disease that can cause disability and is still stigmatized in the community. Based on WHO data (2019), leprosy cases are found in more than 120 countries with around 30 – 40% of new cases accompanied by disability and Indonesia ranks third as the country with the highest prevalence of leprosy cases, after Brazil and India. Approximately 68% of leprosy patients have damage to the posterior tibial nerve resulting in loss of sensation in the foot and cause ulceration, especially in the plantar area.[3,10,11] In these serial cases, ten leprosy patients who had completed leprosy treatment (Release From Treatment/RFT) developed foot ulcers, three patients in the *cruris* region, two patients in the *anterior pedis* region, and five patients in the *posterior pedis* (plantar) region.

The etiology of foot ulcers varies with loss of protective sensation such as the inability to feel pressure and deformity of the foot. The most common ulcer is in the plantar region near the toes. Ulcers in the lateral area are rare and usually associated with drop foot due to fibular muscle paresis. In addition, ulcers near the fifth toe metatarsal are also common. The loss of intrinsic muscle volume of the hypothenar region of the foot causes the *styloid processus* or the base of the fifth metatarsal to protrude, resulting in callus formation and ulceration of the fifth metatarsal. While calcaneal ulcers are less common and more difficult to treat. Usually, ulcers in the calcaneal region occur due to trauma caused by sharp objects or uneven footwear shape, long walking activities, thus increasing the frictional force on the calcaneal region.[3,11] This is consistent with this case series. Ulcers in the calcaneal region have insignificant results on wound tissue repair.

In addition, occupation and daily activities are risk factors for the development and healing process of

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foot ulcers.[3,11] They will affect the wound healing process, as pressure on the ulcer is associated with wound healing, especially for ulcer locations on the plantar pedis. Patients usually come to the health facility after the ulcer appears, rarely patients who seek treatment in the early stages of ulcers, so the examination of disability prevention in ulcer patients is very important. The identification of risk factors can be used as a basis for providing interventions, such as wound care education, customized footwear modification, and provision of foot orthoses.[3] In this case series, there were two patients who had ulcers in the plantar pedis region and had significant results for wound tissue repair, namely granulation and re-epithelialization. After further identification, these patients underwent footwear modification by perforating the footwear in the area of the ulcer so that there was less pressure on that area.

Based on demographic data, the prevalence of leprosy cases is higher in males than in females (63% vs. 37%), so complications in the form of ulcers are also higher in males.[3,12] In this case series, 7 male patients and 3 female patients were reported. In addition, age is also one of the risk factors for ulcers. In older age, there are changes in the function and structure of the foot, which becomes more pronated and fattier. So, if accompanied by a loss of sensation, it will cause excessive pressure which results in an ulcer.[3] In this case series, the patient's average age was 53 years.

In general, healing process of leprosy ulcers is very slow due to vascular and nerve disorders in the lesion area. This is related to the pathogenesis of leprosy ulcers. Chronic inflammatory process on subcutaneous with infiltration macrophages, lymphocytes T, tumor necrosis factor, reactive oxygen species cause ulceration and necrosis.[2] Treatment of wound ulcer involving methods that aim to accelerate healing process and identify the underlying cause. Ulcer healing time varies from a few weeks to 6 months, depending on the type of ulcers and the effectiveness of the treatment given, as follows:[13,14]

a. Wound cleaning and dressing.

Cleaning and dressing the wound is a crucial first step in wound care of ulcers. This involving disappearance dirt and necrotic tissues from the ulcers to create an environment conducive to wound healing. After cleaning, the wound is dressed with a special dressing that functions to protect against infection and absorb moisture or excess fluid.

b. Compression and antibiotic therapies.

Compression therapy using stockings or bandages is the primary method for improving blood circulation and reducing swelling, especially in venous leg ulcers. Studies show that 87.3% - 88.9% of ulcer cases are treated with therapy compression between year 2009 – 2012. For infected ulcers, antibiotics are used either orally or topically. However, antibiotics do not speed healing of ulcers and only recommended for short-term treatment of confirmed infections.

c. Healing times for ulcers.

Ulcer healing time varies, depending on the type of ulcers, the underlying conditions, and the therapeutic approach. Factors that influence wound healing time include the size and severity of the ulcer, larger and deeper ulcers take longer to heal; the age of the patient, older patients typically have slower healing; the presence of infection or other complications; and the consistency of treatment and follow-up care.

In retrospective and analysis study which has reported in 2023, therapy in ulcers can be done by corrective surgery on the bone prominence and closure of the primary lesion with a local *bipediculated flap*. After surgery, wound healing can occur in an average of 12 weeks. Obesity is one of the factors associated with delayed wound healing time. Therefore, patients with younger age can use orthopedics shoe or sole regularly and wound dressing before surgery.[14] Meanwhile, based on a study in Sweden (2013), healing of ulcers with antibiotics takes about 63 days.[15] In general, wound care which is done in Indonesia still using wound dressing with moist gauze. Based on a meta-analysis conducted by Liang Z, et al (2023), the time needed for healing of diabetic patient ulcers with moist gauze is around 37 days.[16] Currently, there is innovation in wound healing, one of which is with use exosomes with hope time healing faster and better outcomes. In this case series, seven patients had experienced complete wound closure on the 28th day after exosome injection.

