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Comparison between the Nano-Silica Extracted from Date Palm Ash and Silicone Gel to Treat the Induced Second Degree Burn in Albino Rats Model

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Abstract

This study explores the healing effects of nanosilica extracted from date palm on second-degree burns in white rats, comparing its efficacy with that of silicone gel, a commonly used treatment for burn injuries, and a control group that received no treatment. A total of 30 white rats were randomly divided into three groups, with each group consisting of 10 animals. The first group was treated with a 5% nanosilica, the second group received a topical application of silicone gel (Scarmed®), and the third group served as a control and received no treatment. All treatments were applied once daily for 7 days.

The healing process was evaluated macroscopically and microscopically at 7, 14, and 21days post-treatment. The nanosilica-treated group showed accelerated wound healing, faster re-epithelialization, reduced inflammation, and minimal scarring compared to both the silicone gel-treated and control groups. The silicone gel-treated group showed moderate improvement in wound healing but lagged behind the nanosilica group, while the control group exhibited the slowest healing, with prolonged inflammation, less organized collagen deposition, and noticeable scarring.

Histological examinations revealed enhanced collagen deposition, fibroblast activity, and tissue regeneration in the nanosilica-treated wounds, which were significantly better than those observed in the other two groups. These findings indicate that nanosilica, with its biocompatibility and regenerative properties, holds great promise as a natural, effective treatment for burn injuries, offering a viable alternative to traditional silicone-based treatments.

Keywords: *Nanosilica, silicone gel, Nanoparticles, Healing, Burn, Cutaneous*

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Manufacture new therapy for burns by extraction of Nano silica from date palm. Compare the efficacy of Nano silica extracted from date palm and synthetic silicone gel on 2nd degree cutaneous burns

Introduction:

Burn injuries, particularly second-degree burns, are a common type of skin damage that affects both the epidermis and dermis, resulting in pain, blistering, and inflammation(Jeschke et al., 2020). These burns often require prompt and effective treatment to promote healing and prevent complications such as infection and scarring(Rowan et al., 2015). Traditional treatments, such as silicone-based gels, have been widely used due to their ability to provide a protective barrier and facilitate healing. However, there is an increasing interest in exploring alternative therapies with natural properties that may offer enhanced healing benefits(Hussein & Towfik, 2023).

Nanosilica, a nanoparticle with unique physicochemical characteristics, has gained attention for its potential in wound healing due to its large surface area, high reactivity, and ability to stimulate collagen production and cellular regeneration(Kadhim, 2023; Jeon et al., 2024). Derived from natural sources like date palm, nanosilica offers a biocompatible and cost-effective alternative for burn treatment(Isopencu et al., 2023). The sol-gel method, a chemical process used to synthesize nanosilica, allows for the production of high-purity nanoparticles with desirable properties for biomedical applications(Elanthikkal et al., 2023)

Aims of the study

Materials and Methods

Ethical Approval

The study was conducted in accordance with ethical guidelines for animal research, and the experimental protocol was approved by the Scientific Committee of the College of Veterinary Medicine in the University of Al-Qadisiyah, Iraq. All efforts were made to minimize animal suffering and ensure humane handling throughout the experiment.

Study Animals

A total of 30 female white albino rats, aged 8–10 weeks and weighing between 200–250 grams, were used in this study. The rats were obtained from a certified supplier and acclimatized to the laboratory environment for 7 days prior to the experiment. They were housed in standard cages under controlled conditions, maintaining a temperature range of 22–24°C, with free access to food and water. A second-degree burn was induced on the dorsal side of each rat using a solid aluminum bar (1 cm in diameter) that had been immersed in boiling water (100°C) for 15 seconds and then placed on the skin without applying pressure. The animals were randomly divided into three groups of 10 rats each. The first group received treatment with 5% nanosilica, the second group was treated with silicone gel (Scarmed® Sana Pharma), and the third group served as a control group, receiving

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no treatment. All procedures were conducted under the supervision of a trained veterinary professional.

Preparation of Nanosilica from Date Palm

Nanosilica was extracted from Date Palm Biomass Ash (DPBA) using the chemical sol–gel method. This multi-step process was adapted from previously reported protocols(Cui et al., 2015)(Liang et al., 2020) to ensure the removal of impurities and the production of high-purity silica nanoparticles.

Initially, the DPBA was treated with 1.5 M HCl at 85°C for 3 hours. This step aimed to remove metallic impurities present in the ash. After acid treatment, the DPBA was filtered, and the black residue obtained was repeatedly washed with hot deionized water to ensure the removal of residual acid and impurities. The washed residue was then dried, resulting in acid-leached DPBA.The acid-leached DPBA was treated with 2 M NaOH solution at 90°C under continuous stirring for 2 hours. During this process, silica in the ash reacted with sodium hydroxide to form soluble sodium silicate. The soluble sodium silicate solution was then subjected to a precipitation process by the dropwise addition of 2 M HCl. The pH of the solution was carefully monitored, and precipitation began below pH 10. The addition of HCl continued until the solution reached a nearly neutral pH, ensuring complete precipitation of silica. The resulting white silica suspension was allowed to settle overnight to ensure complete precipitation. The suspension was then centrifuged, and the clear supernatant was discarded. The silica gel obtained was washed and centrifuged multiple times (four cycles) to remove any remaining impurities. The purified silica gel was transferred to a petri dish and dried in an oven at 70°C for 24 hours. Finally, the dried silica gel was finely ground using a mortar and pestle to produce a uniform white powder. This biosilica powder, was stored in a glass container for subsequent use(Elanthikkal et al., 2023).

Characterization of Nanoparticles

Before using the nanosilica in the experiment, the particle size and other characteristics of the biosilica were thoroughly analyzed to ensure its suitability for the study.

UV-Vis Spectroscopy: This technique was used to determine the optical properties of the biosilica nanoparticles (Biradar et al., 2021).

X-ray Diffraction (XRD): XRD was The X-ray diffraction pattern of silica nanoparticles shows an intense wide band around 2 θ = 22 degrees, which is the hallmark of their amorphous nature. (Elanthikkal et al., 2023) .

Fourier Transform Infrared Spectroscopy (FTIR): FTIR analyze of different nano silica samples showed as in Fig. (3) These shows absorption bands arising from asymmetric vibration (Si–O and Si–OH) are 1105 cm–1, 965 cm–1 and symmetric vibration (Si–O) at 800 cm–1 respectively. The absorption bands between 800 and 1260 cm-1 have been described as a superimposition of various SiO2 peaks due to residual organic groups

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which corresponds to(Elanthikkal et al., 2023; Imoisili & Jen, 2024) .

Scanning Electron Microscopy (SEM): SEM was utilized to examine the surface morphology and particle size of the nanosilica, the sizes of the nanoparticles were different with an average diameter of 46 Nm(Elanthikkal et al., 2023; Il et al., 2015).

Histopathological Study

Tissue samples from the burn sites were collected on days 7, 14, and 21 post-treatments. Samples were fixed in 10% formalin, embedded in paraffin, and sectioned at 5 µm thickness. Sections were stained with Hematoxylin and Eosin (H&E) to assess tissue morphology(Samaka et al., 2021).

