Original Article

A Comparative Study on the Effect of Two Topical Formulations of Bee Propolis Cream in Ordinary and Nano Form on Wound Healing in Aged Rats (A Histological and Immunohistochemical Study)

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ABSTRACT

Introduction: Aging could result in delayed wound healing. propolis has good effect on healing.

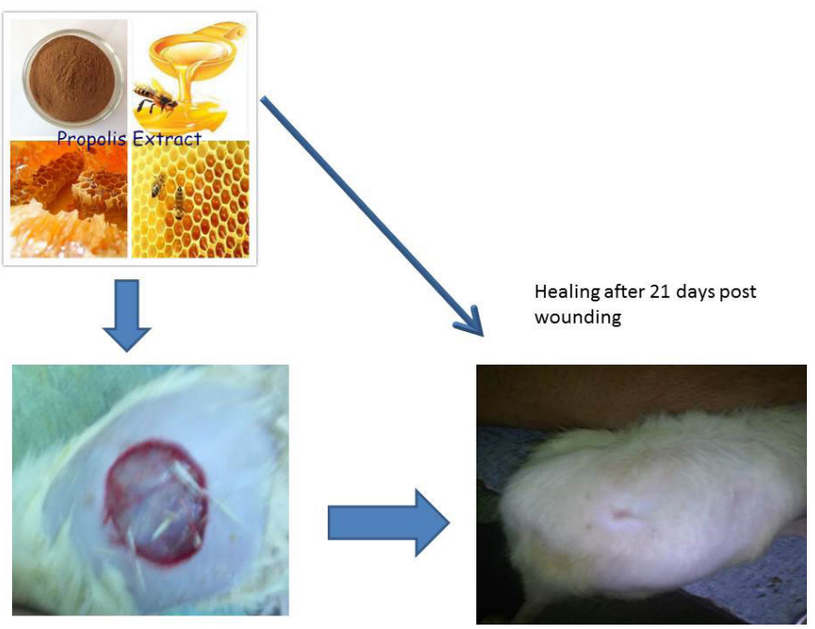
Objectives: This investigation was done to assess whether propolis and nanopropolis creams could aid in the healing of wounds in aged rats.

Materials and Methods: Fifty old-aged albino rats have been separated into four groups: group I (control intact skin) (5 rats); wounded groups: group II (control wounded) (3 subgroups, 5 rats each); subgroup IIa: vehicle cream was topically applied once per day for 7 days; subgroup IIb: once per day for 14 days; and subgroup IIc: once per day for 21 days; group III (propolis group), which has been divided into 3 subgroups with 5 rats each, where propolis cream was topically applied once per day for 7 days (subgroup IIIa); once per day for 14 days (subgroup IIIb); and once per day for 21 days (subgroup IIIc), group IV (nanopropolis group), which has been divided into 3 subgroups with 5 rats each, where nanopropolis cream was topically applied for the same durations as the aforementioned in group III. Hematoxylin and eosin stain, picro sirius red histochemical reaction, and CD 105 immunostain were used in this study.

Results: Wound healing was significantly improved in the propolis and nanopropolis groups, with a significant increase in the rate of wound closure and collagen deposition.

Conclusion: The current study results confirmed the importance of CD105 in the process of wound healing. Additionally, it demonstrated how effectively propolis and nanopropolis formulas treated aged wounds. This technique might be effective for treating chronic wounds safely and efficiently.

Graphical Abstract



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Key Words: Aged, CD 105, nanopropolis, propolis, wound healing.

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INTRODUCTION

Healing of wounds is a complicated process. It targets restoring tissue integrity as well as the skin's barrier role. It begins just after the damage with hemostasis. Then it is followed by three stages named: inflammation, proliferation and remodeling[1]. The inflammatory stage begins with hemostasis, chemotaxis, and increase in vascular permeability. This will consequently decrease more tissue damage by closing the wound to prevent hemorrhage, removing dead tissues and bacteria, and accelerating migration of different cells. The inflammatory stage usually continues for many days[2]. Granulation tissue deposition, angiogenesis, and re-epithelialization are characteristics of the proliferative stage. This phase may continue for many weeks. Wound attains its full strength and maturation in the third stage; maturation and remodeling stage[3].

Skin function deterioration is related to aging as a result of morphological and structural changes. A reduction in the quantity of Langerhans cells and melanocytes as well as a flatness of the dermal-epidermal Junction are characteristics of aging-related epidermal alterations. Moreover, the turnover time is increased by 50%, as keratinocyte proliferation is decreased. Fewer fibroblasts, decreased vascularity, a loss of collagen, reduced mast cells and macrophages, elastin, and glycosaminoglycan were revealed in the dermis of older skin. The remaining collagen quality and the morphology of elastic fibers are also disturbed[4].

Alterations in skin due to aging not only affect the healing of wounds but also increase the skin susceptibility to harm. The development of chronic wounds is caused by immunosenescence, which is shown by a decline in number of Langerhans cells and fibroblasts[5].

Wounds with impaired healing have not progressed through the normal physiological phases of healing. Usually, these wounds enter a stage of pathological inflammation that results in a delayed, incomplete, or uncoordinated process of healing[6].

Nanocream is one of the medicinal topical preparations used externally[7,8]. Nano creams can be set by different techniques like ultrasonic generators, high pressure homogenizers, or high shear stirring[9]. The significance of using nanocream in cosmetics is owing to the droplets' small size, which falls between 100 and 600 nm[10]. This permits them to deposit evenly onto the skin with consequent rise in transfer of active ingredients through the skin[11,12].

Propolis is a sticky material produced by honeybees (bee glue). The extract is a complex blend of many substances. It comprises aromatic aldehydes, flavonoids, terpenes, esters, phenolic chemicals, and beta-steroids. Antifungal, antiviral, antibacterial, anti-inflammatory, antioxidant, immunomodulatory, and anticarcinogenic qualities are among propolis' natural and pharmacological properties. It displayed faster healing ability by quickening healthy cell growth[13].

The aim of this study was to histologically assess the potential healing impact of bee propolis natural form on wound healing in aged adult male albino rats and to compare it with that of its nano-form which is designed to improve delivery of active ingredient into tissue.

MATERIALS AND METHODS

Materials

Animals

The present investigation used fifty aged male albino rats (24 months old). Animals were obtained from the animal house of Faculty of Science, Fayoum University. The animals have been kept at regular room temperatures, subjected to everyday natural light-dark cycles, and given access to water and food ad libitum. The Animal Ethics Committee at Fayoum University supervised the care and usage of the animals, which have been handled with care and in accordance with international guidelines and regulations for animal research in laboratories[14].

Rats have been split into four groups at random:

Unwounded group

Group I (control intact skin) (5 rats)

Wounded groups: divided into:

Group II (control wounded skin): subdivided into 3 subgroups, 5 rats each:

- Subgroup IIa: vehicle cream was applied once a day for 7 days.
- Subgroup IIb: vehicle cream was applied once a day for 14 days.
- Subgroup IIc: vehicle cream was applied once a day for 21 days.

Group III (propolis group): subdivided into 3 subgroups, each with 5 rats:

- Subgroup IIIa: propolis cream was applied once a day for 7 days.

- Subgroup IIIb: propolis cream was applied once a day for 14 days.
- Subgroup IIIc: propolis cream was applied once a day for 21 days.

Group IV (nanopropolis group): subdivided into 3 subgroups, each with 5 rats:

- Subgroup IVa: nanopropolis cream was applied once a day for 7 days.
- Subgroup IVb: nanopropolis cream was applied once a day for 14 days.
- Subgroup IVc: nanopropolis cream was applied once a day for 21 days.

Chemicals

Bee propolis extract powder: was purchased from Bulk Supplements Company (USA, Nevada).

Methods

preparation of propolis cream

Propolis extract powder was used to make 10% propolis cream by mixing the powder with ready-made cold cream (Pond's cold cream, Pond's Institute, USA)[15].

Preparation of nanopropolis cream

Nanocream was prepared by mixing the dry nanoparticles in cold cream at concentration of 10%.

Copper nanoparticle synthesis using dry bee propolis extract

For the synthesis of copper nanoparticles, 25 ml of dissolved propolis (10 gm in 100 ml ethanol) was added drop wise into 25 ml of 1 mM solution of copper sulphate under constant stirring for reduction of copper ions and incubated in dark place at room temperature for 2-3 hours[16]. The color changed from dark green to straw yellow, indicating that copper sulphate had been reduced to copper ions. The undesirable biological components were then removed from the solution by centrifugation for 15 minutes at 10,000 rpm and dispersing it in double-distilled water. The formation of copper nanoparticles was confirmed by using UV-Visible spectrometer in the range between 300-600 nm. Thereafter, the nanoparticles were allowed to dry and ground so as to be used for further analysis[17].

Droplet size measurement

The Zeta Sizer 1000 HSA, (Malvern Instrument, UK) has been used to measure the formulation's droplet sizes. Its basis is photon correlation spectroscopy. Prior to measuring the droplet size, the sample had been diluted using the phosphate buffer solution (PBS) to obtain a K count that was between 50 and 200, as needed by the machine's consistency.

