# Formulation Of Aloe Vera Nano Pseudo Gel for Topical Antibacterial And Anti Inflammatory Therapy: In Vitro And In Vivo Assessment

Tasbeeha Saeed[1] , Muhammad Mubashar[2], Anam Wahid[5], Faheem Abbas[7], Aqib Javed[4], Faiza Hameed[4], Shumaila Umar[4] , Dr.Nouman Bashir[6], Abdul Mateen[1] , Kainat Fatima[5], Inam Ullah4[4], Farwa Shoukat[1], Hira Khalid[1], Javeria Ahmed[1], Anam Zahra[5]

1. Quaid e Azam University Islamabad, Pakistan
2. University of Agriculture Faisalabad
3. .University of Sargodha, Sargodha Main campus
4. Bacha Khan University Charsada
5. Government College University, Faisalabad
6. FVAS Gomal University DI Khan
7. University of Veternary and Animal Sciences,Lahore

**A B S T R A C T**

The current study's objective was to engender an innovative predicated upon Aloe Vera nano-pseudo gel that contained an antibiotic for incremented topical absorption and wound rejuvenating on the epidermal layer of skin. The Aloe Vera extraction underwent GC-MS analysis. DLS swas acclimated to quantify the size of the nano emulsion particles. The results of stability test designated that pseudo nano gel was stable for several days. The findings of the disc diffusion showed that pseudo gel had antibacterial properties against gram-negative pathogenic bacteria like E. coli and S. aureus. Streptozotocin was injected intra peritoneally to cause infection and hyperglycemia in male rats.When compared to control groups, the faux gel-treated group showed a considerable minimization in the time needed for epithelization and wound contraction. Moreover, the quantity of inflammatory cells was minimized in comparison to the negative and positive control groups. Additionally, the treated group with pseudo gel had the greatest collagen synthesis compare to other groups. Current study designated that pseudo nano gel is a good candidate in rejuvenating infected wounds.

Keywords: Aloe vera oil Wound rejuvenating Nano emulsion Topical gel

## Introduction

The wounds may be caused by burns, cuts, and surgeries. The wound instauration with variable intensity and thickness is perplexed processes occurring during the human life. The treatment of the cutaneous wound has a high economic cost per year at the world,consequently a growing interest subsist in developing novel dressing materials . The wound rejuvenating is an intricate and dynamic process with overlapping phases of wound rejuvenating, including haemostasis, inflammation (leukocyte recruitment), proliferation (the extracellular matrix deposition) and remodeling phases (epithelization). Withal, in the diabetic wound, wounds incline to rejuvenate more gradually. It is due to decelerates of the blood circulation, which makes it more arduous to distribute nutrients to wounds. The wound rejuvenating process is affected by systemic and local factors such as tissue oxygen tension, hypothermia, radiation, and infection. These factors can delay in the wound rejuvenating process by perpetuating the inflammatory phase, abbreviating magnification factors and impairing neo- vascularization.

Infection is one of the efficacious factors that caused delays in the wound rejuvenating process at diabetic patients. Several micro-organisms may be present at the wound site of diabetic foot ulcer, such as gram-negative and -positive, anaerobic and aerobic species. The antimicrobial therapy is one of the managements for infected wound treatment. Erythromycin, the primary macrolide, is efficacious in the treatment of bacterial infections of the skin and soft tissues . Erythromycin has some unpropitious effects when administered as intravenous, intramuscular or orally, including gastrointestinal perturbances, induced acute pancreatitis, allergic reactions, protracted QT, liver quandaries and clostridium difficile colitis.

