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Possible Impact of Jojoba Nano-Emulsion and/or Vitamin-D on Experimentally- Induced Cutaneous Lesion in Adult Male Albino Rats

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ABSTRACT

Background: The body's greatest organ is the epidermis. This is the initial defense against the external environment.

Objective: This study aimed to investigate the possible effect of jojoba nano-emulsion with or without vitamin D on experimentally-induced cutaneous lesion in adult male albino rats.

Materials and methods: A strain of 28 adult male albino rats was employed. Their body weight ranged between 130-100 g. They were left 2 weeks for accommodation to environment of the laboratory in Al-Azhar Medical Physiology Department. The rats were separated randomly into 4 equal groups as follows: Group I [control group], Group II [vitamin D-treated group], Group III [jojoba nano-emulsion-treated group], Group IV [vitamin D and jojoba nano-emulsion-treated group].

Results: There were significant variances in IL-10 levels among groups, with G2 having non-significant higher levels than G1, G3 and G4 having higher levels than G1, and G3 having higher levels than G2. Vitamin D plus Fucidin did not significantly increase IL-10 compared to Fucidin alone, but jojoba alone or with vitamin D increased IL-10 levels. Vitamin D plus Fucidin led to a mild decrease in TNF-α, but jojoba significantly reduced it.

Conclusion: The current study showed that jojoba nano-emulsion either with or without vitamin D were promising on cutaneous lesions healing, but jojoba nano-emulsion with vitamin D showed the most promising results. The combination of jojoba nano-emulsion and vitamin D treatment had a potent anti-inflammatory effect. This was demonstrated by the low levels of pro-inflammatory mediators. The combined treatment also showed superior antioxidant capacity.

Keywords: Jojoba Nano-emulsion; Vitamin D; Skin Lesion; Albino rats.

INTRODUCTION

The skin is the biggest organ in the body. It serves as the primary defense against the external environment. It provides a protective barrier against mechanical, thermal, physical and chemical injury. A wound is a break in the anatomical as well as functional continuity of the epidermis that can be induced by physical, thermal, chemical, microbial, or immunological insults [1]. Keratinocytes, fibroblasts, endothelial cells, neurons, inflammatory cells, and immune cells all play intricate roles in the healing process of cutaneous lesions [2]. Ischemic illnesses such as arteriosclerosis obliterans [ASO], Buerger's disease, diabetes mellitus, blue toe syndrome, as well as persistent decubitus affect some individuals with chronic disease [3] must have special care to avoid complications as infection, osteomyelitis, tissue necrosis, gangrene and diabetic foot [4]. Impaired release of growth factors and cytokines, inadequate recruitment of stem cells, and inadequate macrophage switching are known to occur in chronic refractory skin ulcers. For proper healing of cutaneous lesion, we need cytokines and cells [5].

Vitamin D is crucial for the regulation of glucose metabolism, calcium metabolism, as well as the immune system. Vitamin D is responsible for the regulation of cellular proliferation, apoptosis, differentiation, as well as angiogenesis in the epidermis [6]. A lack of vitamin D has been linked to a number of health problems, such as osteoporosis, diabetes, and slowed wound healing [7]. The jojoba plant is native to arid and semiarid regions, including Egypt's Ismailia Desert. The liquid known as jojoba oil [JJBO] has a faint yellow liquid. Flavonoids, alkaloids, and polyphenols are all present [8].

Kidney problems, sunburn, chaffed skin, headaches, and thinning hair are all treated with JJBO. Repairing the skin's barrier function and promoting wound healing are the primary traditional therapeutic uses of JJBO [9]. The present work aimed to examine the possible effect of jojoba nano-emulsion with or without vitamin D on experimentally-induced cutaneous lesion in adult male albino rats.

MATERIALS AND METHODS

Twenty-eight adult male albino rats of a local strain were used. Their body weight ranged between 130-100g. They were left 2 weeks for acclimatization [and had free access to water and food] to environment of the laboratory in Al-Azhar Medical Physiology Department. Rats were kept in suitable cages [50 × 60 × 50 cm for every 7 rats]. The rats were separated randomly into 4 equal groups as follows: Group I [control group]: Superficial skin incision was induced and treated with topical antibiotic [2.0% fusidic acid], Group II [vitamin D-treated group]: Superficial skin incision was induced and treated with topical antibiotic [2.0% fusidic acid] and oral vitamin D3 once daily at a supplementary dose of 400 IU/Kg body weight [10], Group III [jojoba nano-emulsion-treated group]: Superficial skin incision was induced and treated with topical antibiotic [2.0% fusidic acid] and topical application of jojoba nano-emulsion and Group IV [vitamin D and jojoba nano-emulsion-treated group]: Superficial skin incision was induced and treated with topical antibiotic [2.0.% fusidic acid], topical application of jojoba nano-emulsion and oral vitamin D3 at a supplementary dose of 400 IU/Kg/D.

Induction of cutaneous lesion: Under mild ether anesthesia, using sterile surgical scalpel, a superficial surgical wound was produced on shaved backs and disinfected with 70.0% alcohol making a longitudinal midline one incision 1 ± 0.2 cm in length on the dorsal side and extending down to the panniculus carnosus [part of the subcutaneous tissues in vertebrates] [11].

Ethical aspects: The study protocol was reviewed and approved by the local research and ethics committee [Damietta Faculty of Medicine] [Branch of "Ethics of Animal Use in Research]. The manuscript was prepared according to guidelines for research conduction and reporting.

Drugs and chemicals: Vidrop, [Medical Union Pharmaceuticals, Egypt] is a liquid vitamin D3 supplement. The drop of vitamin D3 [cholecalciferol] is 100 international units [IU], or 0.036 milliliters of the medication. It was demonstrated that the bioavailability of vitamin D is higher when administered in an olive oil vehicle in contrast to when administered alone. Each rat was given one milliliter of pure olive oil intraorally once daily [12]. Jojoba nano-emulsion was obtained in the form of cream [Science Way Company, Elwaha, Nasr City] to be applied in wound twice daily for 15 days [13]. Fusidic acid cream 2.0%: All rats were treated twice daily for 15 days [PHARCO Company] [14]. The body weights of rats were documented every five days during the study period to ascertain the dosage of the additional vitamin D3.