Statistical Analysis

The significance between groups was determined using statistical analysis of the morphometric data. The data were subjected to one-way analysis of variance (ANOVA) to assess the overall differences between the groups at each time point (7, 14, and 21 days). To determine which specific groups differed, post-hoc comparisons were made using the Least Significant Difference (LSD) test. A p-value of ≤ 0.05 was considered statistically significant.

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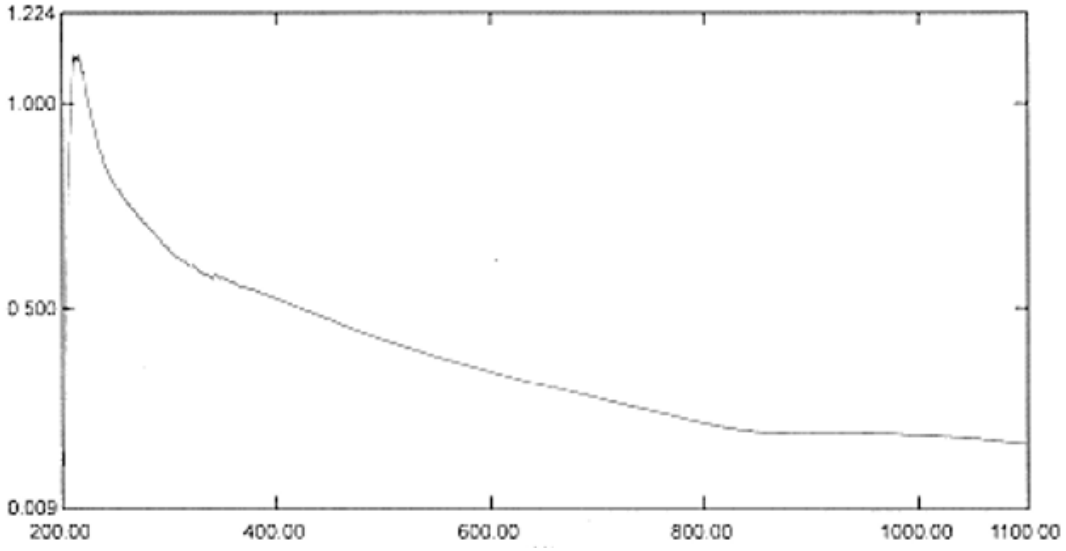

Figure 1: UV-visible absorption spectrum of silica nanoparticles

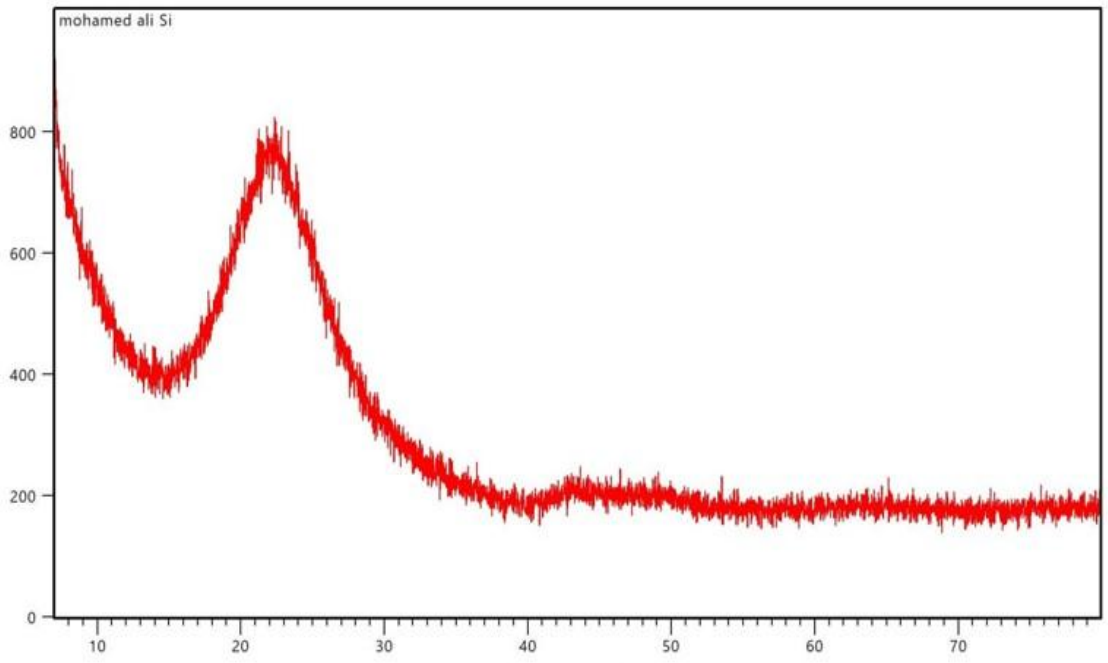

Figure2: XRD analysis of silica nanoparticles synthesized from DPBA

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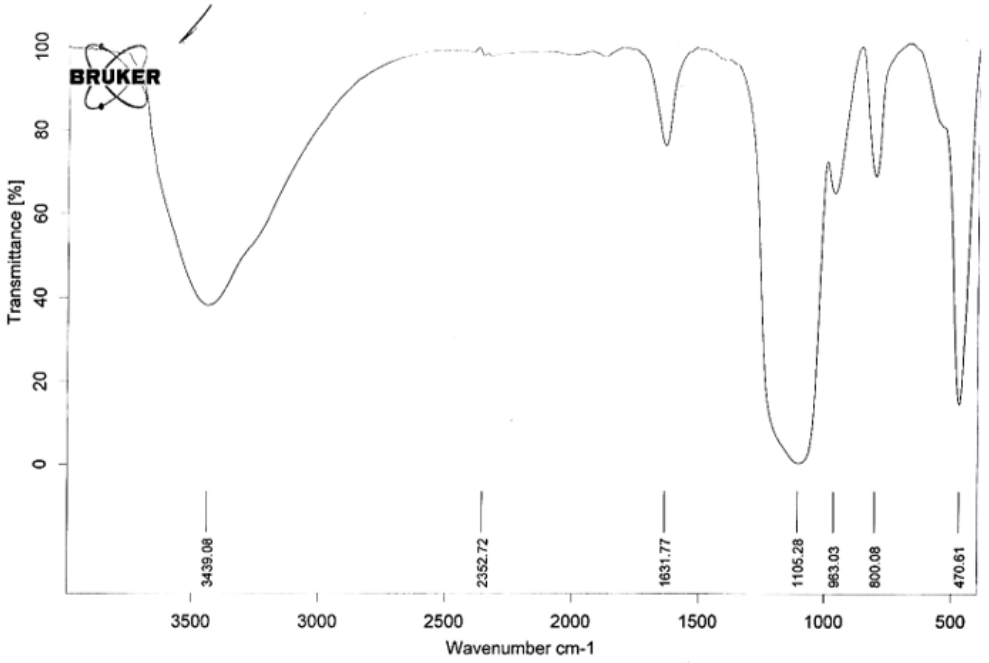

Figure 3: FT-IR spectra silica nanoparticles synthesized from DPBA.