Zeta potential measurement

The Nano ZS (Malvern, UK) has been used to measure the formulation's zeta potential. As the droplets don't coalesce within this range of ±30, the formulated Nano cream zeta potential has been measured to make sure it is within the limit. To stabilize the ionic strength and lower the droplet number, the formulations have been diluted using the same buffer solution that was employed as the external stage of the formula. In order to prevent changes in the droplets' mobility in the samples, bubbles have been removed before measurements.

Transmission electron microscopy

The FEI CM 12 high resolution TEM (Philips, Electron Optics, Eindhoven, Netherlands) was used to study the propolis nanocream's size and morphology. Using self-locking fine forceps, the propolis nanocream specimen has been put on a 400 mesh copper grid coated with collodion formvar carbon film. A 2% methylamine tungstate drop has been added to the grid's surface as a negative stain solution. Filter paper was used to carefully wipe away the extra stain solution from the specimen. Prior to getting examined under the microscope, the grid has been put on a Petri dish lined with filtering paper and allowed to dry for roughly 10 minutes at room temperature.

Rat Excision Wound Model and Treatment

Hair eradication cream has been used to shave the anaesthetized rats' back fur, and alcohol swabs were used to clean it. Under ketamine anesthesia, one full-thickness excision wound (2.5 x 2.5 cm) was created on each rat's dorsum utilizing dissection scissors and forceps. Daily saline cleaning was performed on all wounds. Wounds have been left uncovered and medicated topically once a day following cleansing using vehicle, propolis, or nanopropolis cream in accordance with their respective subgroups for 7, 14, and 21 days.

Wound-size measurements

A digital camera has been used to take photographs of every wound on days 1, 7, 14, and 21. Image J software has been used to measure the area of the wound. The wound closure rate (% of wound decrease from the initial wound) has been determined as follows:

(wound region day 1-wounds region days (7,14 and 21) x 100)/(wound region day 1)

Histological Studies

On the 7th, 14th, and 21st days after the initiation of the treatment of topical cream in the various groups, rats were sacrificed by using an overdose of ether. The wound sites were excised with a 5mm rim of normal skin around them. Tissue samples have been preserved in 10% neutral buffered formalin solutions before being prepared for paraffin sections. Five µm thickness sections have been cut and stained using hematoxylin and eosin (H & E) as well as the picro sirius red reaction. For the purpose of

identifying thick mature (type I) and thin freshly generated (type III) collagen fibers, polarized light and bright field microscopy images of picro sirius red stained sections have been used. Immunohistochemical staining has been carried out to detect the expression of CD 105 (Labvision, Thermo Scientific, CA, USA) using rabbit polyclonal antibody, catalogue no. RB-9291-P0, both cytoplasmic and membranous. The tonsil served as the positive control. Negative control in each run, an extra slide of a skin sample has been treated using buffer solution instead of primary antibody.

Morphometric study

Image analyzer computer system (Leica Qwin 500C) (Leica, England, UK) was used to obtain the data. The number of inflammatory cells including neutrophils and macrophages at x1000 magnification, the average area percent of collagen fibers at x100 magnification, the average area percent of CD 105 immunoexpression in dermis and epidermis, the number of immunostained blood vessels at x200 magnification, and epidermal thickness were measured All measurements were made in 10 non-overlapping fields.

Statistical analysis

The data was presented as group mean ± SD. After checking the distribution's normality, one-way ANOVA test has been performed in the statistical analysis employing SPSS version 10 (SPSS, Chicago, IL, USA). Employing Post-hoc analysis as well as Tukey's range test, the findings have been compared one by one with those of the other groups and with those of the control group. A value of $p \leq 0.05$ has been considered statistically significant[18].

RESULTS

Transmission electron microscope results

It presented the image of droplets of propolis nanocream employing HR-TEM. The nanocream preparation's droplets were dark in color. It had a sphere-shaped average size < 300 nm. Therefore, the results of the Zeta Sizer 1000 HSA were further reinforced by this outcome (Figure 1).

Wound closure

At 7, 14 and 21 days of induced wounds, a significant increase in wound closure rate has been noticed in the groups that received propolis and nanopropolis cream as compared with the wounded control group. There have been significant differences between propolis and nanopropolis groups at 7 and 14 days post wounding by 15.1% and 13.3% respectively, while at the 21st day, the difference in wound reduction rate has been statistically non-significant (*P value* = 0.1) (Figure 2, Table 1).

Histological results

Hematoxylin & eosin stain

Unwounded group (group I)

Normal skin exhibited a surface epidermis and dermis containing hair follicles as well as sebaceous glands (Figure 3).

Wounded groups

After 7 days

Untreated group (group IIa) showed wound area with loss of covering epidermis and accumulation of more cellular connective tissue in the wound bed. Newly formed blood capillaries and inflammatory cellular infiltration in the crust and dermal stroma were observed (Figure 4a). Both Propolis treated (group IIIa) and nanopropolis (group IVa) treated groups showed wound area with loss of covering epidermis and accumulation of both cellular connective tissue and thin collagen fibers in the wound bed. Nano group revealed also thick collagen fibers besides thin ones. The newly formed blood capillaries and inflammatory cellular infiltration were also revealed in the crust and dermal stroma (Figures 4b, c).

After 14 days

Untreated group (group IIb) showed wound area with loss of covering epidermis and accumulation of thin and thick collagen fibers in the wound bed. The newly formed blood capillaries and inflammatory cellular infiltration in the crust and dermis were noted (Figure 5a). Propolis treated group (group IIIb) showed wound area with loss of covering epidermis in some parts and re-epithelialization at the side of wound. Accumulation of collagen in the form of thin fibers in wound bed and thick bundles under the newly formed epidermis were also revealed. Wound bed showed parts with more cellular connective tissue. The newly formed blood capillaries, lymphatic vessels and inflammatory cellular infiltration in the crust and dermal stroma were evident (Figures 5b,c). Nanopropolis treated group (group IVb) showed wound area with loss of covering epidermis in some parts and re-epithelialization at the side of wound. Accumulation of collagen in the form of thick fibers in wound bed was noted. The newly formed blood capillaries and less inflammatory cellular infiltration in dermis were observed (Figure 5d).

After 21 days

Untreated group (group IIc) showed wound area with loss of covering epidermis and accumulation of thin and thick collagen fibers. There were congested blood vessels and apparently less inflammatory cellular infiltration in dermis (Figure 6a). Propolis treated group (group IIIc) showed filling of the wound area with more mature collagen in the form of thick fibers and thick bundles. Nearly complete re-epithelialization in the form of thick epidermis with deficient small area was noticed. New hair follicles were also revealed (Figure 6b). Nanopropolis treated group (IVc) showed the same findings as propolis group but with apparently thinner epidermis (Figure 6c).

Picro sirius red reaction

Unwounded group (group I)

Normal skin showed thick collagen bundles stained

deep red under bright field microscope (Figure 7a). While under polarized light microscope, predominantly thick orange collagen fibers (collagen I) and few thick yellow fibers (collagen III) were revealed (Figure 7b).

Wounded groups

After 7 days

Under a bright field microscope, all experimental groups showed collagen accumulation in wound sites to varying degrees and fibers disposition, which stained red (Figures 8a-c), while under a polarized microscope, the untreated group (group IIa) showed some orange fibers (collagen I) and many thin green fibers (collagen III) (Figure 9a). The Propolis treated group (group IIIa) revealed predominantly thin orange fibers (collagen I) and a few thin green-yellow fibers (collagen III) (Figure 9b), while the nanopropolis treated group (group IVa) had predominantly thick orange fibers (collagen I) with a few green-yellow ones (collagen III) (Figure 9c).

After 14 days

Under bright field microscope, all experimental groups showed collagen accumulation in wound sites at different intensity and disposition of fibers which stained red. Untreated group (group IIb) showed predominantly red fine collagen fibers (Figure 10a). Propolis treated group (group IIIb) showed both thin and thick fibers (Figure 10b), while nanopropolis treated group (group IVb) revealed predominantly thick collagen fibers (Figure 10c).

Under polarized microscope, untreated group (group IIb) showed orange (collagen I) and thin green fibers (collagen III) (Figure 11a). Propolis treated group (group IIIb) revealed predominantly thin orange fibers (collagen I) and few thin green-yellow fibers (collagen III) (Figure 11b), while nanopropolis group (group IVb) revealed predominantly thin and thick orange fibers (collagen I) with few thin green-yellow ones (collagen III) (Figure 11c).

After 21 days

Under bright field microscope, all experimental groups showed collagen accumulation in wound sites at different intensity and disposition of fibers which stained red. Untreated group (group IIc) showed predominantly red thick collagen fibers (Figure 12a). Propolis treated group (group IIIc) showed red thick fibers and bundles (Figure 12b), while nanopropolis group (group IVc) had predominantly red thick collagen bundles (Figure 12c). Under polarized microscope, Untreated group (group IIc) showed some thin orange fibers (collagen I) and thick yellow fibers (collagen I) (Figure 13a). Propolis treated group (group IIIc) revealed predominantly thin and thick orange fibers (collagen I) and few yellow fibers (collagen III) (Figure 13b), while nanopropolis group (group IVc) had predominantly thick red fibers (collagen I) with few thick green-yellow ones (collagen III) (Figure 13c).