Methods

For all groups, the following parameters were measured: Kidney function tests, calcium level [total and ionized], [MG kits], liver function test, oxidant state, anti-oxidant state, Inflammatory marker: Tumor necrosis factor alpha [TNF alpha] [Rat TNF alpha ELISA Kit], anti-inflammatory marker: Interleukin 10 [IL10] [Rat IL-10 ELISA Kit]. All kits were obtained from BIODIAGNOSTIC and ELGOMHOURIA Company and occurred in Tumor Markers Oncology Research Center in Faculty of Pharmacy [Boys] Al-Azhar University.

Collection of blood samples: Blood was drawn from the retro-orbital venous sinus utilizing a heparinized capillary tube after anesthetizing the rats with ether on day fifteen. Approximately, 2 ml of blood was collected into a labeled sterile sample vial containing 1 mg of Na-EDTA powder as an anticoagulant. Samples were counted no longer than five hours after blood withdrawal for measurement of WBCs, neutrophils, lymphocytes, and platelets. To get the serum, three milliliters of blood was transferred into a clean, dry, graduated glass centrifuge tube and spun at 5,000 revolutions per minute for 10 minutes. We transferred approximately half of the serum supernatant to Eppendorf tubes and kept frozen at -20 ºC until needed.

Rate of wound healing: In order to document the wounds' healing progress, they were photographed every four days using a high-power camera and a digital camera. At various intervals after wounding, the area of the incision was measured; the percentage area of the wound was then determined by comparing the changed size of the wound to the initial wound [11]. The percentage [%] of Wound contraction = healed area/total area ×100. After healing, tissue samples were isolated from each group for histopathological and ultrastructure examination. Animals were sacrificed and 5-mm punch biopsy specimens were taken and preserved in phosphate-buffered formalin. The histopathological examination was conducted using hematoxylin and eosin stain. The specimens were examined under Leica Application Suite [LASEZ] microscopy The ultrastructure examination entailed the slitting of the epidermis into extremely thin sections and their placement on copper grids for electron microscopy. Uranyl acetate and lead citrate were employed to color the meshes.

Statistical analysis: The Statistical Package for Social Sciences [SPSS], version 20 [IBM®SPSS® Inc., Armonk, USA] was used to compile, tabulate, and analyze the data that was collected. The data were exclusively quantitative. Consequently, the mean, standard deviation [SD], minimum, and maximum were determined. The one-way analysis of variance [ANOVA] test was employed to compare the four groups. Furthermore, the post-HOC least significant differences [LSD] were

computed to facilitate comparisons between the two groups. P value of less than or equal to 0.05 was regarded as a statistically significant.

RESULTS

There was a significant variation among groups [P<0.05]. The highest number of WBCs was reported in the first group [Fucidin] followed by the third group [Fucidin plus jojopa] [Table 1]. There were significant distinctions in lymphocyte and monocyte percentages between groups II and IV which had significantly lower values than groups I and III regarding lymphocyte%, and higher values as regards monocyte and granulocyte percentages [p <0.05]. Vitamin D had a lowering effect on lymphocyte percentages, while increasing it on monocyte and granulocyte percentages. Jojopa did not have a significant effect when used alone [Table 2]. There were significant disparities in IL-10 levels among groups, with group II having non-significant higher levels than groups I, III and IV. In addition, group IV had higher levels than groups I, and group III had higher levels than group II. Vitamin D plus Fucidin did not significantly increase IL-10 compared to Fucidin alone, but jojoba alone or with vitamin D increased IL-10 levels p<0.05. Vitamin D plus Fucidin led to a mild decrease in TNF-α, but with jojoba significantly reduced it [Table 3]. The groups showed a significant disparity in SOD levels, but there was no variance in Catalase levels [Table 4]. The results of the wound healing are presented through histopathological results.

Table [1]: Comparison among study groups concerning WBCs

Group / Parameters		G-I N=7	G-II N=7	G-III N=7	G4-IV N=7	Test	P
WBCS x10^3/cc	Mean ± SD Min.- Max.	16.84 ± 2.39 13.90-21.30	11.47 ±3.19 7.20- 17.30	16.20 ± 2.88 11.10-19.20	11.34 ± 2.57 8.70- 15.70	7.319	P1=≤0.001* P2= 0.007* P3= 0.972 P4= 0.005*
P5= 0.01*			P6= 0.99		P7= 0.01*		

F: ANOVA test. G-I [Fucidin], G-II [Fucidin plus vitamin D], G-III [Fucidin plus jojopa], G-IV [Fucidin, vitamin D and jojopa], Min. for minimum and Max. for maximum. P1: Between 4 groups, P2: Group-I vs group II2, P3: Group-I vs group-III, P4: Group-I vs group –IV, P5: Group -II vs group-III, P6: Group-II vs group -IV P7: Group-III vs group -IV.

Table [2]: Comparison among study groups concerning lymphocyte, monocyte as well as granulocytes percentages

Group / Parameters		G-I N=7	G-II N=7	G-III N=7	G4-IV N=7	F	P
Lymphocyte %	Mean ± SD Min.- Max.	78.60±3.92 72.20 – 83.50	**66.77±6.25** 59.90 –77.40	78.30±4.99 68.40-83.10	**60.27±3.12** 55.30-63.90	**25.601**	P1=≤0.001* P2= ≤0.001* P3= 0.99 P4= ≤0.001*
		P5= ≤0.001*	P6= 0.07		P7= ≤ 0.001*		
Monocyte %	Mean ± SD Min.- Max.	11.74±2.19 8.40-14.10	**15.54±1.73** 12.60-17.30	12.27±2.01 10.40-16.20	**19.53±2.15** 16.80-22.30	**21.918**	P1=≤0.001* P2= 0.009* P3= 0.96 P4= ≤0.001*
		P5= 0.02*	P6= 0.006*		P7=≤0.001*		
Granulocyte %	Mean ± SD Min.- Max.	9.66±2.38 7.20-13.70	**17.69±5.64** 10.00-24.00	9.43±3.46 6.20-15.40	**20.20±3.81** 14.20-25.00	**13.353**	P1= ≤0.001* P2=0.005* P3= 0.99 P4=≤0.001*
		P5= 0.003*	P6= 0.64		P7= ≤0.001*		

F: ANOVA test. G-I [Fucidin], G-II [Fucidin plus vitamin D], G-III [Fucidin plus jojopa], G-IV [Fucidin, vitamin D and jojopa], Min. for minimum and Max. for maximum. P1: Between 4 groups, P2: Group-I vs group II2, P3: Group-I vs group-III, P4: Group-I vs group –IV, P5: Group -II vs group-III, P6: Group-II vs group -IV P7: Group-III vs group -IV.