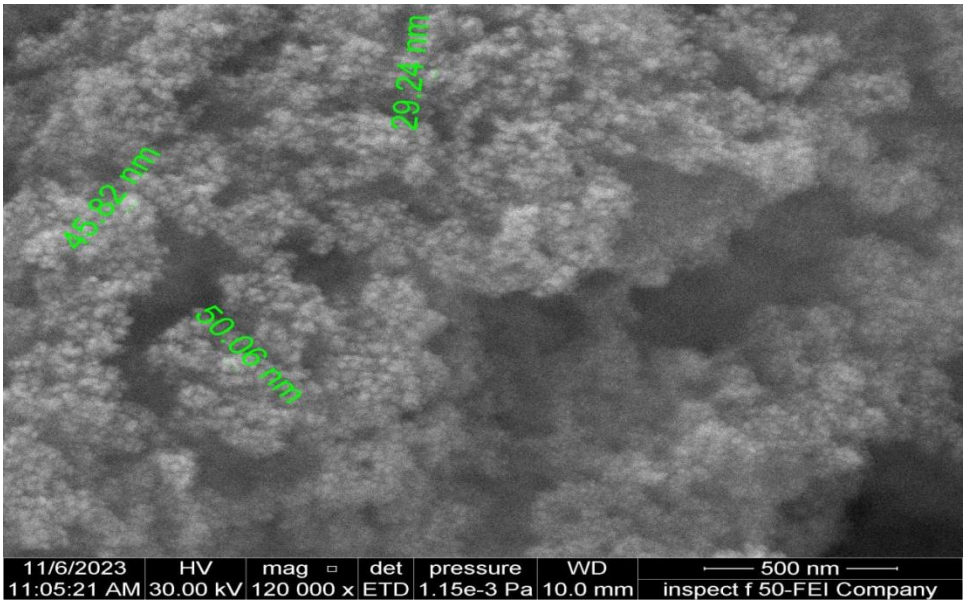

Figure 4: XRD analysis of silica nanoparticles synthesized from DPBA

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Results

The healing process of second-degree burns in rats treated with 5% nanosilica, silicone gel, and a control group (no treatment) was assessed by monitoring the surface area of the wound over three weeks. The surface area measurements, presented as mean ± standard deviation, revealed significant differences among the three groups at various time points.

On day 0 after burn, the initial surface area was consistent across all groups at 75.42 ± 0.00 mm². After first week, rats treated with 5% nanosilica showed a substantial reduction in wound surface area to 42.17 ± 0.88 mm² ($p \leq 0.05$). By second week, the surface area further decreased to 18.79 ± 0.62 mm², and by third week, it was minimal at 0.90 ± 0.12 mm².

In contrast, rats treated with silicone gel also showed wound healing, but at a slower rate. At day 7, the surface area was reduced to 55.08 ± 1.21 mm². By day 14, it decreased to 21.72 ± 0.60 mm², and by day 21, it was 5.77 ± 0.38 mm². The control group, which received no treatment. After 7 days, the surface area had reduced to 62.63 ± 1.13 mm², while at day 14, it was 30.98 ± 1.03 mm². By day 21, the wound area was 8.45 ± 0.35 mm², considerably larger than both the nanosilica and silicone gel groups.

Morphological Appearance

The morphological appearance of the burn wounds in rats treated with 5% nanosilica, silicone gel, and the control group (no treatment) was observed throughout the three-week study period. The groups displayed distinct differences in wound healing, as observed macroscopically. In the nanosilica-treated group, the wounds began to show visible signs of healing by the end of the first week, with reduced erythema (redness) and no signs of infection. By the second week, the wound edges contracted significantly, and the tissue appeared more regenerated with minimal redness. By the third week, the burn wound was almost completely healed, with minimal scarring. The skin in this group regained a healthy and s mooth appearance.

In the silicone gel-treated group, the healing process was noticeably slower. At first week, the wounds showed moderate

Table 1 Surface area (mm^2)

Periods / Groups	0 day	7 days	14 days	21 days
G1(n=10)	75.42 ± 0 Aa	42.17 ± 0.88 Db	18.79 ± 0.62 Cc	0.90 ± 0.12 Cd
G2(n=10)	75.42 ± 0 Aa	55.08 ± 1.21 Bb	21.72 ± 0.60 Bc	5.77 ± 0.38 Bd
G3(n=10)	75.42 ± 0 Aa	62.63 ± 1.13 Ab	30.98 ± 1.03 Ac	8.45 ± 0.35 Ad
LSD	2.9			

• Capital letters denote to the vertical statistical reading
• Small letters denote to the horizontal statistical reading
• Different letters denote to the significant difference, whereas similar letters refer to the no significant difference at P<0.05

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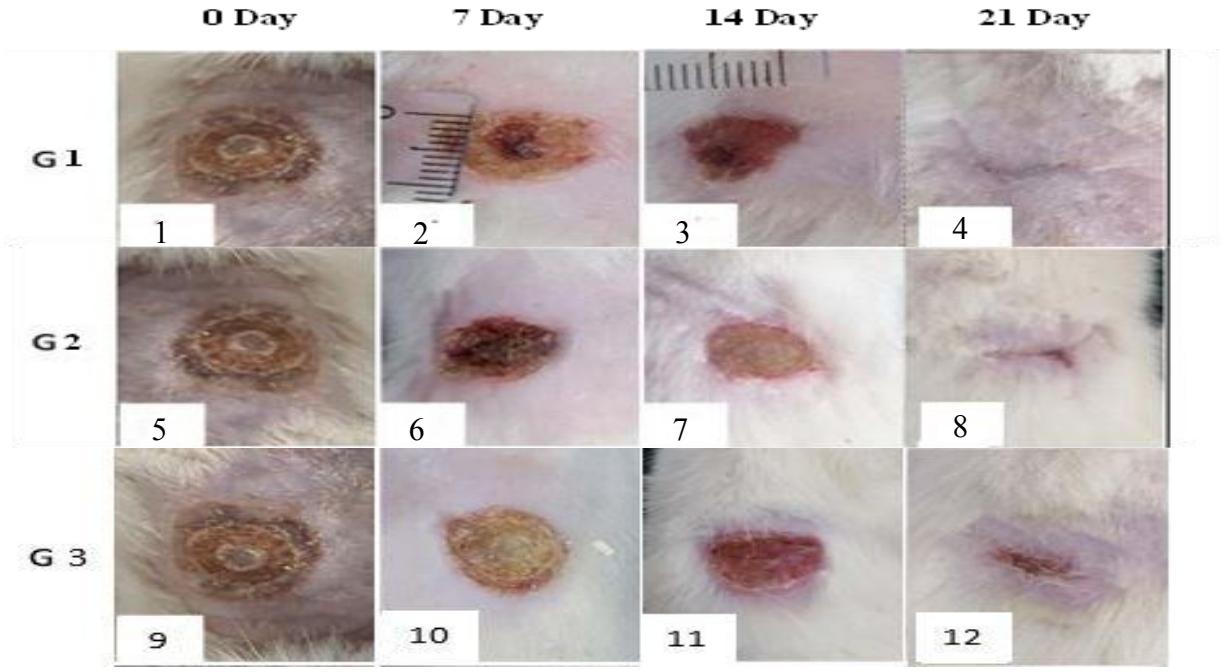

Figure 5: Morphological appearance of burn in groups at different periods

erythema, inflammation, and no significant contraction. By second week, the wound edges contracted modestly, but the healing process was accompanied by more visible redness and some scarring. By week 3, although the wounds had contracted.