Immunohistochemical results of CD 105

Unwounded group (group I)

Normal skin showed negative immunoexpression in epidermal keratinocytes and positive reaction in dermal stromal cells (Figure 14).

Wounded groups

After 7 days

The untreated group (group IIa) showed positive immunoreaction in proliferating endothelial cells and inflammatory cells in the crust (Figure 15a). Both the propolis (group IIIa) and nanopropolis (group IVa) treated groups revealed positive immunoreaction in proliferating endothelial cells and an intense reaction in inflammatory cells in the crust as well as dermal stromal cells. Negative reactions in the newly formed lymphatic vessels have been observed in the propolis treated group (Figures 15b-f).

After 14 days

Untreated group (group IIb) showed marked positive immunoreaction in proliferating endothelial cells and moderate reaction in dermal stromal cells (Figure 16a). Both propolis (group IIIb) and nanopropolis (group IVb) treated groups revealed positive immunoreaction in proliferating endothelial cells, dermal stromal cells and inflammatory cells in the crust. Negative reaction in the newly formed lymphatic vessels was observed in propolis treated group (Figures 16b-f).

After 21 days

Untreated group (group IIc) showed marked positive immunoreaction in proliferating endothelial cells and dermal stromal cells (Figure 17a). Both propolis (group IIIc) and nanopropolis (group IVc) treated groups revealed positive immunoreaction in epidermal keratinocytes and few dermal stromal cells (Figure 17b, c).

Morphometric results

Number of neutrophils and macrophages

The wounded untreated group exhibited a significant increase in neutrophil number and a reduction in macrophage number as compared to other treated groups all through the experimental periods. The propolis treated group revealed a significant reduction in neutrophils and an increase in macrophages on day 7 as compared with the nano group. On day 14, there has been a significant increase in neutrophils and a decrease in macrophages in comparison to the nano group. On day 21, there had been no significant differences between both treated groups (Table 2).

Number of new blood vessels

The number of new microvessels in the wounded untreated group increased significantly over time. Despite that, there was a significant decrease in the number at days 7, and 14 as compared to the treated groups. Both

treated groups also showed increased numbers in a time-dependent manner confined to 7 and 14 days post wounding. On day 21, this parameter returned to its normal level as compared with the normal control. There had been no significant differences between both groups through all the experimental periods (Table 3).

Area % of collagen fibers

All subgroups revealed a significant rise in collagen content with the increased duration after wound induction. When compared to the wounded control group, the average area percent of collagen fibers in the propolis and nanopropolis treated subgroups increased significantly ($P \leq 0.05$) regarding all durations. Propolis and nanopropolis groups didn't show a significant difference following 7 days. When compared to the propolis group, there has been a significant rise in collagen content in the nanopropolis groups following 14 and 21 days. All subgroups showed a significant decrease in collagen content as compared to the normal group, except the nanopropolis group after 21 days, where it revealed a non-significant difference with the normal control (Table 4).

Area % of CD 105 expression

All subgroups showed a significant rise ($P \leq 0.05$) in the average area percent of CD 105 immunoreactivity in the dermis for all durations when compared with normal control. When compared to the wounded control group after 7 and 14 days post-wounding, there had been a significant rise ($P \leq 0.05$) in immunoreactivity in the propolis and nanopropolis treated groups. While after 21 days, a significant decrease was detected. Propolis and nanopropolis groups didn't show a significant difference following 7 days. While there had been a significant rise in immunoreactivity in the nanopropolis group following 14 days and a significant decrease following 21 days when compared with the propolis group. Side wounds from all groups showed a significant increase in immunoreactivity in the epidermis as compared to the normal group. Treated groups showed increased immunoreactivity in time-dependent manner. Only on day 21 post wounding, the propolis and nanopropolis groups did differ significantly (Table 5).

Epidermal thickness after 21 days

The propolis-treated group revealed a significant rise in epidermal thickness following 21 days when compared with the normal, wounded control, and nano propolis groups. When compared to other groups, the wounded control group revealed a significant decrease. There was no significant difference between the normal and nano propolis groups (Table 6).

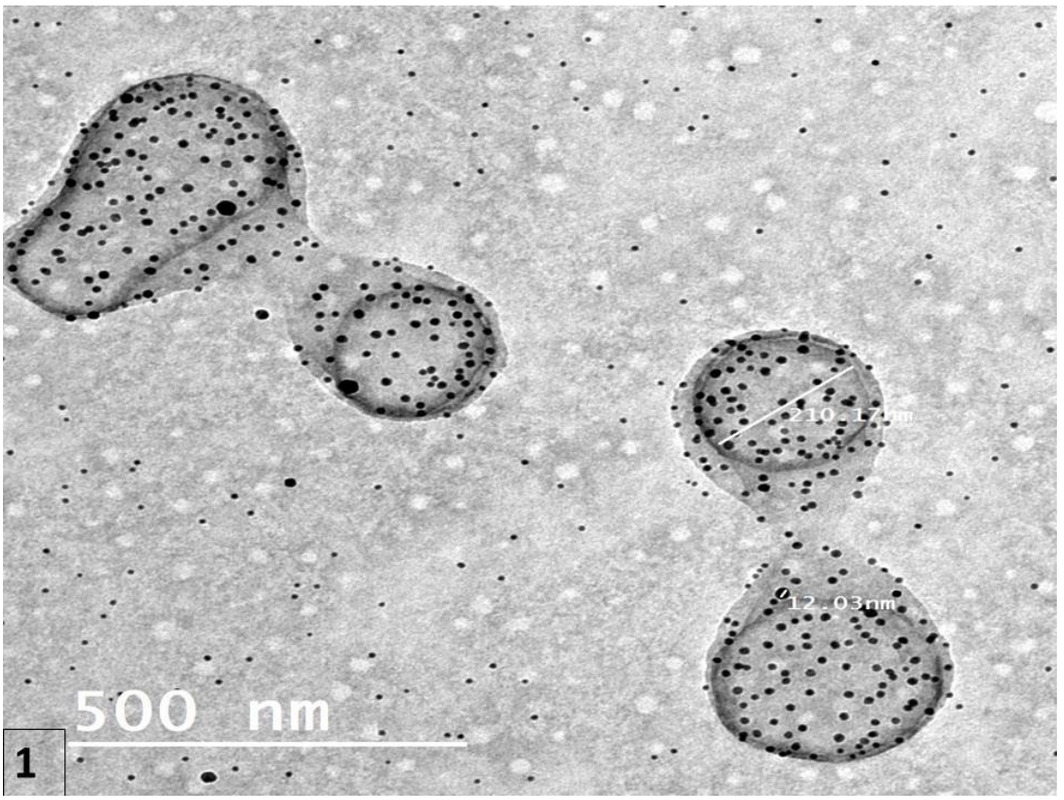

Fig. 1: Transmission electron photomicrograph of particle propolis nanocream under 40000 magnification showing the size of nano particles within its normal range