Table [3]: Comparison between study groups regarding IL-10 and TNF-α

Group / Parameters		G-I N=7	G-II N=7	G-III N=7	G4-IV N=7	Test	P
IL-10	Mean ± SD Min.- Max.	5.70±0.57 4.90 – 6.40	6.56±0.79 5.40- 7.70	7.11±0.58 6.40- 8.20	8.11±0.94 6.50- 9.10	**13.292**	P1=≤0.001* P2= 0.15 P3=0.007* P4=≤0.001*
		P5= 0.51	P6= 0.003*		P7= 0.07		
TNF-α	Mean ± SD Min.- Max.	2.93±0.50 2.10- 3.50	2.91±0.74 1.90-4.20	2.76±0.33 2.30- 3.20	2.17±0.38 1.50- 2.60	**3.397**	P1= 0.03* P2= 0.99 P3=0.92 P4= 0.04*
		P5= 0.94	P6= 0.056		P7= 0.16		

Table [4]: Comparison between study groups regarding catalase and SOD

Group / Parameters		G-I N=7	G-II N=7	G-III N=7	G4-IV N=7	Test	P
Catalase	Mean ± SD Min.- Max.	28.43±2.76 25- 33	28.43±3.51 24 – 33	31.71±3.73 27 - 37	27.14±4.10 22 - 33	**2.111**	0.125
SOD	Mean ± SD Min.- Max.	2.87±0.34 2.40 – 3.30	3.41±0.51 2.60 – 4.10	3.50±0.35 2.90 -3.80	4.09±0.58 3.50- 5.10	**8.351**	P1=≤0.001* P2=0.14 P3= 0.07 P4=≤0.001*
	P5= 0.98		P6= 0.04*		P7= 0.1		

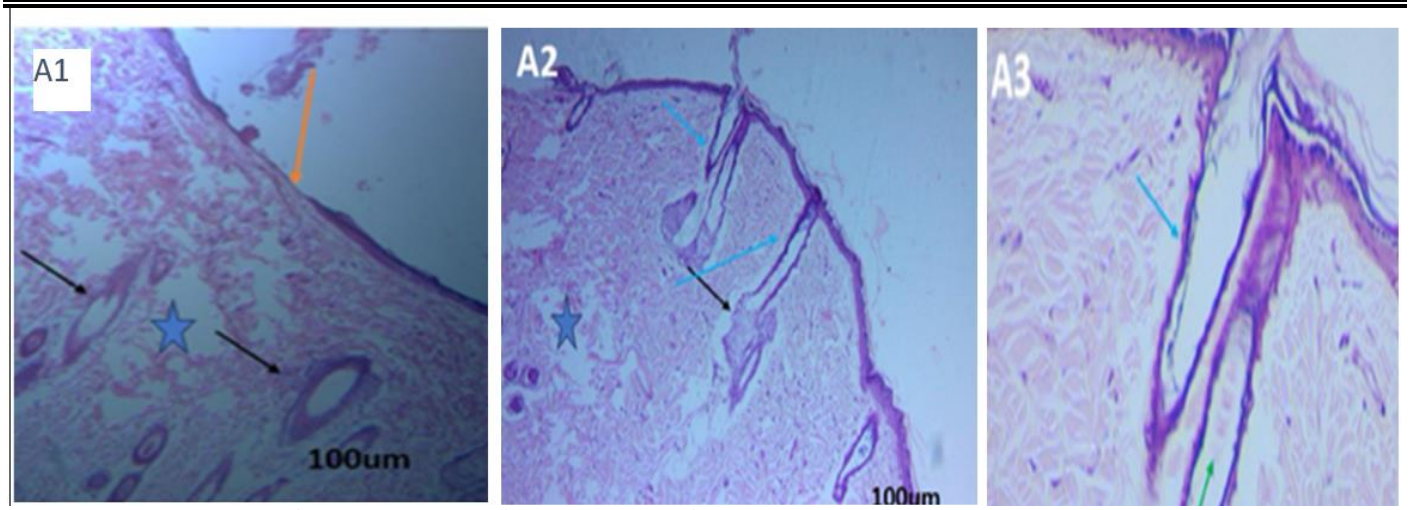

Figure [1]: Photomicrographs of sections in the skin of an adult male rat of group -I [Control group]. In [**A1 and A2**]**,** skin showed incomplete epidermal epithelialization and differentiation [**orange arrow**]. Dermis showed a marked decrease in collagen and fibroblast formation oriented by wide spaces [**blue star**] and skin appendages [**black arrow**]**.** Higher magnifications [**A3**] showed thin keratinized squamous epithelium with no morphological signs of cutaneous appendages differentiation, V shape permanent scar tissue with collagen fiber penetration [**green arrow**] [**H & E, X100 & X400**]**.**