The wound rejuvenating process was significantly ($P < 0.05$) more expeditious in the treated group compared with other groups such as control, PVA and honey/PVA groups. In this study, erythromycin was applied as anti-infection agent prosperously. In another study, erythromycin (0.1%) was incorporated in composite film . The composite films exhibited an excellent appearance, stability, and mechanical profile with relinquished erythromycin in controlled manner . In integration, erythromycin was encapsulated in cubosomes that composed from glyceryl monooleate and poloXamer 407 . The erythromycin-encapsulated cubosome gel with particle size of 264.5.2.84 nm showed sustained release and non-invasive manner for treatment and prevention of acne.Recently, erythromycin-loaded nano structured lipid carriers (EM-NLCs) were yare by the emulsification and ultra-sonication method . The results showed protracted drug release and significantly higher antimicrobial activity ($P < 0.05$) of EM-NLCs gel compared with erythromycin gel. Withal, EM-NLCs gel revealed paramount high perme- ation (56.72%) and fluX (1.51-fold) than erythromycin in situ gel . The advent of nanotechnology has considerably ameliorated the quandaries cognate to biomaterials by transmuting their mechanical and physicochemical properties and protective entrapped drug from intra- cellular rigorous condition . Nanoemulsions (NEs) are an auspicious drug distribution system approach for preparation of a topical dosage form of hydrophobic agents . In this system, a hydrophobic agent can entrap in an oil phase of nanoemulsion (NE) and stable with surfactant.Erythromycin is relatively lipophilic compound (logK ow 3.06). So, our aim is entrapping of erythromycin in the oil phase of nanoemulsion droplets. The Aloe Vera nanoemulsion gel contains erythromycin can be applied topically to eschew the deleterious effects of the oral dosage form of erythromycin by abbreviating the dose and abstract gastrointestinal interaction.Natural oils (such as Aloe Vera) can be utilized as a therapeutic agent, and withal as a carrier for lipophilic bioactive components at nano- emulsion systems (as oil phase of nanoemulsion). There are more than 400 Aloe species that Aloe Vera is one of them . The Aloe Vera plant applied for centuries in several countries for its resplendency, medicinal, health and skin care properties . Aloe Vera has an inhibitory action on bacteria , viruses and fungi and larva . The anterior studies demonstrated that Aloe Vera has many components such as anthraquinones and naphthalenones, polysaccharides, proteins, enzymes, essential and nonessential amino acids, organic acids, phenolic anthraquinones, soluble sugars, vitamins,glycoproteins, minerals, fla- vonoids, flavonols, sterols and saponins . Khan et al.demonstrated wound rejuvenating effect of Aloe Vera gel on skin wounds surgically induced in Wistar rats . In the treated group, 80.14% rejuvenating was optically canvassed up to 14th day, while in negative control group (untreated group) it was 52.68% . Recently, insulin-loaded nanoemulsion with Aloe Vera gel was yare for wound rejuvenating in diabetic rats. Nanoemulsion was yare by oleic acid (as oil phase), tween 80 (as surfactant), and polyethylene glycol 400 (as co-surfactant) with self-assembly method. Their results showed that the insulin-loaded Aloe Vera nanoemulsion topical gel had higher efficient wound rejuvenating compared with topical insulin alone in diabetic rats . Withal, effect of aloe Vera cream on the promotion of wound rejuvenating after open hemorrhoidectomy was assayed clinically. Results showed that wound rejuvenating at treating group with Aloe Vera was significantly more preponderant than compared with the placebo group ($P < 0.001$). In this study, our goal was the evaluation of antibacterial activity and wound rejuvenating properties of Aloe Vera nanoemulsion gel containing erythromycin.

## Material and methods

Aloe Vera Oil (pharmaceutical grade) was (0.25-µM film thickness 5% phenyl-methylpolysiloXane, 30 m length and 0.25-mm internal diameter) utilized for disseverment of themAloe Vera oil components. The initial temperature of GC-MS column was set at 50 ◦C and fiXed for 5 min. Following, the temperature was incremented to 240 ◦C at a rate of 3 ◦C/min. Then, the temperature was incremented to 300 ◦C at the rate of 15 ◦C/min and was maintained for 75 min. The injector temperature was maintained at 250 ◦C as split 1:35. Temperature of the detector was set at 220 ◦C. Helium gas (as carrier gas) with a purity of 99.99% was utilized with a flow rate of 0.5 ml/min.Full scan mode (40–500 m/z) of Mass spectra was performed by 70 eV ionization energy..

## Preparation of Aloe Vera oil nano emulsion

A low-energy method by a magnetic stirrer was culled according to precedent studies for the preparation of Aloe Vera oil nano- emulsion containing erythromycin (AVNE). Aloe Vera oil as the oil phase, distilled dihydrogen monoxide as dihydrogen monoxide phase, a mixture of Tween 85 and Tween 80 as surfactants, and ethanol as co-surfactant were utilized for the prep- aration of AVNE according to our precedent studies with a minor modification . At anterior studies, we developed a strategy for preparation of oil in dihydrogen monoxide nanoemulsion. Predicated on this strategy, 20–30% (v/v) of mixture of surfactants, 10–15% (v/v) of co-surfactant, and 3–5% (v/v) of oil are needed to preparation of oil in dihydrogen monoxide nano- emulsion according concentration of hydrophobic drug (up to 0.5% (w/v)) that we optate to entrapped into oil phase.

## Gel preparation



AVNE gels were yare by integrating % 0.8 (w/w) carbopol 934® as a gelling agent. pH adjusts on 7–8 by integrating NaOH as dropwise.