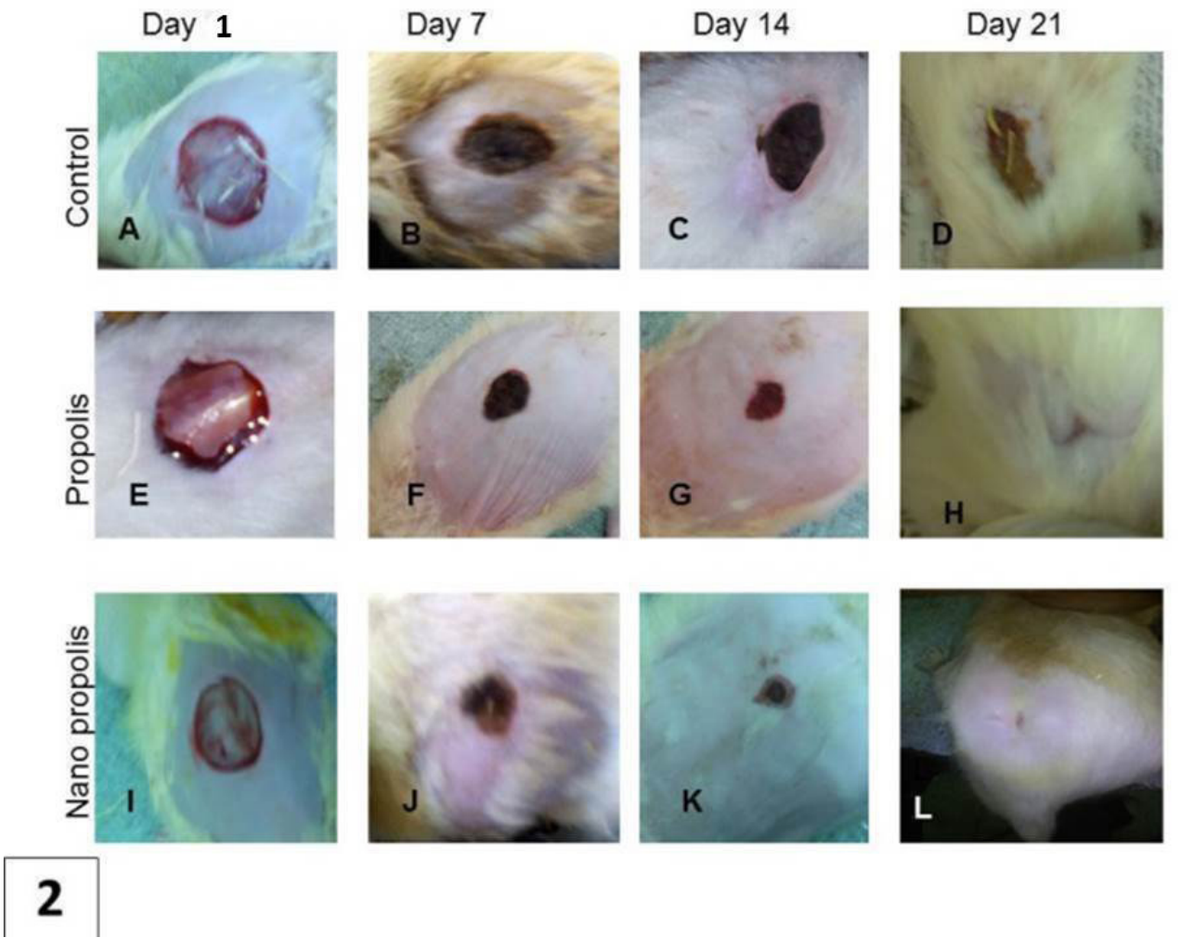

Fig. 2: Wound closure of wounded groups at different periods of healing

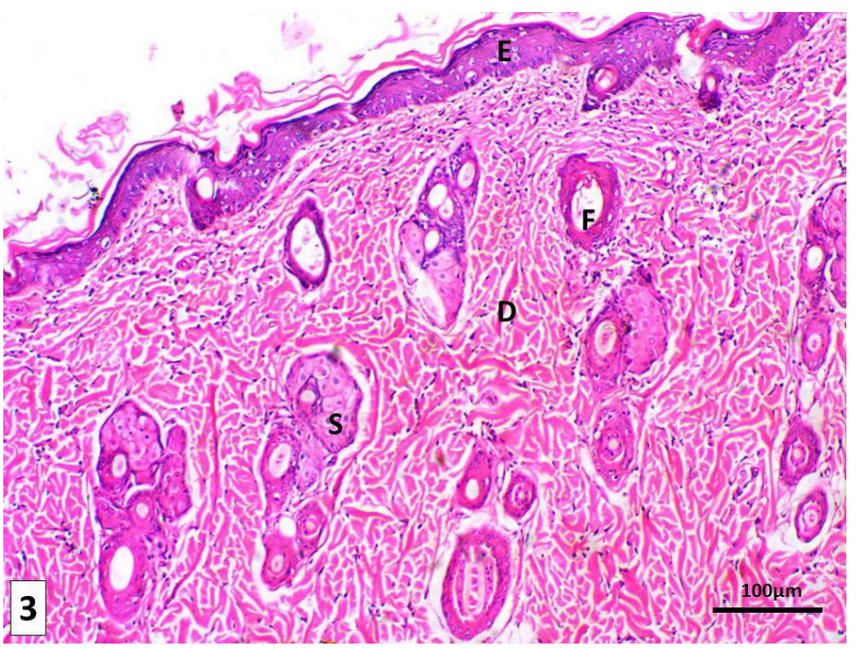

Fig. 3: A photomicrograph of a section in normal skin showing surface epidermis (E) and dermis (D) containing hair follicles (F) and sebaceous glands (S). (H & E x 100)

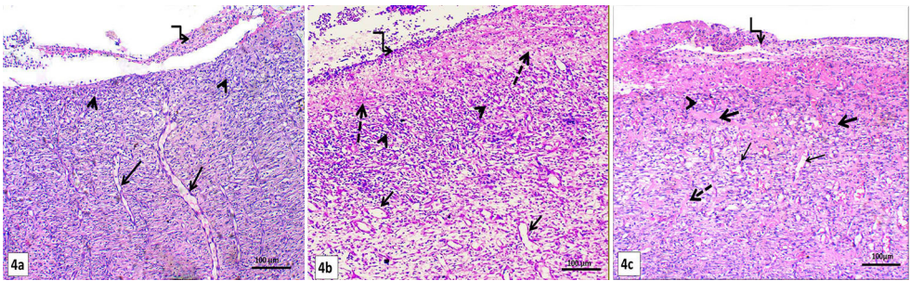

Fig. (4a-c): Photomicrographs of skin of rat in (a) subgroup IIa showing wound area with loss of covering epidermis and accumulation of more cellular connective tissue in the wound bed. Note the newly formed blood capillaries (thin arrows) and inflammatory cellular infiltration in the crust (wavy arrow) and dermis (arrow heads). (b) subgroup IIIa showing wound area with loss of covering epidermis and accumulation of both cellular connective tissue and thin collagen fibers (dotted arrows) in the wound bed. Note the newly formed blood capillaries (thin arrows) and inflammatory cellular infiltration in the crust (wavy arrow) and dermis (arrow heads). (c) subgroup IVa showing wound area with loss of covering epidermis and accumulation of both thin (dotted arrow) and thick (thick arrows) collagen fibers in the wound bed. Note the newly formed blood capillaries (thin arrows) and inflammatory cellular infiltration in the crust (wavy arrow) and dermis (arrow head). (H & E x100).

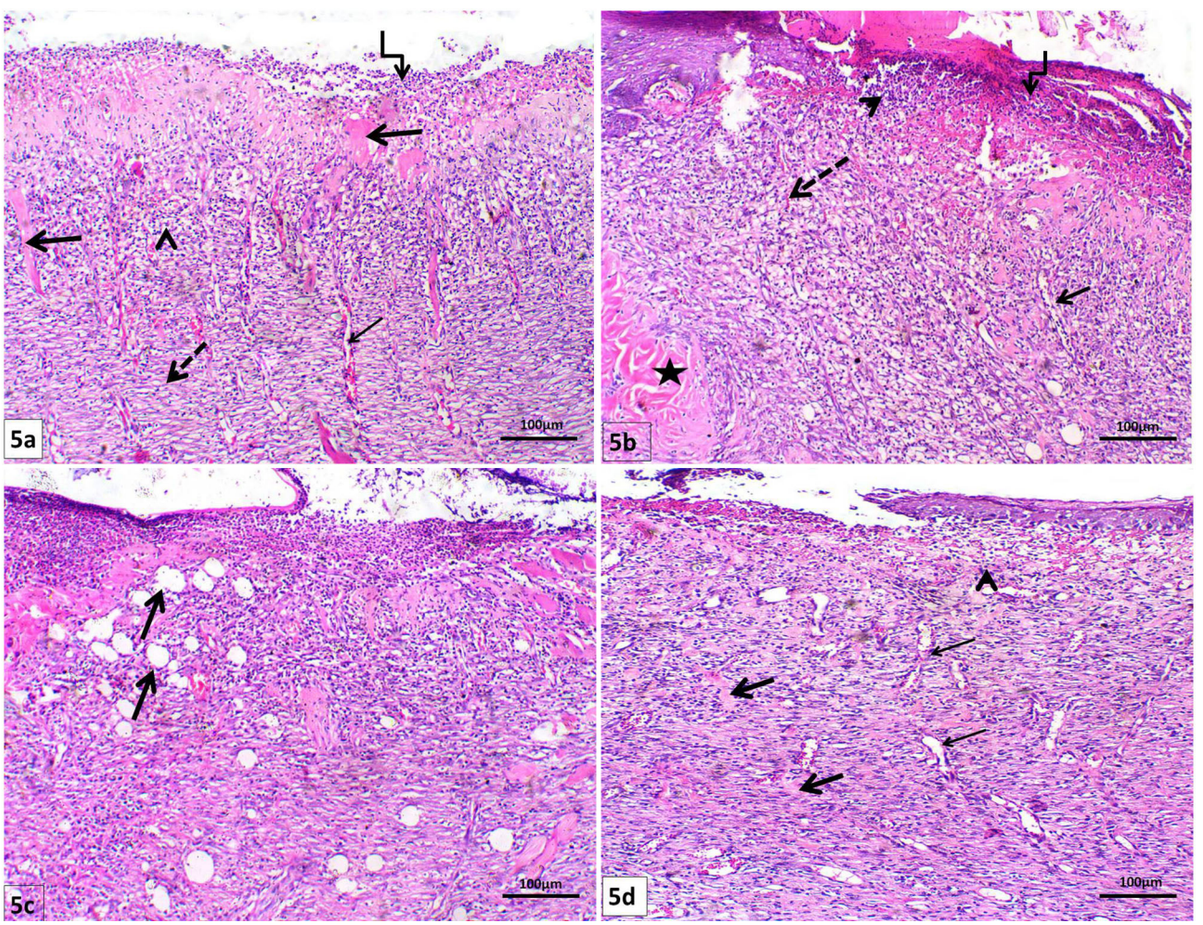

Fig. (5a-d): Photomicrographs of skin of rat in (a) subgroup IIb showing wound area with loss of covering epidermis and accumulation of thin (dotted arrow) and thick collagen (thick arrows) fibers in the wound bed. Note the newly formed blood capillaries (thin arrow) and inflammatory cellular infiltration in the crust (wavy arrow) and dermis (arrow head). (b, c) subgroup IIIb showing wound area with loss of covering epidermis in some parts and re-epithelialization at the side of wound. Note accumulation of collagen in the form of thin fibers (dotted arrow) in wound bed and thick bundles (star) under the newly formed epidermis. Wound bed shows parts with more cellular connective tissue. Note the newly formed blood capillaries (thin arrow) and inflammatory cellular infiltration in the crust (wavy arrow) and dermis (arrow head). (c): lymphatic vessels were evident in the dermis (thick arrows) and near the wound edge. (d): subgroup IVb showing wound area with loss of covering epidermis in some parts and re-epithelialization at the side of wound. Note accumulation of collagen in the form of thick fibers (thick arrows) in wound bed. Note the newly formed blood capillaries (thin arrows) and less inflammatory cellular infiltration in dermis (arrow head). (H & E x100).