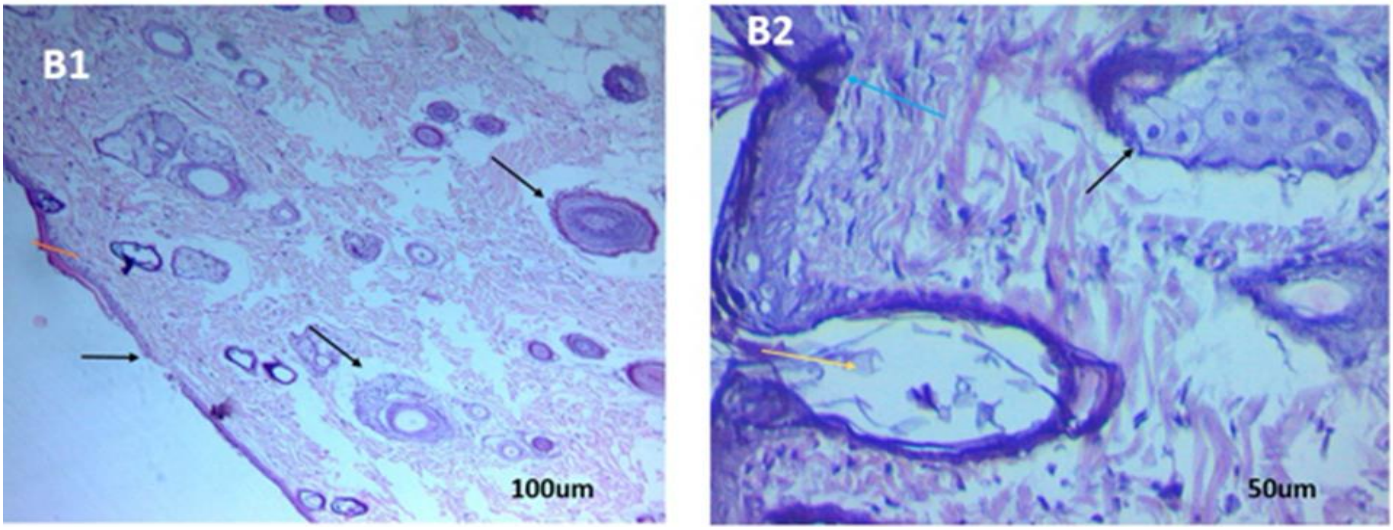

Figure [2]: Photomicrographs of sections in the skin of an adult male rat of group-II [Vitamin D-treated group]**.** In [B1] the epidermis was thin and relatively atrophic with a flattened basement membrane [orange arrow]. There was an area of dis-continuous epidermis [black arrow]. The dermis showed no clear distinction among a superficial and a deep zone that contain collagen fibers, which are both thick and hyalinized with neogenesis of skin appendages [black arrows]. [B2] exhibited bulbous buds, most of them with central keratinization [yellow arrow], also X -shape mature scar compatible with hair follicles and loosely arranged collagen bundles [blue arrows] [H & E, X100 & X400].

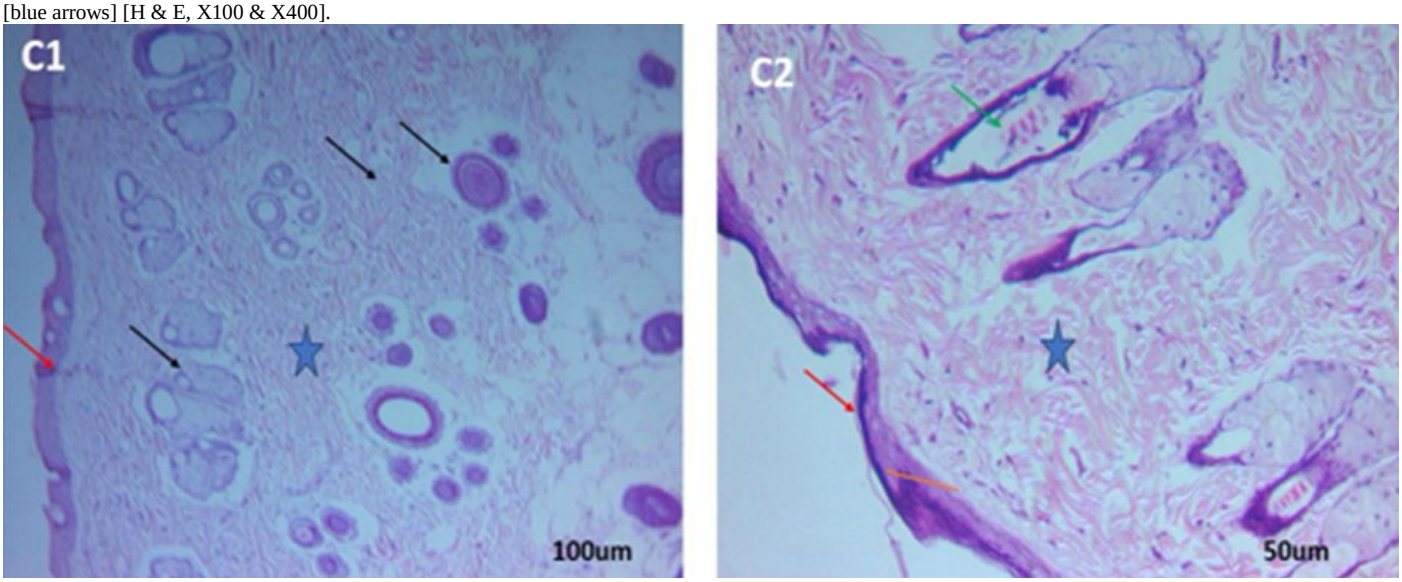

Figure [3]: photomicrographs of sections in the skin of an adult male rat of group-III [jojoba nano-emulsion-treated group]**.** In [C1] there was full re-epithelialization of the wound surface [red arrow], plentiful hair follicles and sebaceous glands [black arrows]. [C2] showed U shape permanent scar tissue. The epidermis was thin with a flattened basement membrane [orange arrow]. Dermis showed densely arranged collagen bundles [blue star] with collagen fiber penetration of skin appendages [green arrow] [H & E, X100 & X400].