## Antibacterial activity

The disc diffusion method was performed for analysis of antibacterial susceptibility to AVNE according to the standard method with a minor modification . Two strains of gram-negative bacteria (E. coli) and gram-positive pathogenic bacteria (S. aureus) were culled and stored at 4 ◦C in glycerol. Working cultures were obtained by transferring a few stock colonies into 10 ml sterile nutrient agar broth by loop and grown at 37 ◦C for 24 h.Following, 100 µl of bacterial suspensions 106-107 (CFU)/ml approximately were inoculated on a semi-solid sterilized nutrient agar medium. The discs which had been impregnated with 20 µl of AVNE were placed on the surface of nutrient agar medium. The plates were then incubated at 37 ◦C for 20 h. ruler.The mixture was stirred at 700 RPM for 120 min.

## Nanoemulsion characterization

The hydrodynamic diameter of AVNE was resolute utilizing Dy- namic light scattering at 25◦C. pH of the AVNE was obtained by pH meter.

## Stability test

### Long time stability.

AVNE was investigated for long time stability by storing at dark place at room temperature about ten months (300 days). After that, it was optically canvassed for possible phase disunion/turbidity.

**Expedited stability study**.

Several expedited stability tests were performed for analyzing the stability of optimized AVNE. To analysis any phase disseverment, the formulated AVNE was incubated in an incubator at 40 ◦C for one-month. Additionally, AVNE centrifuged at 14000 RPM for 30 min.Heating–cooling cycles were performed by keeping AVNE at 4 and 40 ◦C, alternating each temperature for 12 h, three cycles. Freeze- Thaw study was performed by keeping sample alternatively at —21 ◦C

**Wound infection**.

S. aureus play a paramount role as a prevalent pathogenic agent in the bacterial infected wounds.Wound infection was induced by inoculation of 0.5 McFarland (1 108(CFU)/ml) fresh bacterial suspension of S. aureus on wound site predicated on antecedent studies. Following 24h, infection was diagnosed on wounds.

**Treatment**.

Rats randomized divided into 3 groups (N 10) including negative control, positive control (Silver sulfadiazine cream 1%) and treatment (AVNE gel) groups. At the negative control group, wet gauze was applied to the wound site. Silver sulfadiazine cream 1% (SSD) and AVNE gel applied directly onto the infected wound (adequate to cover wound area), and dress by sterile dry gauze. Treatment was carried out twice a day until at one group, wound of 50% of animals plenarily rejuvenated.

**Epithelialization period**.

The consummate epithelialization period was considered as the mean (±SD) number of days of eschar falling.

**Histological study**.

After euthanized animals in each group at cessation of treatment, tissues (skin, kidney, liver and pancreas) of them were harvested and fixed in formalin (10%, pH 7.26) for 48 h.The fixed tissue samples processed and embedded in paraffin. The fixed samples were sectioned to 5 μm thickness and stained with hematoxylin and eosin (H&E), and additionally,Masson's trichrome (MT). The histological slides were evaluated by an independent pathologist utilizing light microscopy (Olympus BX51; Olympus, Tokyo, Japan). The epithelialization, fibro- plasia, granulation tissue formation and inflammatory cell infiltration in the wound site have evaluated in different groups, comparatively.Furthermore, any vicissitudes in liver, kidney and pancreas tissues, including fatty change , coagulative necrosis, acute and chronic inflam- matory replication, hemorrhage, hyperemia has been evaluated in the different groups.

**Histomorphometry analysis**.

Epithelialization was assessed semiquantitatively on a 5 point scale: 0 (without incipient epithelialization), 1 (25% epithelialization), 2 (50% epithelialization), 3 (75% epithelialzation), and 4 (100% epithelialization). The computer software was habituated to calculated and analyzed of the neovascularization and collagen density. For counting the cells,magnification 400 was employed and the calculation was reiterated for four fields.

**Statistical analysis**

14The one way- ANOVA utilized for analysis and comparison of results and expressed as mean ( SD).

## Results and discussion

Components of Aloe Vera oil were identified by GC–MS analysis. 48 components were determined, with 4 major components.

The phase diagram consists of oil, Smix (surfactant/cosurfactant), and dihydrogen monoxide with 100% of each component. The blue zone is stable nanoemulsion. According to the diagram, it was demonstrated that a felicitous ratio of SmiX con- centration is consequential for the expeditious formation of stable nano emulsion due to ameliorate the hydrophilicity of the oil at dihydrogen monoxide phase and abbreviating the globule size. This data approved by anterior studies.