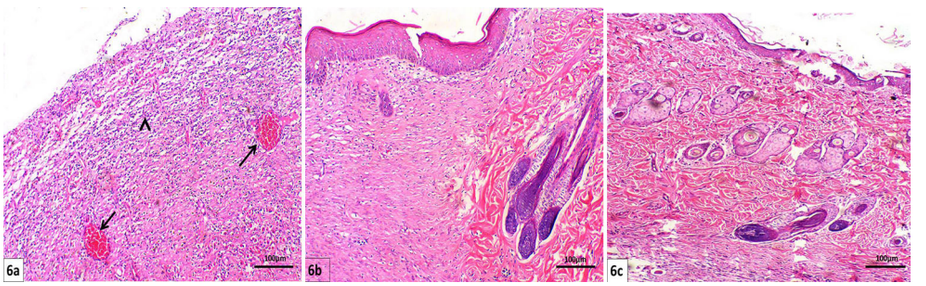

Fig. (6a-c): Photomicrographs of skin of rat in (a) subgroup IIc showing wound area with loss of covering epidermis and accumulation of thin and thick collagen fibers. Note the congested blood vessels (thin arrows) and less inflammatory cellular infiltration in dermis (arrow head). (b) subgroup IIIc showing filling of the wound area with more mature collagen in the form of thick fibers and thick bundles . Note the nearly complete re-epithelialization in the form of thick epidermis with deficient small area. New hair follicles are revealed. (c): subgroup IVc showing filling of the wound area with more mature collagen bundles. Note the nearly complete re-epithelialization in the form of thin epidermis with deficient small area. Many new hair follicles and sebaceous glands are revealed. (H & E x100).

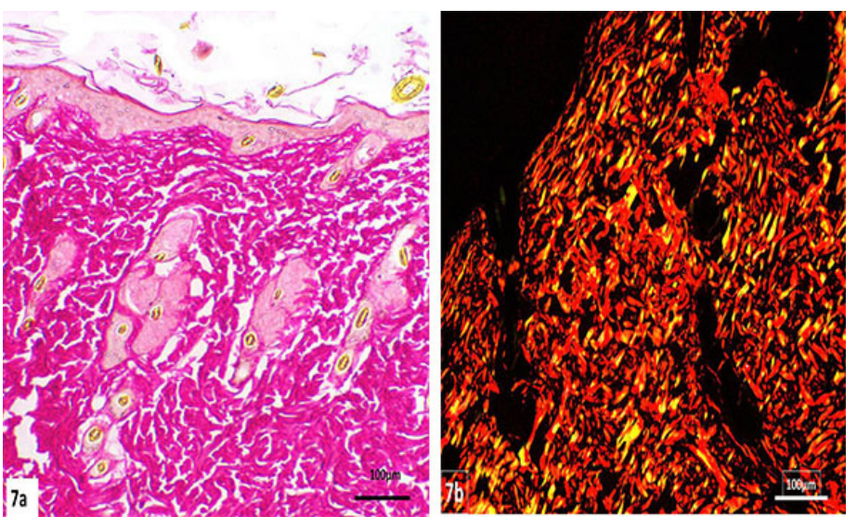

Fig. (7a, b): A photomicrograph of a section in normal skin showing (a) thick collagen bundles stained deep red under bright field microscope (b) predominantly thick orange collagen fibers (collagen I) and few thick yellow fibers (collagen III) under polarized light microscope. (picro sirius red x100)

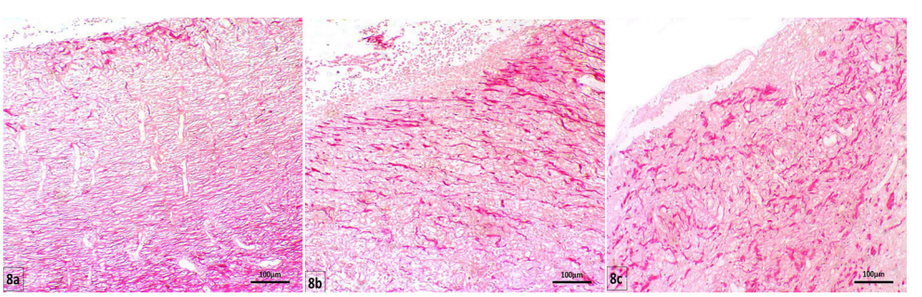

Fig. (8a-c): Photomicrographs of skin of rat in subgroups IIa, IIIa, IVa showing (a-c) Collagen accumulation in wound areas at day 7 post wounding. Note the collagen intensity and disposition of fibers stained red under bright field microscope. (picro sirius red x100)

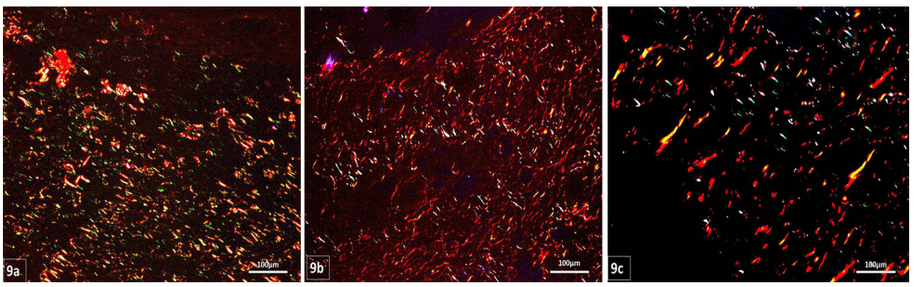

Fig. (9a-c): Photomicrographs of skin of rat in subgroups IIa, IIIa, IVa showing (a-c) Collagen accumulation in wound areas at day 7 post wounding. Note the collagen intensity and disposition of fibers with yellow to green birefringence under polarized microscope. (a) Some orange fibers (collagen I) and many thin green fibers (collagen III). (b) Predominantly thin orange fibers (collagen I) and few thin green-yellow fibers (collagen III). (c) Predominantly thick orange fibers (collagen I) with few green-yellow ones (collagen III). (picro sirius red x100)

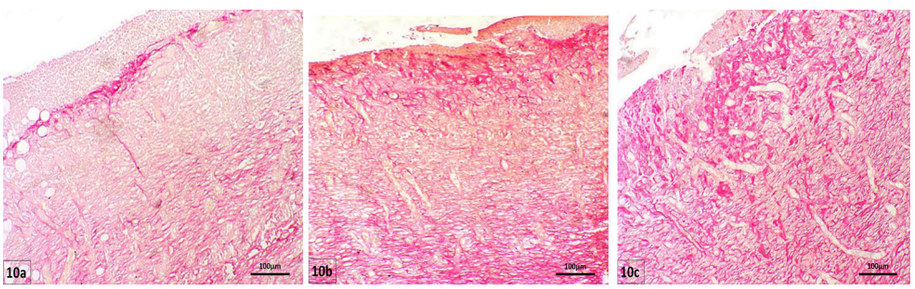

Fig. (10a-c): Photomicrographs of skin of rat in subgroups IIb, IIIb, IVb showing (a-c) Collagen accumulation in wound areas at day 14 post wounding. Note the collagen intensity and disposition of fibers stained red under bright field microscope. (a) Show predominantly red fine collagen fibers. (b) Show both thin and thick fibers. (c) Show predominantly thick collagen fibers. (picro sirius red x100)

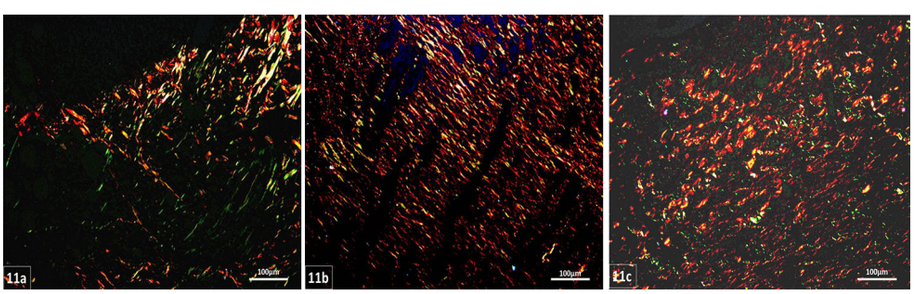

Fig. (11a-c): Photomicrographs of skin of rat in subgroups IIb, IIIb, IVb showing (a-c) Collagen accumulation in wound areas at day 14 post wounding. Note the collagen intensity and disposition of fibers with yellow to green birefringence under polarized microscope. (a) Orange (collagen I) and thin green fibers (collagen III). (b) Predominantly thin orange fibers (collagen I) and few thin green-yellow fibers (collagen III). (c) Predominantly thin and thick orange fibers (collagen I) with few thin green-yellow ones (collagen III). (picro sirius red x100)