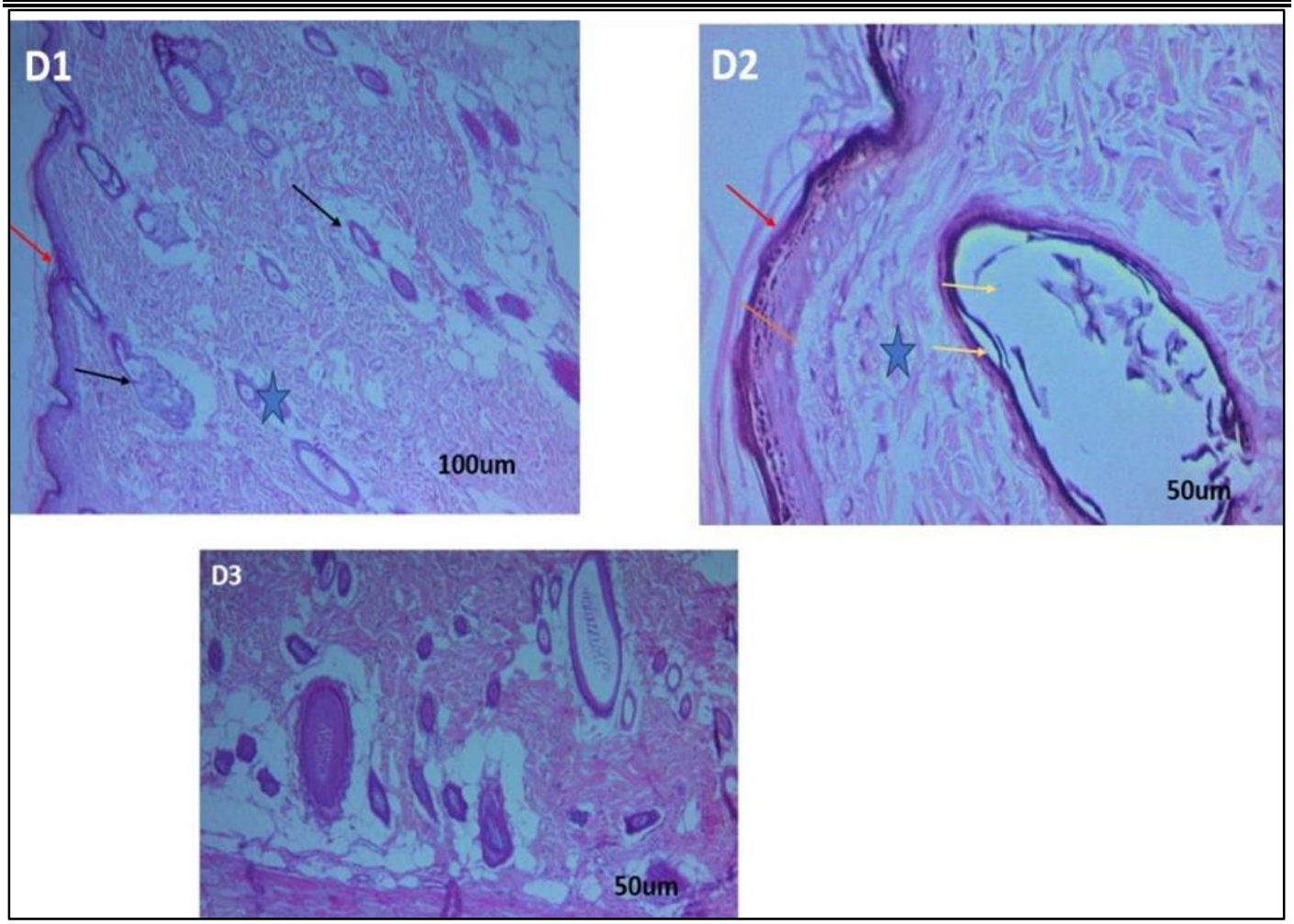

Figure [4]: Photomicrographs of sections in the skin of an adult male rat of group -IV [Vitamin D and jojoba nano-emulsion-treated group]. D] Represents a skin sample of G4 [Vitamin D and jojoba nano-emulsion-treated group]. shows normal histological features of skin layers. mature epidermis [**red arrow**] with complete epithelialization in the form of closure of the basal layer with spinous and granular epidermal differentiation. The dermis shows plentiful hair follicles and sebaceous glands [**black arrows**]. [**D2**] An increase in collagen fiber [**blue star**] and fibroblast formation with exhibiting bulbous buds, with central keratinization, is noticed [**yellow arrows**]. [**D3**] neoformation of cutaneous appendages [hair follicles] with Collagen fiber penetration [**H&E, X100, X400**].