In the control group, the wounds showed the slowest healing progress. At first week, the burn wounds remained inflamed with significant erythema, and there was no visible contraction. By second week, the wound edges had only slightly contracted, and the tissue still appeared red and inflamed. By third week, while some wound contraction was observed, the area remained discolored with prominent scarring and poor tissue regeneration. The control group's wounds exhibited the least favorable healing outcomes, with delayed recovery and persistent inflammation.

Histopathological Assessment of the Burn Healing

W1-G1. Note narrow scar tissue. Downward hyperplasia of epidermis with thin keratinized layer above it. In the dermis, there is infiltration of inflammatory cells profuse fibrosis with formation of new small blood vessels (Figure 6).

W2-G1. Note complete and normal epidermal layers. In the dermis, there are large blood vessels filled with blood with fibrosis and regular network of collagen fibers and formation of new hair follicles, showing a wide blood vessel filled with blood and thick network of collagen fibers with proliferation of fibroblasts (Figure 7).

W3-G1. There is thickening and normal epidermis with keratinized layer and normal dermis with profuse hair follicles with large blood vessels. Note all layers of the epidermis stratum basale, Stratum spinosum, Stratum granulosum, and the stratum corneum and dense and thick network of collagen fibers and wide blood vessels in the dermis (Figure 8).

W1-G2. epidermis shows slight thickening, while the dermis exhibits significant scarring with new blood vessel

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formation and inflammatory cell infiltration (Figure9).

W2-G2. Slightly hyperplasia and thin epidermis along the scar tissue. In the dermis, incomplete healing there are wide spaces with thin and branched collagen fibers (Figure 10).

W3-G2. Showing mild thickening of epidermis. Wide scar tissue and thin and branched collagen fibers, and formation of new blood vessels. Few proliferations or hyperplasia of stratum basale in the epidermis. Note new formation of small blood vessels with fibroblasts proliferation. Note coarse and branched network of epidermis and presence of crust and abscessation above the site of wound. Profuse of granulation tissue and inflammatory cells infiltration in the dermis. Profuse granulation tissue with small new blood vessels formation, proliferation of fibroblasts and mononuclear cells infiltration (Figure 12).

W2-G3. Histopathological changes following burn injury after second week showed mild hyperplasia of epidermis with presence of abscess and crust above the site of wound. Thick and coarse collagen fibers network and thickening of subcutaneous tissue with profuse adipose tissue in the dermis. Thick and branched collagen fibers and thickening of profuse adipose tissue in the subcutaneous. Note hyperplasia of stratum basale of epidermis with presence of abscessation and tissue debris. Branched collagen fibers, granulation tissue with infiltration of inflammatory cells were seen (Figure 13).

W3-G3. Note hyperplasia of stratum basale of epidermis with presence of keratinized layer. Incomplete healing, there collagen fibers. Newly-formed blood vessels and infiltration of mononuclear cells mainly macrophages (Figure 11).

W1-G3. Superficial thickness burns are injuries that cause partial destruction of nerve endings, hair follicles and sweat glands. On the seventh day post burn injury the histopathological changes were observed. In control group there was presence of purulent exudate above the site of wound characterized by dead neutrophils and tissue debris. Profuse of granulation tissue and inflammatory cells infiltration with congestion of blood vessels in the dermis, showing complete sloughing of
are marked spaces in the epidermis and irregular network of branched collagen fibers (Figure14) (Figure 15).

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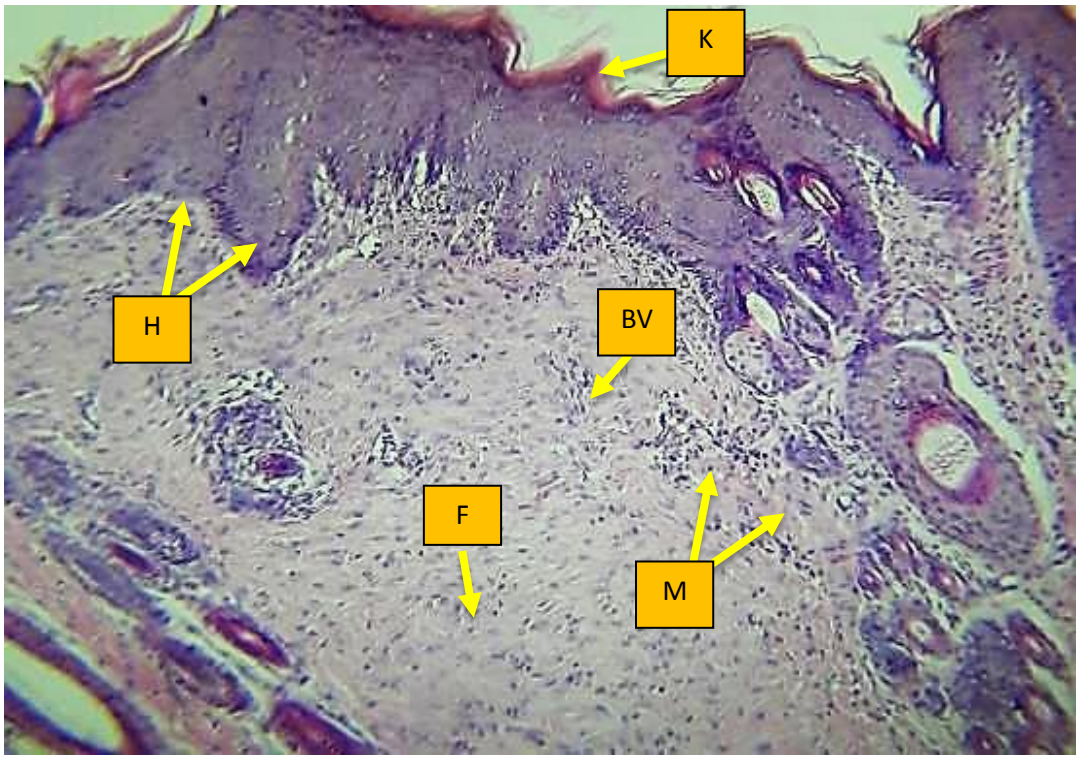

Figure 6: Skin (Nano silica 5% group 7 days). Note narrow scar tissue. Downward hyperplasia(H) of epidermis with thin keratinized layer(K) above it. In the dermis, there is infiltration of inflammatory cells(M) profuse fibrosis(F) with formation of new small blood vessels (BV). 10X H&E.