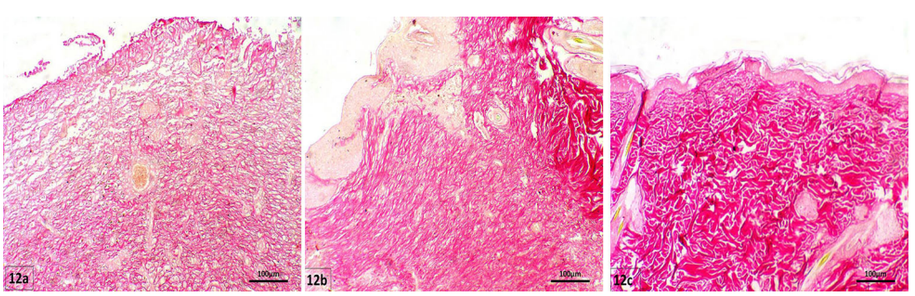

Fig. (12a-c): Photomicrographs of skin of rat in subgroups IIc, IIIc, IVc showing (a-c) Collagen accumulation in wound areas at day 21 post wounding. Note the collagen intensity and disposition of fibers stained red under bright field microscope. (a) Show predominantly red thick collagen fibers. (b) Show red thick fibers and bundles (c) show predominantly red thick collagen bundles. (picro sirius red x100)

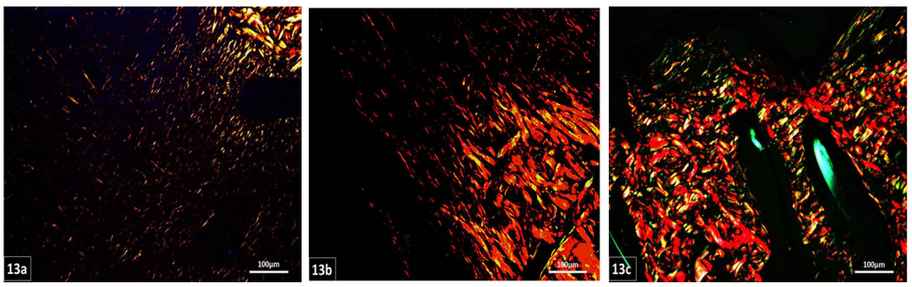

Fig. (13a-c): Photomicrographs of skin of rat in subgroups IIc, IIIc, IVc showing (a-c) Collagen accumulation in wound areas at day 21 post wounding. Note the collagen intensity and disposition of fibers with yellow to green birefringence under polarized microscope. (a) Some thin orange fibers (collagen I) and thick yellow fibers (collagen I). (b) Predominantly thin and thick orange fibers (collagen I) and few yellow fibers (collagen III). (c) Predominantly thick red fibers (collagen I) with few thick green-yellow ones (collagen III). (picro sirius red x100)

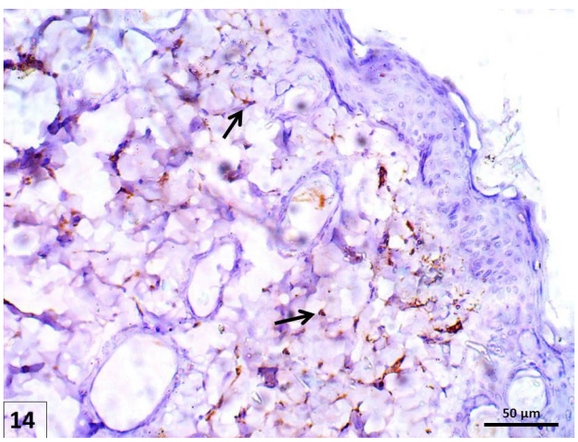

Fig. (14): A photomicrograph of a section in normal skin showing negative immunoexpression in epidermal keratinocytes and positive reaction in dermal stromal cells (arrows). (CD105 x 200)

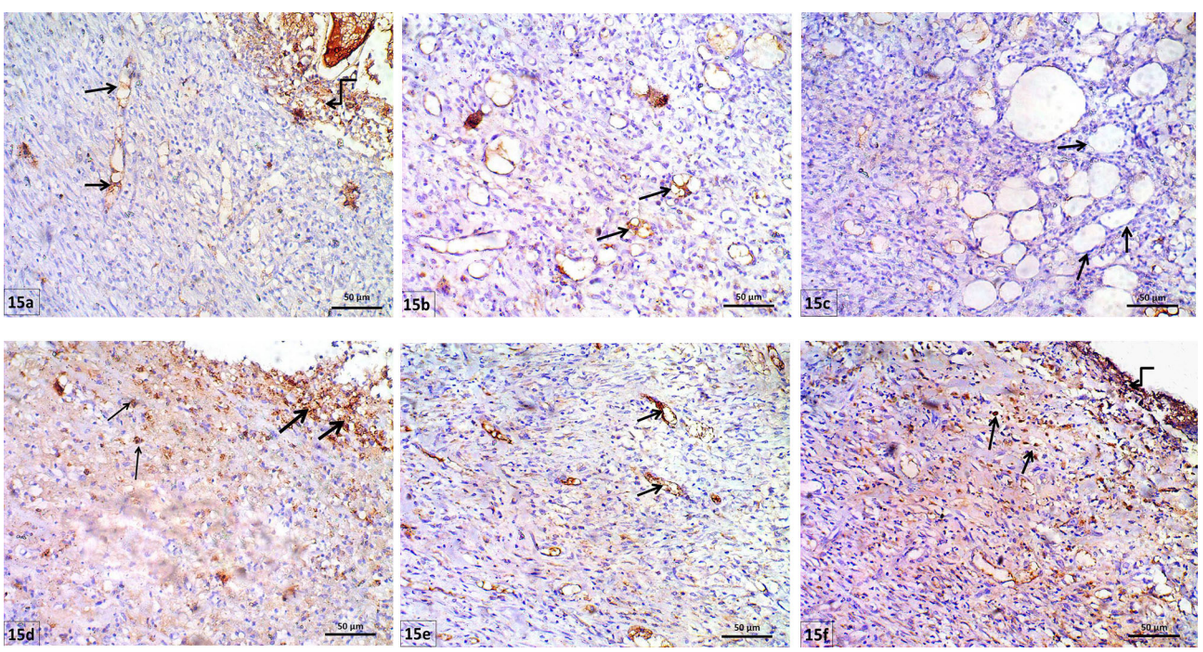

Fig. (15a-f): Photomicrographs of skin of rat in (a) subgroup IIa showing positive immunoreaction in proliferating endothelial cells (arrows) and inflammatory cells in the crust (wavy arrow). (b-d) subgroup IIIa showing (b): positive immunoreaction in proliferating endothelial cells and mild reaction in dermal stromal cells. (c): negative reaction in the newly formed lymphatic vessels (arrows). (d): intense immunoreactivity in inflammatory cells in the crust (thick arrows) and dermal stromal cells (thin arrows). (e, f) subgroup IVa showing (e): positive immunoreaction in proliferating endothelial cells (arrows). (f): intense reaction in inflammatory cells in the crust (wavy arrow) and dermal stromal cells (arrows). (CD105 x200).