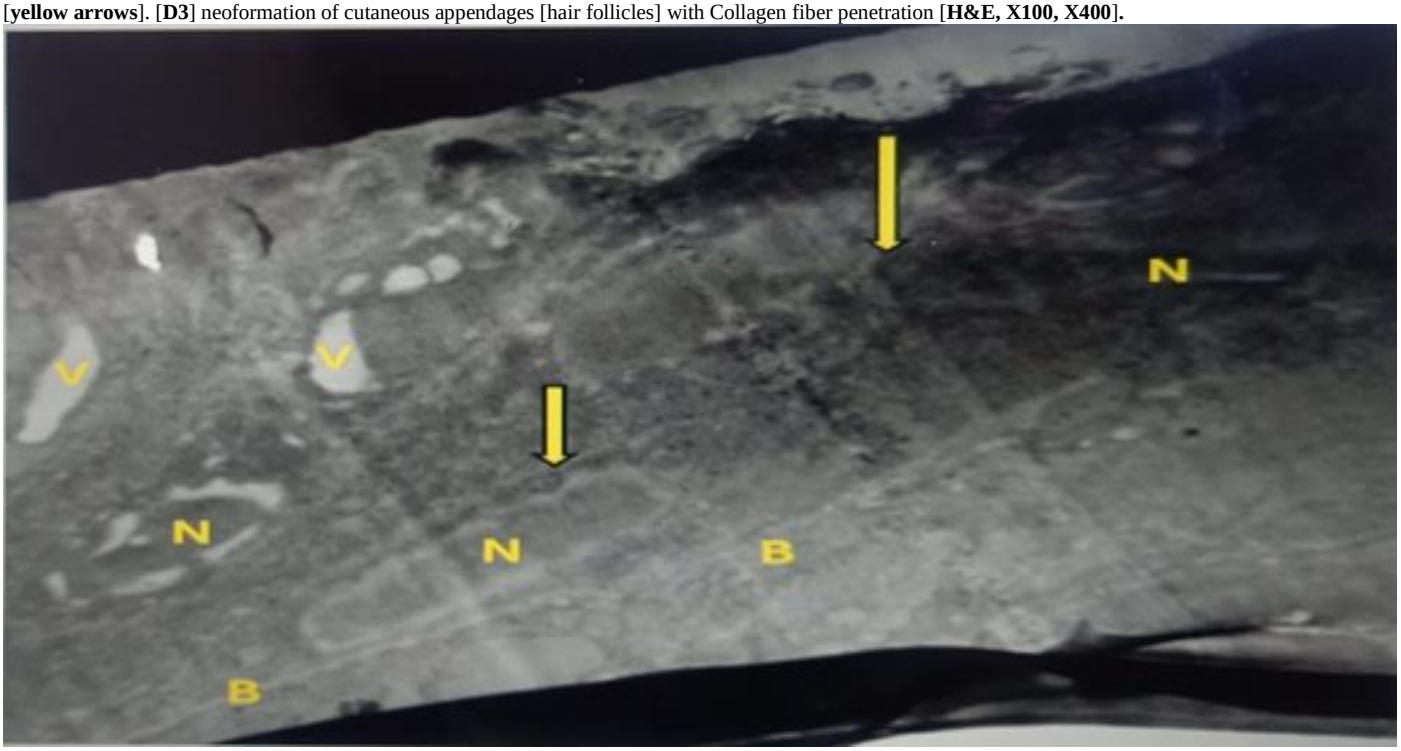

Figure [5]: Transmission electron microscopic image of the rat skin sections of Group-I [Control group]. It showed stratum basale keratinocytes resting on irregular basement membrane [**B**], partially destroyed desmosomes [**yellow arrows**], vacuolations [**V**], and irregularly elongated nuclei with clumped chromatin [**N**] [**X5000**].

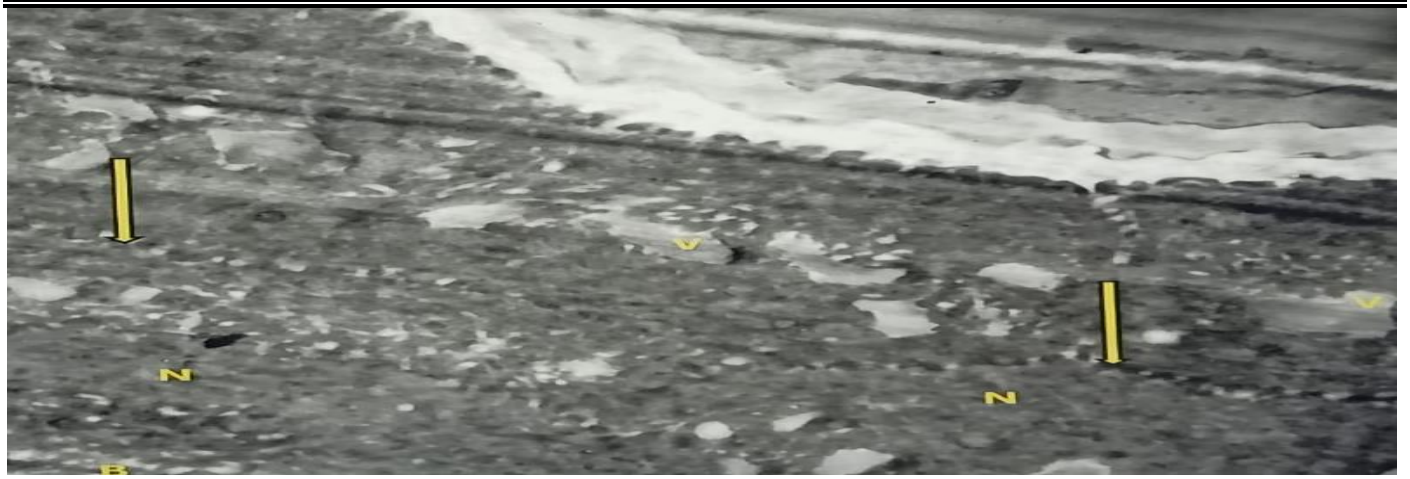

Figure [6]: Transmission electron microscopic images of the rat skin sections of group-II [Vitamin D-treated group]. **B**] **Group-II** [**vitamin D-treated group**] showed stratum basale keratinocytes resting on irregular basement membrane [**B**], partially destroyed desmosomes [**yellow arrows**], vacuolations [**V**]**,** and irregular elongated nuclei with clumped chromatin [**N**] [**X5000**]**.**

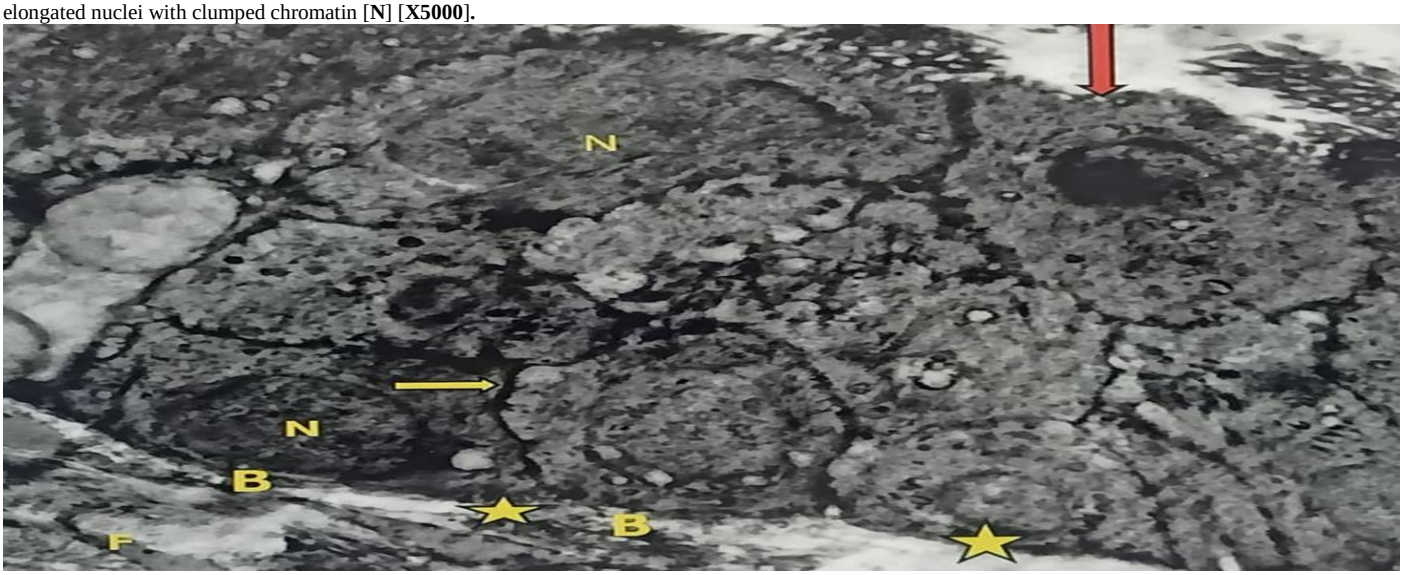

Figure [7]: Transmission electron microscopic images of the rat skin sections of group-III [**jojoba nano-emulsion-treated group**]**. C**] **Group-III** [**Jojoba nano-emulsion-treated group**] showed intact surface epithelium with thin overlying keratin layer [**red arrow**] associated with remarkable improvement in epidermal cell desmosome junction [**yellow arrow**], and rounded nuclei with irregular nuclear membrane and prominent nucleoli. Stratum basale keratinocytes resting on discontinuous basement membrane [**B**], attached to it by slightly organized hemidesmosomes [**star**]. Fibroblasts [**F**] in the papillary dermis was observed [**X5000**].