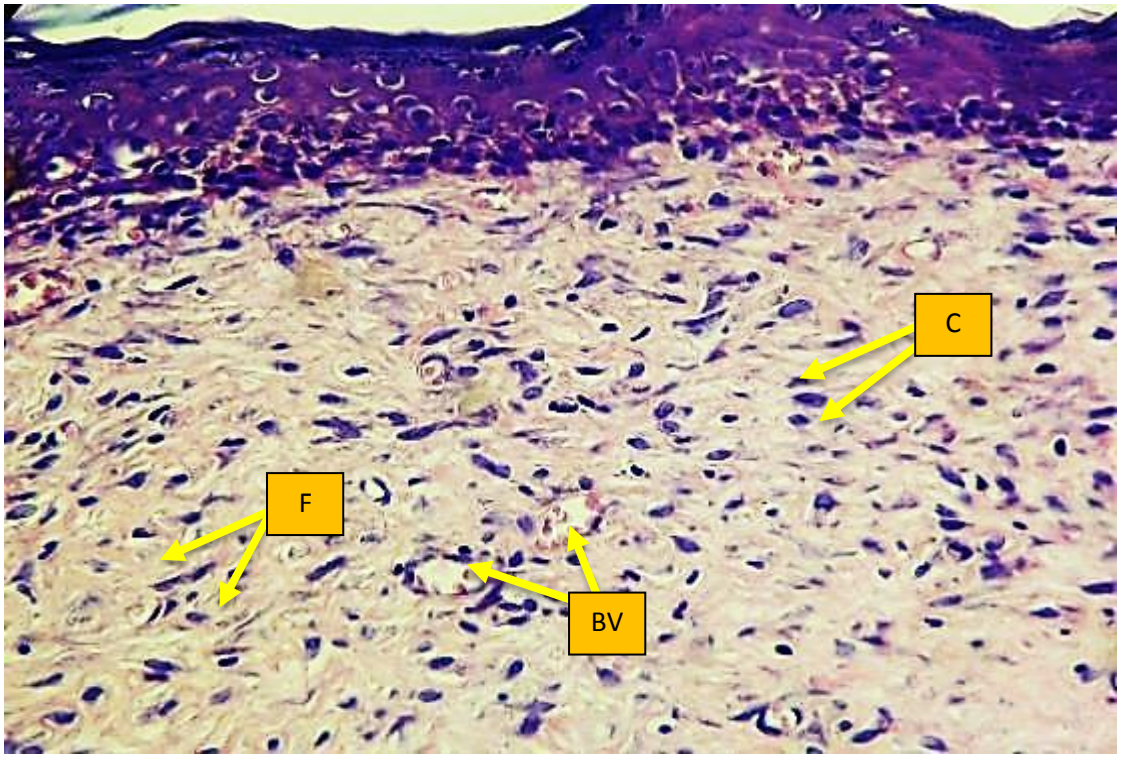

Figure 7: Skin (Nano silica 5% group 14 days). Note there are large blood vessels (BV) filled with blood with fibrosis(F) and regular network of collagen fibers(C). 10X H&E.

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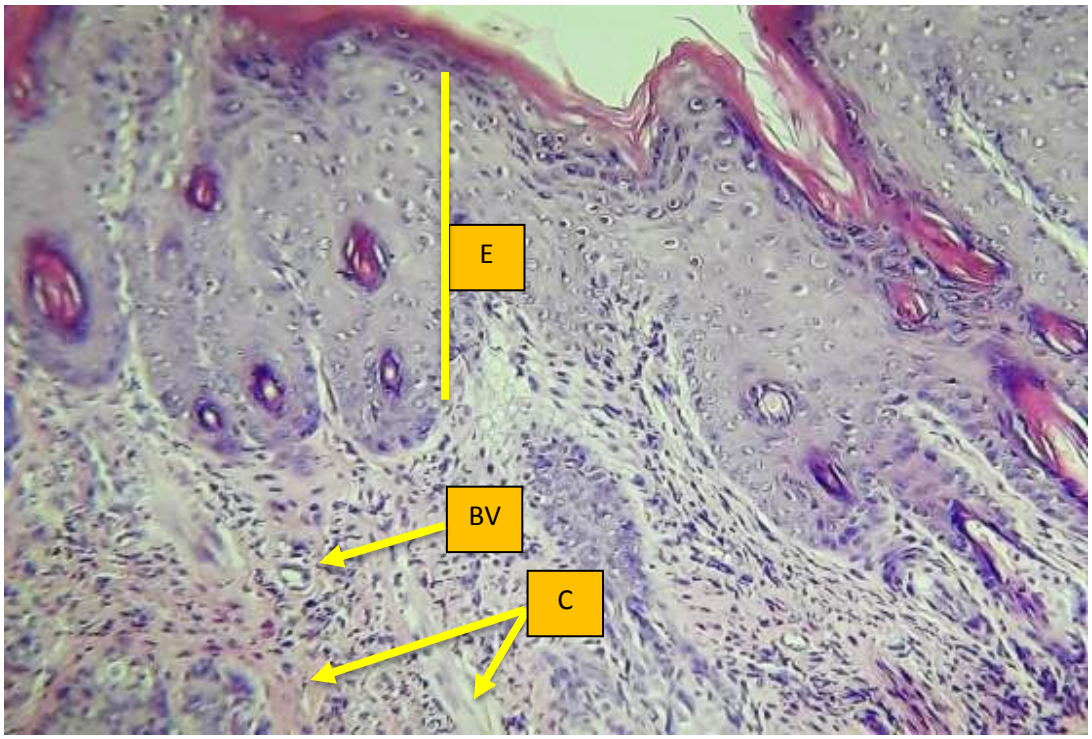

Figure 8: Skin (Nano silica 5% group 21 days). Note all layers of the epidermis(E) stratum basale, Stratum spinosum, Stratum granulosum, and the stratum corneum and dense and thick network of collagen fibers(C) and wide blood vessels (BV) in the dermis. 10X H&E.