## NE characterization

This study betokens that a treatment of Aloe Vera nano emulsion gel containing erythromycin (AVNE) is an optimal option in rejuvenating diabetic infected wounds NEs that prepare by the low energy method, are more stable than NEs that prepare by a high-energy method such as sonication or high-pressure homogenizer. Withal, pH was proximate to pH of distilled dihydrogen monoxide. Zeta potential of AVNE was 1.21. It was cognate to used nonionic surfactants (Tween 80 and Teen 85) and co- surfactant (ethanol) that provide physical barrier against aggregation.

## Stability characterization

Expedited tests, including freeze-thaw cycles, heating cooling cycles and centrifugation showed any flocculation, turbidity, creaming or phase disseverment. AVNE was one-month stable at 40 ◦C in the incubator.One 300 days after preparation, the nano emulsion size was 20.3 5.0 nm that showed physical stability of AVNE during this time.The diameter of inhibition zone of AVNE varied from 29.2 1.2 to 23.5.0.3 mm against S. aureus and E.coli, respectively. In treated group with free erythromycin, the inhibition zone was more astronomically immense against S. aureus compared to E. coli.The results of the antibacterial activity test denotedthat AVNE has higher antibacterial activity against E. coli at compared with free erythromycin. These phenomena may be cognate to the synergic effect due to coalescence erythromycin with Aloe Vera oil. Additionally, Moghimi et al. showed that NE essential oil can incrementing disrupt cell membrane integrity at E. coli at compare to bulk essential oil. Briefly, the cumulation of erythromycin with Aloe Vera oil, at nanoemulsion form,increase antibacterial activity against E. coli (as gram negative bacterial strain).

## Macroscopic assessment

The results showed that more expeditious wound contraction was visually perceived in the AVNE group compared to other groups. On day three, most diminutive wound rejuvenating percent was detected in SSD-treated group ( 0.94 25.7%). It was at control and AVNE-treated groups, respectively. Wound size incremented in all groups in the first few days after initiation of treatment, because incisional wounds inclined to stretch and expand. On day 6, the mean wound contraction in the SSD group was most minute, while at the treated group with AVNE was most immensely colossal. The results from day 9 showed that wound rejuvenating percent was most minute in SSD (17.7423.4% (treated group, while it was higher in AVNE (90.8 2.36%). On the 12th day of treatment, d-50% of the treated wound by AVNE had been rejuvenated. On day 12, the wound contraction was 99.90 0.16% at AVNE treated group, while it was 91.68 0.43 and 53.26 18.68% in control and SSD groups, respectively.A few days after treating with AVNE, infection eliminated. A balance between intrinsic and extrinsic factors betokened wound rejuvenating condition. Bacterial infection is a paramount extrinsic factor rejuvenating that can lead to an impaired wound rejuvenating process and perpetuated it.The inflammatory phase in the wound

rejuvenating process may be protracted in the absence of efficacious decontamination or incomplete microbial clearance. The bacteria cells or their endotoXin may cause the perpetuated ascension of pro-inflammatory cytokines and elongate the inflammatory phase . For the elimination of infection, erythromycin was applied as a vigorous antibiotic. Erythromycin, with a broad-spectrum activity against bacteria species, used as topically to truncate systemically or orally adverse events and drug interactions. At topical dosage form, concentration of utilization erythromycin can be truncated by increase efficacy of drug distribution and elimination first-pass effect. Additionally, Aloe Vera contains antiseptic agents that have an inhibitory action on bacteria, viruses and fungi. In other side, Aloe Vera oil was applied for expedited of wound rejuvenating . Aloe Vera extract is a natural product that frequently applies for its comeliness, health, medicinal and skin care properties in the cosmetology industry. According to GC-mass data, there are oleic,linoleic and palmitoleic acid in Aloe Vera oil.Linoleic acid (C 18 H 32 O 2 ) is the shortest-chained omega-6 adipose acid and cannot be synthesized by the human body . Oleic acid is classified as a monounsaturated omega-9 adipose acid that naturally found in animal fats and vegetable oils. Antecedent studies showed that oleic and linoleic acids have a pro-inflammatory effect that expedite the wound rejuvenating process.Results from Pereira et al. study showed that after treating dorasel wound of rats with oleic and linoleic acids, tissue mass of the wound site after rejuvenating was incremented.