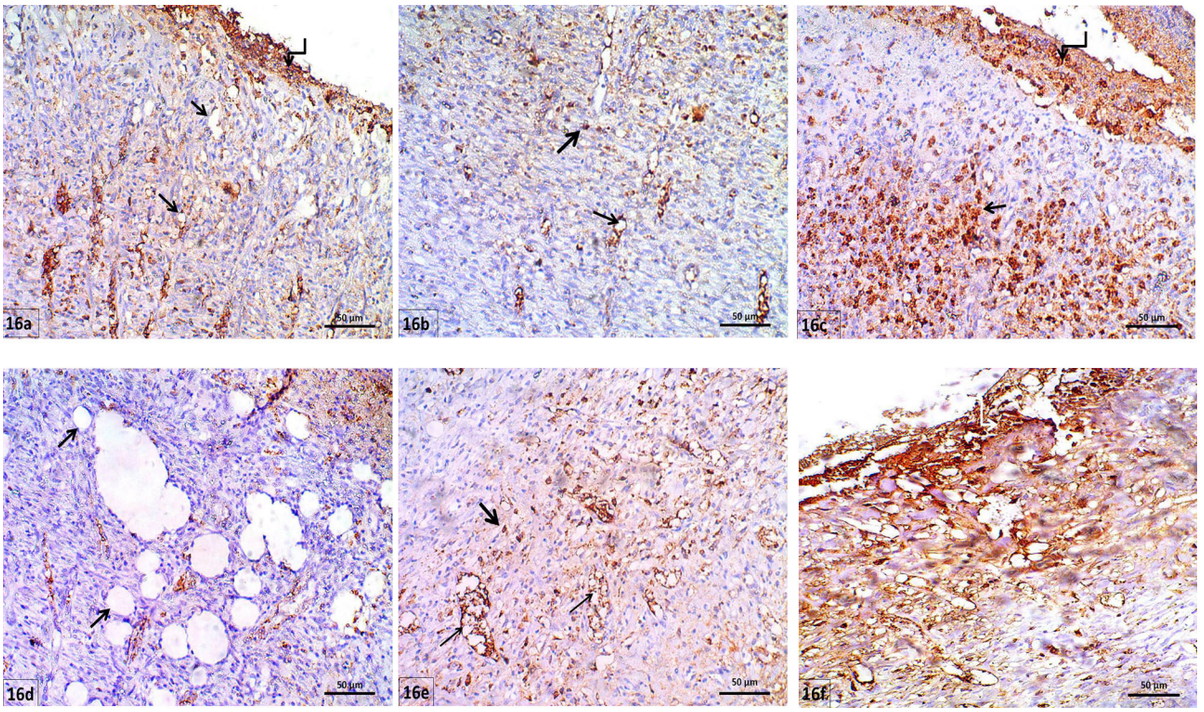

Fig. (16a-f): Photomicrographs of skin of rat in (a) subgroup IIb showing marked positive immunoreaction in proliferating endothelial cells (arrows), inflammatory cells in the crust (wavy arrow) and moderate reaction in dermal stromal cells. (b-d) subgroup IIIb showing (b): positive immunoreaction in proliferating endothelial cells (thin arrow) and dermal stromal cells (thick arrow). (c): intense immunoreaction in inflammatory cells in the crust (wavy arrow). Note the intense reaction in dermal stromal cells (arrow). (d): negative reaction in the newly formed lymphatic vessels. (e, f) subgroup IVb showing (e): positive immunoreaction in proliferating endothelial cells (thin arrows) and dermal stromal cells (thick arrow). (f): intense reaction in inflammatory cells in the crust (wavy arrow). (CD105 x200).

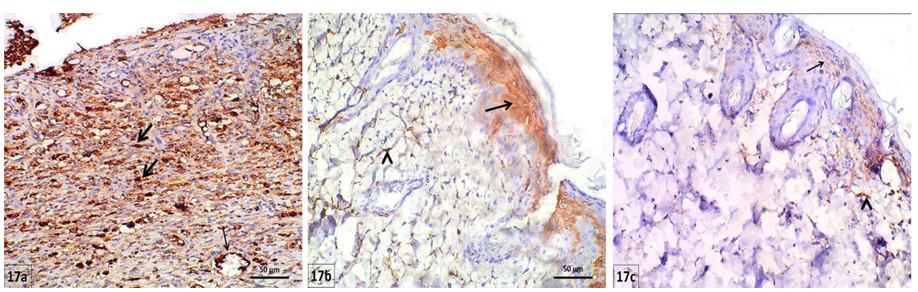

Fig. (17a-c): Photomicrographs of skin of rat in (a) subgroup IIc showing marked positive immunoreaction in proliferating endothelial cells (thin arrow) and dermal stromal cells (thick arrows). (b) subgroup IIIc showing positive immunoreaction in epidermal keratinocytes (arrow) and few dermal stromal cells (arrow head). (c): subgroup IVc showing positive immunoreaction in epidermal keratinocytes (arrow) and few dermal stromal cells (arrow head). (CD105 x200).

Table 1: Mean ± SD of rate of wound size reduction (%)

Groups	Day 7	Day 14	Day 21
Group II(wounded control group)	26.2±3.8	55.7±3.02	76.7±5.9
Group III (propolis group)	48.2±5.2*#	75.3±2.4*#	96.4±2.08*
Group IV (nano propolis group)	63.3±8.6*	88.6±1.3*	99.6±0.75*

*$P \leq 0.05$, significant difference compared to wounded control group
#$P \leq 0.05$, significant difference compared to nano propolis group

Table 2: Mean number ± SD of neutrophils and macrophages

Groups	Day 7		Day 14		Day 21	
	neutrophils	macrophages	neutrophils	Macrophages	neutrophils	macrophages
Group I (normal control)	1.4±0.5	20.8±1.1				
Group II (wounded control)	24.6±0.5*	17.2±1.3*	31.4±1.1*	16.2±8.3*	33.1±1.2*	44.8±0.83*
Group III (propolis group)	10.8±0.7*#@	45±0.7*#@	7.8±0.4*#@	40.8±0.8*#@	1.4±0.5#	21.2±0.8#
Group IV (nano propolis group)	15±1.2*#	25.4±0.5*#	4.8±0.8*#	48.2±0.7*#	1.6±0.5 #	22.6±1.6#

*$P \leq 0.05$, significant difference compared to normal control group

#$P \leq 0.05$, significant difference compared to wounded control group

@ $P \leq 0.05$, significant difference compared to nano propolis group

Table 3: Mean number ± SD of number immunostaind blood vessels

Groups	Day 7	Day 14	Day 21
Group I (normal control)	1.4±0.6		
Group II (wounded control)	4.6±0.5*	9.4±0.8*	15±1.5*
Group III (propolis group)	13.2±0.8*#	20.4±0.5*#	1.4±0.5#
Group IV (nano propolis group)	14.4±0.5*#	22.2±0.8*#	1.3±0.5 #

*$P \leq 0.05$, significant difference compared to normal control group

#$P \leq 0.05$, significant difference compared to wounded control group

Table 4: Mean area% ± SD of collagen fibers

Groups	Day 7	Day 14	Day 21
Group I (normal control)		55±1.01	
Group II (wounded control)	12.5±0.7*	14.7±0.6*	23.4±0.5*
Group III (propolis group)	15.1±0.7*#	21.3±0.32*#@	41.1±3.6*#@
Group IV (nano propolis group)	15.6±0.3*#	25.1±0.2*#	50.4±1.4 #

*$P \leq 0.05$, significant difference compared to normal control group

#$P \leq 0.05$, significant difference compared to wounded control group

@ $P \leq 0.05$, significant difference compared to nano propolis group

Table 5: Mean area% ± SD of CD 105 immunoreactivity in dermis and epidermis

Groups	Day 7		Day 14		Day 21	
	dermis	epidermis	dermis	epidermis	dermis	epidermis
Group I (normal control)	2.03±0.3	0.12±0.001				
Group II (wounded control)	4.04±0.6*	4.3±0.35*	7.9±0.5*	4.5±0.21*	22.7±0.9*	4.4±1.07*
Group III (propolis group)	5.9±0.5*#	2.4±0.14*#	16.14±0.2*@	3.2±0.35*#	2.6±0.4#	11.9±1.3*#@
Group IV (nano propolis group)	6.5±1.02*#	2.6±0.2*#	21.9±0.96*#	3.6±0.23*	2.4±0.3#	9.3±0.4*#

*$P \leq 0.05$, significant difference compared to normal control group

#$P \leq 0.05$, significant difference compared to wounded control group

@ $P \leq 0.05$, significant difference compared to nano propolis group

Table 6: Mean ± SD of epidermal thickness after 21 days post wounding (µm)

Groups	Group I (normal control)	Group II (wounded control)	Group III (propolis group)	Group IV (nano propolis group)
Day 21	20.5±2.13	9.9±1.2*	28.9±2.23*#@	19.3±1.14#

*$P \leq 0.05$, significant difference compared to normal control group

#$P \leq 0.05$, significant difference compared to wounded control group

@ $P \leq 0.05$, significant difference compared to nano propolis group

DISCUSSION

Aging is a physiological process where a decline in cell functions and a deficit of some organs' renewing responses happens[19]. Many physiological pathways are triggered following tissue damage in order to stop infection and repair the injured tissues. Monocytes, neutrophils, lymphocytes, and dendritic cells, as well as endothelial, keratinocyte, and fibroblast cells, are some of the cells that are activated during the healing of wounds. These cells have central role in cell migration, differentiation, and proliferation[20]. Regrettably, ageing has an imperfection in gene expression regulation in those directing cells[19].

Non-healing wounds and infected wounds are common problems. So, numerous studies have been conducted to look at various compounds that can accelerate wound healing. Glycerol, platelet-rich plasma (PRP), zinc compounds, tripeptide copper complex (TCC) hydrogel, sildenafil, tocopherol, Lantana (Lantan camara), pomegranate (Punica granatum), chitosan, stem cell treatment, honey, and ribwort plantain leaf hydroethanolic extract were among these compounds[21].

The best topical formula for wound healing has to be biocompatible, nontoxic, and able to increase the healing with no side consequences[22]. Because of its biological and pharmacological properties, propolis has attracted the attention of many researchers[23]. Therefore, propolis was selected as a natural product in the current research to assess its impact on complete thickness skin wound healing due to its helpful features and absence of negative impacts. The Nanopropolis formula was also prepared and compared with the ordinary formula.

The current investigation showed a significant enhancement in healing of wounds in the propolis and nano propolis treated groups in a time-dependent manner. This was confirmed by measuring the rate of wound reduction as an assessment tool for measuring the progress of healing. It is worthy to mention that propolis and nano propolis treated groups showed almost complete closure after 21 days with appearance of skin appendages. These were in agreement with Abu-Seida,[15] who used propolis paste on excisional wounds, Sarhan & Azzazy,[24] who revealed antibacterial and wound healing activity of nanofibers loaded with propolis; Haghighian et al.[25] who revealed increased wound healing in diabetic rats by topical propolis extract; and Sahib et al.[26] showed faster healing of wounds with propolis ointment. On the other hand, the untreated group's wounded sites did not heal till day 21, with no appearance of skin appendages. These findings were consistent with those of Gushiken et al.[27] and Samdavid Thanapaul et al.[28], who observed decreased wound healing in aged skin.