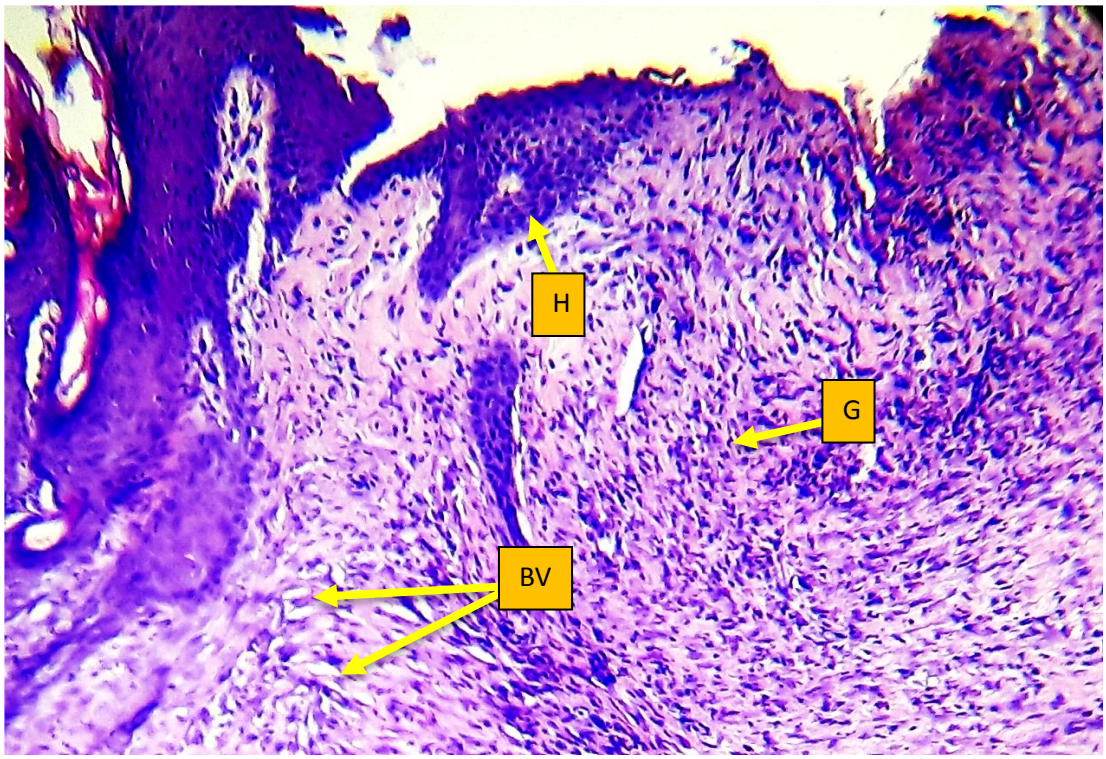

Figure 9: Skin (Silicone gel group 7 days). Slightly hyperplasia of epidermis(H). In the dermis, wide scar tissue and granulation(G) tissue in which there is profuse fibrosis and new blood vessels formation (BV). 10X H&E.

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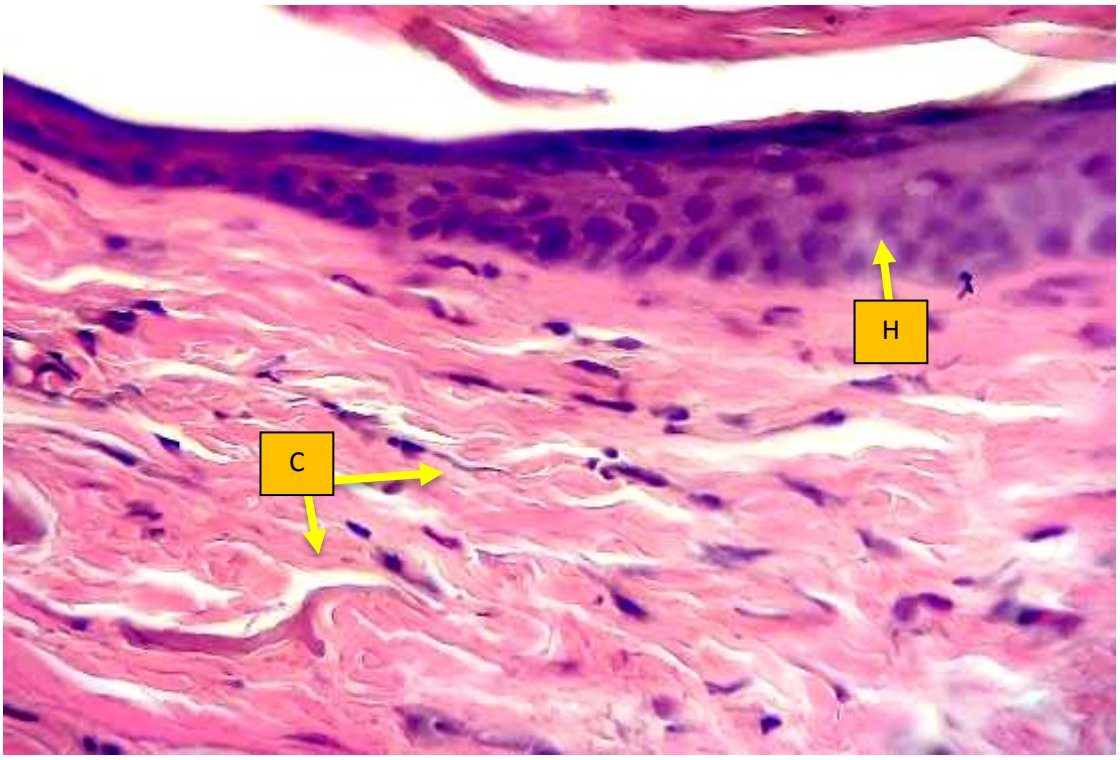

Figure 10: Skin (Silica gel group 14 days). Mild hyperplasia of the epidermis(H) with branched and irregular network of collagen fibers(C) in the dermis. 10X H&E.

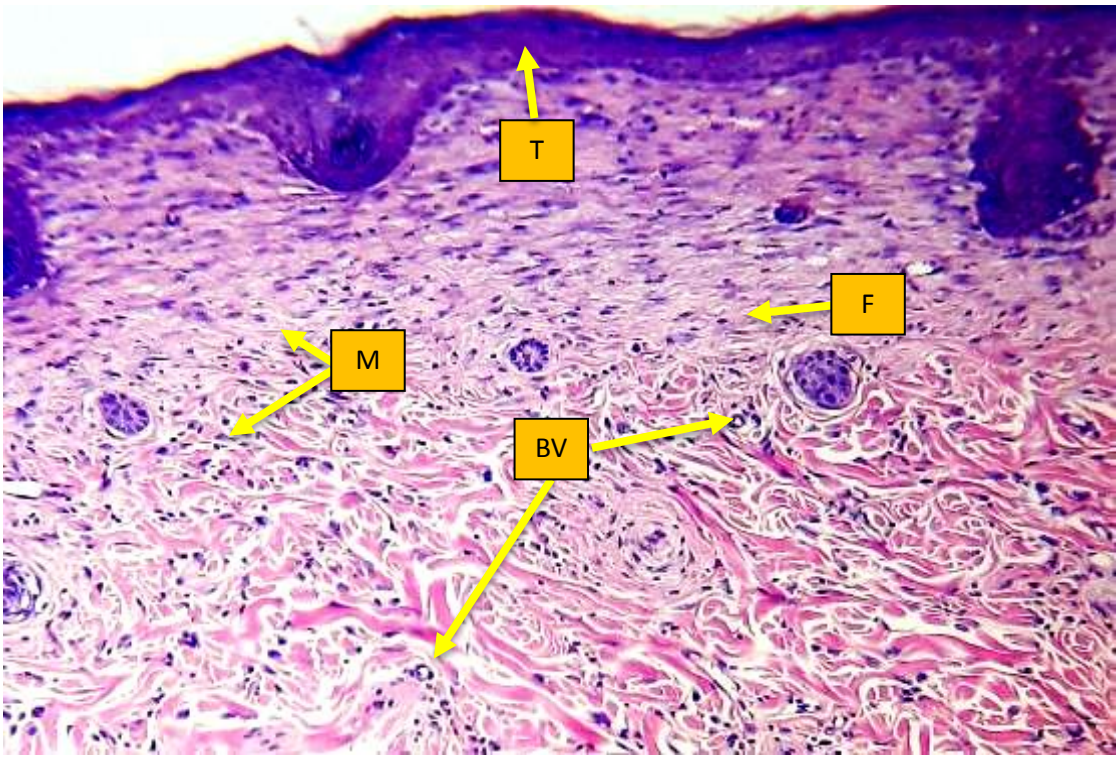

Figure 11: Skin (Silicone gel group 21 days). Slightly thickening(T) of epidermis. Newly-formed blood vessels (BV) and profuse fibrosis(F) with infiltration of mononuclear cells(M). 10X H&E.