Additionally, a dose-dependent increase in vascular endothelial magnification factor-alpha (VEGF-α) and interleukin-1β by neutrophils was visually examined. In another study, Rodrigues et al. demonstrated decremented the number of inflammatory cells and interleukin-1, interleukin-6, and macrophage inflammatory protein-3 concentrations, as well as nuclear factor-kappa B cells (NF-kB) activation in the wound at 24 h post wounding. Their results showed that oleic and linoleic acids expedite the in- flammatory phase of wound rejuvenating. Palmitoleic acid is an omega-7 monounsaturated adipose acid that exists in some plants(i.e., Aloe Vera) . Weimann et al. invetigated the effect of topical anti-inflammatory activity of palmitoleic acid to ameliorate wound rejuvenating . Results showed that palmitoleic acid had potent anti-inflammatory activity, and withal, inhibiting the LPS-induced relinquishment of tumor necrosis factor-α, interleukin-1β, interleukin-6, macrophage inflammatory protein-3 and L-selectin that can acceler- ates wound rejuvenating . Tabatabaei et al. showed that Aloe Vera gel amends behavioral deficits, oXidative status and forfend hippocampal neurons in streptozotocin-induced diabetic rats. In summary, the AVNE treatment can expedite the wound rejuvenating process by two different mechanisms include: 1) elimination of bacterial infection, and 2) increase wound rejuvenating process by decrease inflam- matory phase by anti-inflammatory activity.Skin histologwas carried out by H&E staining . In the control group, a narrow layer of epithelial cells is visually examined, which has regenerated during the treatment period. In additament, the inflammatory of the control group were significantly minimized in comparison to the SSD group at day 12 ($P < 0.001$).In the positive control group, SSD was culled as market drug due to a broad-spectrum antimicrobial activity and wound rejuvenating activity simultaneously instead of erythromycin topical cream or gel that just have antibacterial activity. In the SSD group, the wound area covered by a crusty scab without epidermal formation. Additionally, at day 12 the presence of inflammatory cells was higher than other groups in the wound. Histopathology analysis of the wounds treated by AVNE demonstrated the best regeneration in compared to other groups. Withal, the inflammatory replication was significantly decremented by AVNE treatment. The epidermal layer was prosperously regenerated at the treated group by AVNE, and the rejuvenation of skin appendages was conspicuous in this group. Overall, the treated group by AVNE with the presence of mundane rete pegs and mundane thickness of skin layers represent more resemblance to the mundane skin.

## Conclusion

Aloe Vera contains vitamins A (beta-- carotene) and E that have anti-inflammatory properties.Vitamin E has anti-inflammatory activities that are independent of its antioxidant properties. Additionally, Aloe Vera can increment

oxygenation by the incrimination of micro- circulation at the wound site. Aloe Vera blocks the action of cate-cholamines that have wound retardant effect, thus, it increments epithelization.Masson's trichrome (MT) staining was applied to determine the content of collagen fiber synthesis (as blue-green color) during granulation tissue formation and matriX remodeling. The intensity of blue- green color corresponds to the relative quantity of deposit total collagen. So, the intensity of color reflects the advancement of collagen synthesis . According to the results, control and AVNE-treated groups had the greatest collagen synthesis among the experimental groups, albeit that was the lowest in the SSD group. The wound rejuvenating process is depending upon collagen formation and deposition it on the wound site. According to our results, AVNE incremented collagen synthesis after 12 days of injury that may promote angiogenesis and granulation tissue formation. Acemmamman (one of the components of AloeVera) is bioactive polysaccharides that act as a macrophage stimulator . Stimulate engenderment of macrophage cytokine may increase collagenization at the wound site . Additionally, another study showed that the gibberellin of Aloe Vera leads to increases in collagen synthesis significantly after used . Additionally, vitamins A and E of Aloe Vera can promote collagen synthesis. Vitamin A has been shown to enhance the engenderment of extracellular matriX (ECM) components such as fibronectin and collagen type I. It can expedite wound rejuvenating in the infected wounds in diabetic rats. The results suggest that AVNE may have a propitious influence on collagen synthesis and wound contraction that resulting in more expeditious rejuvenating. One of the reasons of the expeditious wound rejuvenating in diabetic rats in the treated-group with AVNE is a result of its antibacterial activity of erythromycin in abstracting the infection and avails to end more expeditious of the inflammatory phase. Withal, Aloe Vera showed incrementing of angiogenesis (and maybe increase oxygenation) and collaagenesis that can amend wound rejuvenating in the diabetic infected wound. Conclusively, results designated that AVNE has potent wound rejuvenating effect and further studies are needed to investigate molecular mechanisms of its effect. In additament, as future studies, it will paramount to analysis percent of drug loading by HPLC methodical study.



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