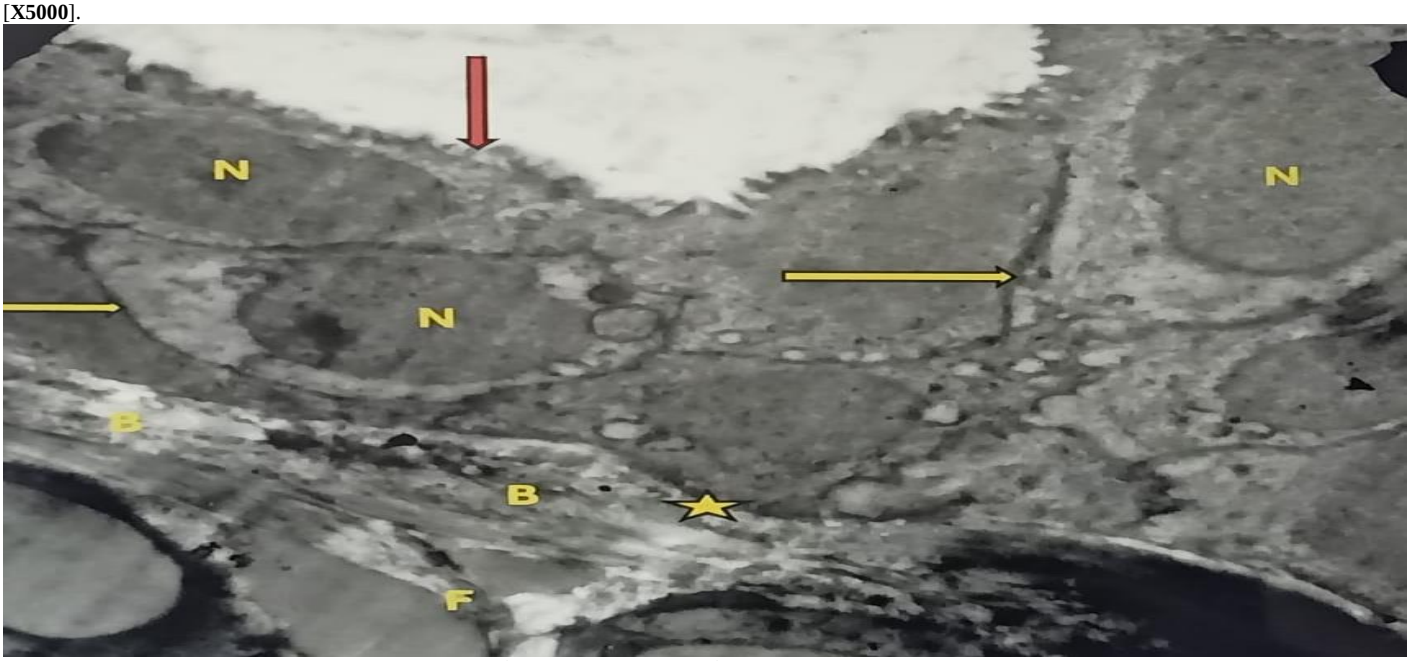

Figure [8]: Transmission electron microscopic images of the rat skin sections of group-IV [Vitamin D and jojoba nano-emulsion-treated group]. D Group-IV [vitamin D and jojoba nano-emulsion-treated group] showed intact surface epithelium with the overlying keratin layer [red arrow] associated with remarkable improvement in epidermal cell desmosome junction [yellow arrow], and rounded nuclei with regular nuclear membrane and prominent nucleoli. Stratum basale keratinocytes resting on regular continuous basement membrane [B], attached to it by hemidesmosomes [stars]. Fibroblasts [F] in the papillary dermis was observed [X5000].

DISCUSSION

Restoring tissue form and function throughout wound healing is accomplished by the coordinated interaction of several components of the extracellular matrix [ECM], involving cells, proteins, growth factors, small molecules, proteases, as well as functioning. Healing and the restoration of tissue function and characteristics are dictated by the complex interplay of stromal, endothelial, and immune cells. There are four distinct but interconnected steps in the skin healing process: hemostasis, inflammation, proliferation, as well as remodeling. There can be a lot of overlap across phases, even while different growth factors, cytokines, and dominant cell types are present at various periods [15]. Each phase has the following durations: hemostasis [0-several hours after damage], inflammation [1-3 days], proliferation [4-21 days], as well as remodeling [21 days-1 year] [16].

Regarding differential count percentages, there was a significant variation among the study groups. Groups II and IV had significantly lower values than groups I and III regarding lymphocyte% and higher values as regards monocyte and granulocyte percentages. In addition, there was a significant increase of lymphocyte percentages and a significant reduction of monocyte percentages in group II than in group IV. No significant differences were recorded between groups I and III. To translate, vitamin D had a lowering effect for lymphocyte percentages, while it had increasing effect on monocyte and granulocyte percentages. Jojoba did not have a significant effect when used alone.

It was reported that WBC count increases in inflammation [17]. The categories of white blood cells include granulocytes [neutrophils, eosinophils, and basophils] and agranulocytes [monocytes and lymphocytes, which encompass T cells & B cells] [18].