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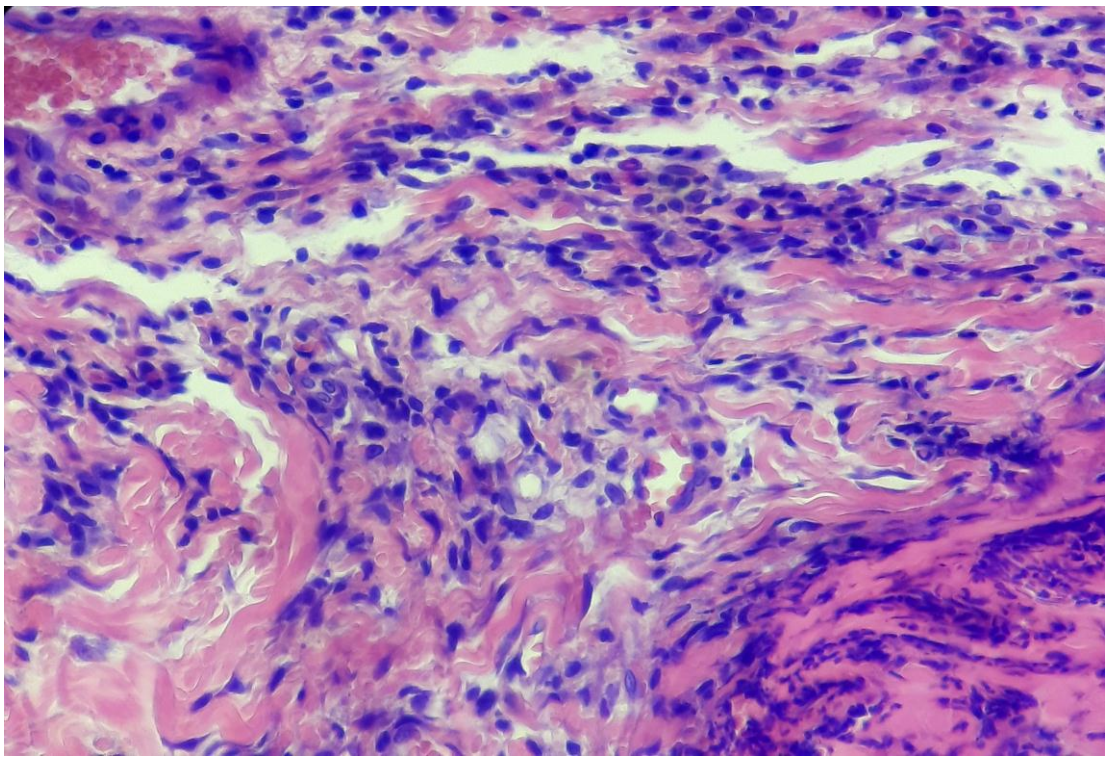

Figure 12: Skin (control group 7 days): Thick abscessation. Profuse granulation tissue with small new blood vessels (BV) formation, proliferation of fibroblasts(F) and mononuclear cells infiltration(M). 10X H&E.

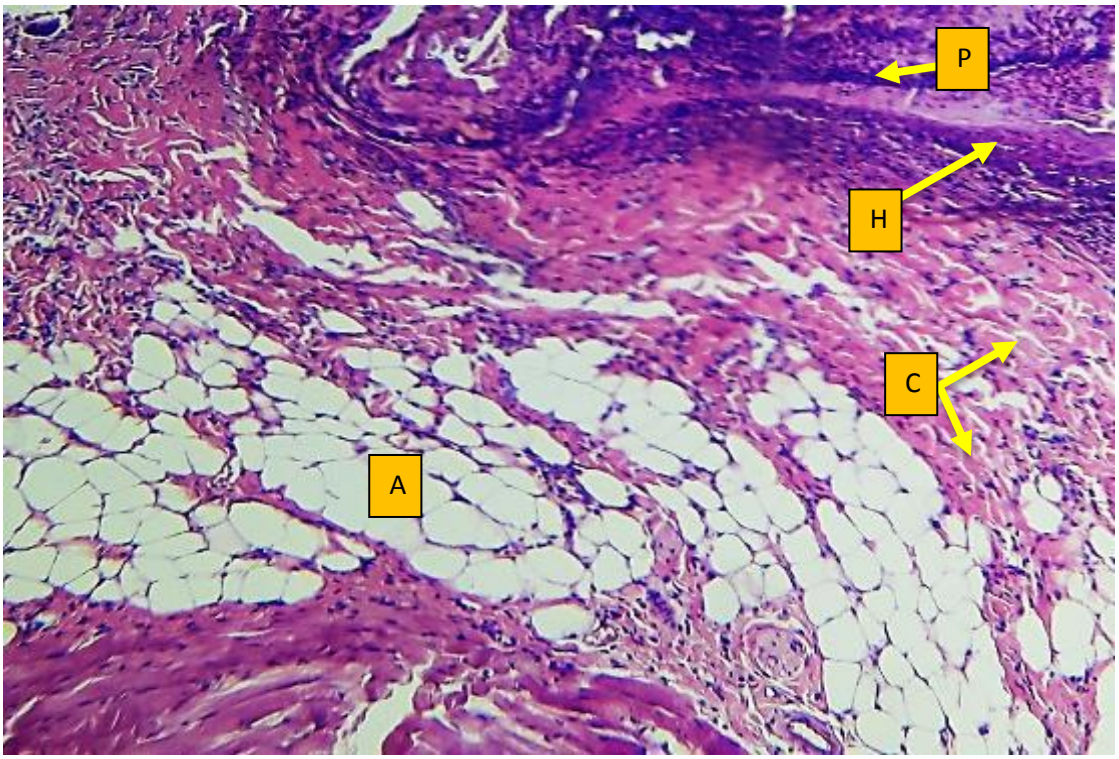

Figure 13: Skin (control group 14 days): Showing mild hyperplasia(H) of epidermis with presence of abscess(P) and crust above the site of wound. Thick and coarse collagen fibers(C) network and thickening of subcutaneous tissue with profuse adipose tissue (A) in the dermis. 4X H&E.