Exosomes are a subpopulation of extravascular vesicles with a diameter of approximately 30 to 150 nm, functioning as a medium messenger macromolecule (DNA, RNA, lipid proteins) and active substances such as drugs. Moreover, the use of exosomes in dermatology has begun much explored, not only for the cosmetic field only, but extends to several skin diseases, such as ulcers, systemic lupus erythematosus, psoriasis, dermatitis atopic, regulation pigmentation, vitiligo, and hair growth. In these serial cases, we will discuss the function of exosomes in wound healing. The therapeutic value of exosomes to modulate the cell microenvironment, regulate gene expression, and induce cell differentiation can have a positive impact on skin health. Exosomes contain pro-inflammatory proteins that stimulate the release of cytokines and chemokines, which then going to location wound and activate process of wound healing. Besides that, exosomes also contain factor growth which stimulate fibroblasts growth so it can help to do re-epithelialization. Exosomes also improve communication between cells because they contain miRNA, so that can organize expression gene in cells around the wound and stimulate the formation of new blood vessels and increase blood flow to the

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wound sites. This increased blood flow will provide oxygen and nutrients to the wound, thereby accelerating the wound healing process. Therefore, exosomes can play an important role in the wound healing process (Figure 3).[17,18]

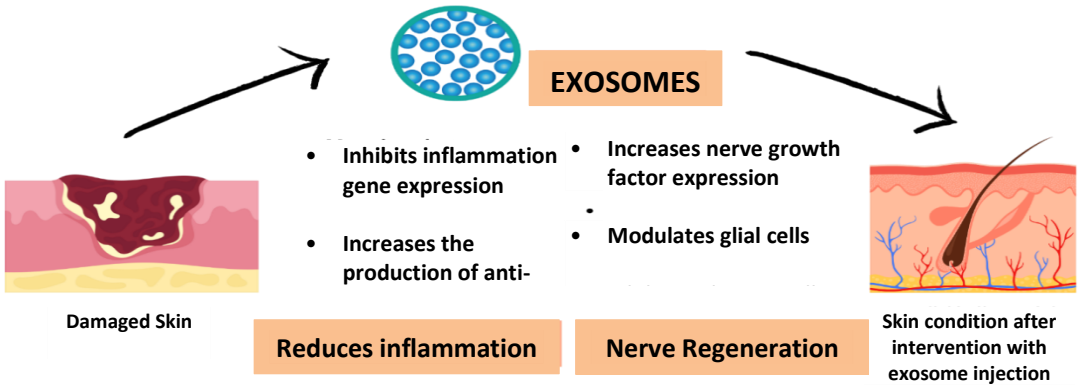

Figure 3. Mechanism of action of exosomes against ulcers.

Besides that, exosomes also can be used for peripheral nerve damage. This is related to the mechanism of restoration of homeostasis in the damaged peripheral nerve environment. The process of axonal regrowth and regenerative effects by exosomes through the paracrine pathway will provide recovery to damaged nerves. The mechanism of axonal regeneration is the specific transfer of exosome content, such as proteins, microRNAs, from *Schwan cells* to axons. Another mechanism is the presence of neurotrophic growth factors that facilitate axon regeneration and axonal growth directly through the *phosphatase and tensin homolog-mechanistic target of rapamycin pathway* (PTEN-mTOR).[19-21] In this study, we did not exam the nerve conduction due to the high cost. Several parameters which used for neuropathy evaluation, namely electrodiagnostic examination with nerve conduction velocity, electromyography (EMG), quantitative sensory examination with *Semmes-Weinstein monofilament*, *Sensory Nerve Action Potential* (SNAP), *Somato Sensory Evoked Potential* (SSEP), *Laser-Evoked Potentials* (LEPs), and *Small Fiber Nerve Conduction Velocity*.[22,23]

There are several limitations in this study. First, the intervention of modification footwear cannot be given to all patients. So, some patients have insignificant wound healing results. Secondly, nerve conduction examination was not checked due to limited time and cost. The use of exosomes in healing wound ulcer can become an innovation in management of ulcers by providing faster and better healing results. Further research about delivery of nerve repair to leprosy patient with neuropathy expected to be carried out.

CONCLUSION

Leprosy ulcers remain a significant cause of morbidity, particularly among patients with residual neuropathy post-treatment. This case series highlights that tailored interventions such as footwear modification and the use of exosomes can significantly improve wound healing outcomes. Exosomes offer a promising regenerative modality by modulating inflammatory responses, promoting fibroblast activity, and enhancing angiogenesis. Their potential in peripheral nerve regeneration also adds value in managing neuropathic complications of leprosy. However, practical barriers such as cost and limited access to diagnostic tools like nerve conduction studies must be addressed to ensure equitable treatment. Future research should focus on large-scale clinical trials and cost-benefit analyses to establish exosome therapy as a standard adjunct in the management of chronic ulcers in leprosy patients.

Conflicts Of Interest

None

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