Regarding anti-inflammatory interleukins IL-10: Significant disparities existed among the research groups. Group IV has significantly increase than group I and has the best effective result. Regarding proinflammatory factor TNF-α: Significant disparities existed among the research groups. Group IV has significantly reduced than group I and has the best effective result. The above results established the superiority of the combined treatment by jojoba and vitamin D, as it led to significant increase in anti-inflammatory cytokine levels [IL-10] and decrease in proinflammatory cytokines [TNF alpha]. But these primary results need to be confirmed with larger research. Vitamin D has been shown to elevate levels of anti-inflammatory cytokines [IL-4, IL-5, IL-10, TGF beta] by boosting T-helper 2 [Th2] cells within the inflammatory system, while concurrently suppressing the generation of Th1 and Th17 cells and proinflammatory cytokines [IL-2, IL-3, IFN-gamma, TNF alpha]. An increase in IL-10, an anti-inflammatory cytokine, suppresses the manufacture of proinflammatory cytokines by T cells and macrophages [19]. Also, in agreement with the current study, **Zhang *et al***. [8] showed that Lung tissues showed low amounts of pro-inflammatory mediators and few total proteins, confirming the significant anti-inflammatory effect of the jojoba oil dry nano-emulsion.

Regarding catalase: There was a non-significant difference between study groups. However, superoxide dismutase [SOD] showed statistically significant differences between groups. The levels were increased in group II than in group I and in groups III and group IV than in group II. Also, in group IV than in group III. So, the use of vitamin D plus fucidin led to an increase of SOD. Jojoba had similar effect but higher than vitamin D. Moreover, when both drugs were combined the increase reached the highest value, which was statistically significant.

Oxygen free radicals are produced after inflammation resulting in increased oxidative stress. The above results showed that both of jojoba and vitamin D had anti-oxidant effect, and their combination led to more beneficial antioxidant capacity. The presence of lipoxygenase inhibitors in jojoba is anticipated to provide protection against oxidative damage caused by free radicals [20].

The antioxidant effect of Jojoba was confirmed by **Zhang *et al***. [8] who showed that jojoba oil dry nano-emulsion had strong antioxidant activity as confirmed by increased level of SOD. Also, in agreement with the current study, **Razzaghi *et al***. [7] showed that supplemental vitamin D have antioxidant effect among cases with diabetic foot ulcer in addition to have beneficial effect on wound healing.

Regarding histopathology of the skin of an adult male rat of group I [Control group], it was found that skin showed incomplete epidermal epithelialization and differentiation. Dermis showed a marked decrease in collagen and fibroblast formation oriented by wide spaces and skin appendages. Higher magnifications showed thin keratinized squamous epithelium with no morphological signs of cutaneous appendages differentiation and V shape permanent scar tissue with collagen fiber penetration. In group II [Vitamin D-treated group]: The basement membrane was flattened, and the epidermis was relatively atrophic and thin. An area of discontinuous epidermis was present. Collagen fibers that were both dense and hyalinized, as well as neogenesis of skin appendages, were present in the dermis, which lacked a clear distinction between a superficial and deep zone. The other section showed bulbous buds, most of them with central keratinization, X-shaped mature scar compatible with hair follicles and loosely arranged collagen bundles. Group III [Jojoba nano-emulsion-treated group] showed full re-epithelialization of the wound surface, mature thin scars, plentiful hair follicles and sebaceous glands. The epidermis was thin with a flattened basement membrane. Dermis showed densely arranged collagen bundles with collagen fiber penetration of skin appendages. Group IV [Vitamin D and jojoba nano-emulsion-treated group] showed normal histological characteristics of the mature epidermis and skin layers, characterized by complete epithelialization in the form of basal layer closure in addition to spinous as well as granular epidermal differentiation. The dermis showed plentiful hair follicles and sebaceous glands. An increase in collagen fiber and fibroblast formation with exhibition of bulbous buds and with central keratinization was noticed. Also, neoformation of cutaneous appendages with collagen fiber penetration

The above results established the beneficial effect on wound healing of jojoba nano-emulsion either with or without vitamin D, but jojoba nano-emulsion with vitamin D showed the most promising results.

Regarding ultrastructure examination of skin of an adult male albino rat of group I [Control group]: It revealed stratum basale keratinocytes perched on an uneven basement membrane, damaged desmosomes, vacuolations, as well as elongated, irregular nuclei with chromatin clumped together.

In group II [Vitamin D-treated group]: The stratum basale keratinocytes were observed resting on an uneven basement membrane, exhibiting partially damaged desmosomes, vacuolations, and irregularly elongated nuclei with clumped chromatin. Group III [Jojoba nano-emulsion-treated group]: The epithelium was intact and covered with a thin coating of keratin. The desmosome connection between epidermal cells was significantly improved, and the nuclei were rounded with an uneven nuclear membrane and conspicuous nucleoli. Furthermore, stratum basale keratinocytes lie on a basement membrane that is discontinuous and is linked to it by hemidesmosomes that are weakly structured. Fibroblasts in the papillary dermis were observed. Group IV

[Vitamin D and jojoba nano-emulsion-treated group]: Round nuclei with regular nuclear membranes and conspicuous nucleoli were present, as was an improved desmosome connection between epidermal cells, an intact surface epithelium, and an overlaying keratin layer. On top of it, keratinocytes in the stratum basale are linked to the normal continuous basement membrane by hemidesmosomes. Fibroblasts in the papillary dermis were observed.

The histological and ultrastructural levels of analysis revealed that the administration of jojoba nano-emulsion and vitamin D improved wound healing by improving the organization and reformation of dermal and epidermal layers, in addition to collagen fibers within cutaneous layers. Consistent with prior reports, **Ayavoo *et al*.** [21] demonstrated the antibacterial and wound-healing properties of garlic extracts. **Hashem *et al*.** [22] showed that the healing activity was enhanced by the use of T. viride extract, a solution of S. boulardii, or their combination with stem cells, all of which demonstrated a successful healing process. In the process of wound recovery, collagen is a critical element. Collagen degradation components that promote cellular migration and epidermis structure remodeling were responsible for the regeneration of granulation tissues.

Conclusion: The current study showed that jojoba nano-emulsion either with or without vitamin D were promising on cutaneous lesions healing, but jojoba nano-emulsion with vitamin D showed the most promising results. The research demonstrated that the combination of jojoba nano-emulsion and vitamin D treatment had potent anti-inflammatory effect. This was demonstrated by the low levels of pro-inflammatory mediators. The combined treatment also showed superior antioxidant capacity.

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