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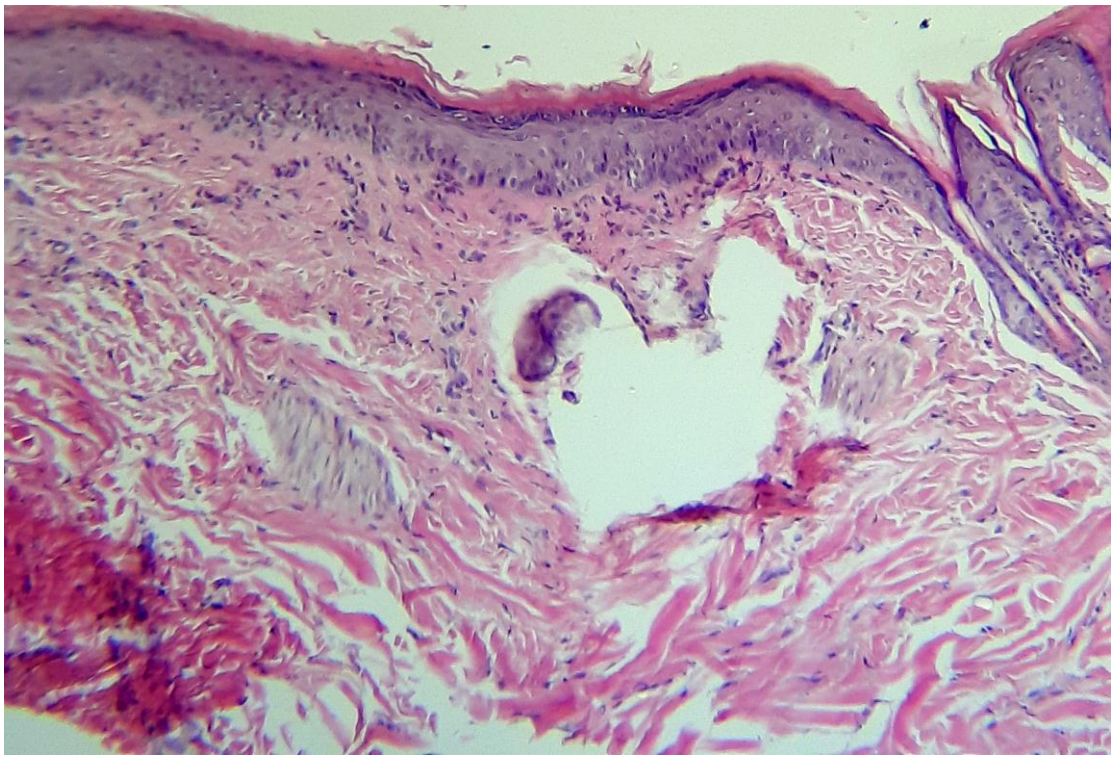

gure 14: Skin (control group 21 days): Note hyperplasia(H) of stratum basale of epidermis with presence of keratinized layer(K). Incomplete healing, there are marked spaces (SP) in the epidermis and irregular network of branched collagen(C) fibers. 10X H&E.

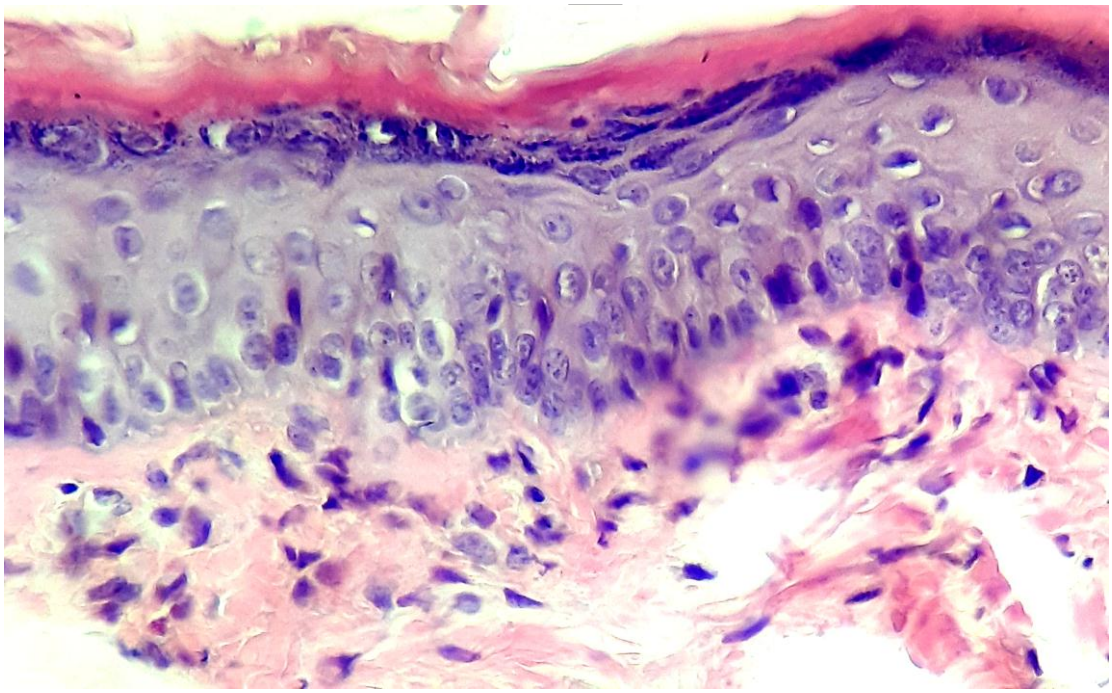

Figure 15: Skin (control group 21 days): Higher magnification of the previous tissue. Note hyperplasia(H) of stratum basale of epidermis with presence of keratinized layer(K) with branched collagen fibers(C). 40X H&E.

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Discussion

The findings of this study demonstrate that 5% nanosilica extracted from date palms significantly enhances the healing of second-degree burns compared to silicone gel. The superior results of nanosilica treatment align with previous research highlighting the advantages of nanomaterials in wound healing due to their unique physicochemical properties.

The observed rapid wound contraction and tissue regeneration in the nanosilica-treated group can be attributed to the nanoparticles' small size, large surface area, and bioactive surface functionalities(Sangnim et al., 2024; Farman & Abood, 2024). These properties enhance cell adhesion, proliferation, and migration, which are critical for tissue repair. Similar findings have been reported by(Pinho et al., 2022), who noted that silica nanoparticles promote fibroblast proliferation and collagen synthesis, facilitating wound healing. Additionally(Paula et al., 2016; Quignard et al., 2017) found that biosilica derived from natural sources exhibited potent antimicrobial and anti-inflammatory properties, further supporting its role in improving wound healing outcomes.

The morphometric data in this study showed a significant reduction in wound surface area in the nanosilica group compared to the silicone gel group. By the third week, the nanosilica-treated wounds achieved near-complete closure, a finding consistent with(Article, 2004; Jeon et al., 2024) , who reported accelerated re-epithelialization and reduced scarring in burns treated with silica-based nanomaterials. Furthermore, the organized collagen deposition observed in nanosilica-treated wounds aligns with the results of (Grotheer et al., 2013), who emphasized the role of nanosilica in enhancing extracellular matrix organization.

In contrast, the silicone gel group demonstrated slower healing, with noticeable scarring and less organized collagen deposition. While silicone gel has been widely acknowledged for its effectiveness in reducing hypertrophic scars, its performance in promoting early-stage wound healing appears limited. These findings are in agreement with(Naskar & Kim, 2020), who highlighted that traditional treatments like silicone gel are often less effective than advanced nanomaterial-based therapies in addressing complex wounds.

In conclusion, this study agrees with prior research in demonstrating that nanosilica accelerates wound healing through enhanced tissue regeneration and reduced inflammation. The nanosilica derived from date palms shows particular promise as a biocompatible and eco-friendly alternative for burn therapy. Future studies should focus on clinical trials and explore the broader therapeutic applications of biosilica in regenerative medicine.

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