Age-related changes in the inflammatory response, like delayed T-cell infiltration into the wound site, may contribute to delayed wound healing. A decrease in the macrophage phagocytic capacity and subsequent changes in the production of chemokines were documented. Aged wounds were also found to exhibit delayed angiogenesis, collagen synthesis, and re-epithelialization[29].

The higher wound closure rate in propolis-treated wounds could be due to the high proliferative activity of fibroblasts and their transformation into myofibroblasts. Increased keratinocyte migration and proliferation to the wound surface could also contribute to accelerated wound healing and re-epithelialization in propolis-treated wounds. Because it provides the wound region with the necessary oxygen and nutrients, angiogenesis plays an important role in the formation of granulation tissue during wound healing[30,31].

The significance of nanotechnology involvement in our study is likely to increase the potential therapeutic properties of this propolis and to increase its solubility, bioavailability, controlled-release, as well as infiltration ability[32]. The preparations that contain nanopropolis can also have the benefits of augmented penetration of flavonoids and phenols through the layers of skin, with consequent better healing results. Given that skin is a multi-layered structure makes it extremely difficult for drugs to infiltrate, nanopropolis may have encouraging effects on wound healing[33]. Additionally, nanopropolis is more water-soluble than traditional propolis[34].

So, the superiority of nanopropolis cream over conventional propolis cream in increased wound closure could be due to the fore mentioned healing properties of propolis, the advantage of nanotechnology intervention, and the presence of copper nanoparticles in the nanopropolis formula. As copper is essential for skin regeneration and angiogenesis, it thus accelerates the healing process through stimulation of VEGF and angiogenesis by HIF-1α [35].

Infiltration of inflammatory cells was observed in the control and treatment groups. However, inflammatory cellular infiltration at day 7 post wounding was less obvious in the control group than in the propolis and nanopropolis groups. This could be due to delayed macrophage and lymphocyte infiltration, decreased macrophage function in the elderly group, resulting in decreased growth factor secretion, late re-epithelialization, impaired angiogenesis and deposition of collagen, decreased collagen turnover, remodeling, and wound strength[5,6]. These reasons were concomitant with the increased neutrophil number with subsequent extended inflammatory phase and the lower number of macrophages in the control group.

The propolis treated group at day 7 showed increased numbers of inflammatory cells as compared to the nanopropolis treated group. In spite of this, the increase in number was confined mainly to macrophages while the number of neutrophils was significantly lower. This indicated that propolis started the proliferation stage with the subsequent ending of the inflammatory stage. The less inflammatory cell number of the nano treated group might be due to increased pharmacological activity, bioavailability, solubility, and stability. The advantages

of nanodrugs are sustained delivery, improved tissue macrophage distribution, toxicity prevention, and protection against chemical and physical deterioration[36], besides the effect of the copper base in the nanocream formula as an antimicrobial[37]. It was worthy to mention that nanopropolis group showed significant increase in macrophages number and less neutrophil number, which was a good indication of starting proliferation and ending inflammation.

When compared to day 7, inflammatory cells in the control group significantly increased at days 14 and 21. This may be attributed to the progression of the healing process of the wound and may indicate an extended inflammatory phase of the healing process. The wounds managed with propolis and nanoproplis cream revealed decreased inflammatory cellular infiltration at day 14 and a return to normal levels at day 21. This may support the hypothesis that propolis possesses an immune-modulatory effect that has a role in reducing inflammation as reported by Magnavacca et al.[38]. However, nanopropolis cream was still showing significantly less inflammatory cells at day 14 as compared with conventional cream. This was most probably due to the mixed anti-inflammatory effect of both propolis and copper base in the nano formula as mentioned before.

It is important to mention that macrophages have very essential functions in all stages of the adult healing process, including inflammation, proliferation, and remodeling. As per the stage of the process of healing, the local macrophages change from being primarily pro-inflammatory (M1-like phenotypes) to anti-inflammatory (M2-like phenotypes) as wounds heal. Non-healing chronic wounds, as well as ageing, cause macrophages to remain inflamed (the first phase of wound healing). Therefore, during this time, local macrophages exhibit pro-inflammatory properties[39]. So, the phenotype of macrophages and its correlation with the healing stage were more important than their number.

At days 7 and 14 post-wounding, the propolis and nanopropolis treatment groups showed more new blood vessels. This was concomitant with some investigators' findings[40] that revealed new microvessels. Furthermore, propolis-treated groups showed dilated lymphatic vessels. This was in accordance with Martínez-Corral et al.[41] and Ibrahim[42], who discovered a rise in angiogenesis and lymphangiogensis during the normal process of wound healing. Asai et al.[43] showed that simvastatin was shown to increase lymphangiogenesis and angiogenesis in rats with genetic diabetes when it was applied topically during the healing of wounds.

Transient processes that occur during the healing of wounds include lymphangiogenesis and angiogenesis. Like angiogenesis, lymphangiogenesis results from the production of vascular endothelial growth factor, the expression of matrix metalloproteinase, and the migration, proliferation, and organization of endothelial cells into functional vessels. However, a tissue's response to hypoxia is angiogenesis[44]. As a tissue response to the flow of interstitial fluid, lymphangiogenesis occurs[45].

Ji et al.[46] have reported lymphatic vessels extending along the wound edges between 7 and 15 days post wounding. Another study detected new lymphatics in the dermis and wound edges[47]. Debates were found about the source of these new vessels, some mentioned that they were derived in situ from macrophages[48] and others documented that they are derived from preexisting lymphatic vessels in subcutaneous tissue[47,49]. The significance of lymphangiogenesis as a step in the healing of wounds is to accelerate the drainage of stagnant interstitial fluid[47]. The aforementioned data may clarify the dual beneficial effect of propolis regarding angiogenesis and lymphangiogensis during wound healing.

It was remarkable that new lymphatic vessels were not evident in nanopropolis treated group. This could be explained that nano formula containing propolis and copper showed powerful anti-inflammatory effect that may inhibit the process of lymphangiogenesis through preventing fluid stagnation. This was correlated with less inflammatory cellular infiltration in nanopropolis group as compared to propolis group.

There was an interesting finding regarding epidermal thickness of treated groups after 21 days post wounding. Significantly more epidermal thickness was found in the propolis treatment group compared to the nanopropolis treatment group and normal control. The possible explanation for this finding might be related to hypertrophic scar formation[50]. This also declared that nano formula had better effect on normal epithelialization without scar formation. This was concomitant with Borkow & Melamed,[51] who revealed absence of scar formation when using copper.

It is worthy to mention that previous studies used PSR staining for collagen detection. They evaluated PSR stained section by both polarized light and bright-field illumination. By using bright–field illumination, the denser fibers had a deep red appearance, while the tinny fibers had a bright pink appearance[52,53]. Picro sirius red histochemical reaction boosts collagen birefringence, so, collagen could be detected easier by polarized light. Certain collagen fibers cannot always be stained with traditional trichrome in the same color. Some fibers don't even show any stains. With picro sirius red, however, even tiny collagen fibers could be detected. Reviewing the difference in collagen type I and III distribution using PSR is also valuable[54].

Collagen deposition originates as fine fibrils, and then they converted to cross-linked larger fibers and bundles. Thin bundles of newly formed collagen type III molecules look green/yellow, while big ones of mature collagen type I molecules that are firmly packed and well-ordered look red/orange[55].

Concerning the area percentage of collagen content, all experimental groups showed progressive increase in time

dependent manner, but they differed in disposition and pattern of distribution of collagen fibers. In comparison to other subgroups, the non-treated subgroup showed a significant reduction ($P \leq 0.05$) in the deposition of collagen fibers (measured by mean area%). This was in concomitant with Sgonc & Gruber, (5) and Smith et al.[56] who documented decreased collagen synthesis in aged wounds. It was evident that in the non-treated group, immature collagen type III predominated over mature collagen type I.

Ashcroft et al.[57] discovered that matrix metalloproteinase-2 and-9 (MMP-2, -9) were up-regulated in skin wounds of old individuals for 84 days following injury. They also have shown the down-regulation of tissue inhibitors of metalloproteinase-1 and -2. This suggests that elderly skin is prone to tissue breakdown. Because of the unbalance among MMPs and their inhibitors, proteolytic activity increased, resulting in a decreased deposition of collagen. Furthermore, transforming growth factor -1 (TGF-1) decreased in wound tissue of old persons. Increased collagen deposition is known to occur by encouraging its synthesis and decreasing its degradation.

On day 11 following injury, older mice's late-stage wounds displayed delayed collagen remodeling and less organized collagen than those of younger mice. Regardless of the aging delay in wound healing speed during early stages, wounds can show increased maturation and improved quality of scars under ideal care of wounds. But under suboptimal conditions like altered adaptive and innate immune responses, postponed re-epithelialization, ageing of fibroblasts, and late deposition of collagen, the wound may be more vulnerable to secondary offenses like infection and recurrent injury[58].

The average area% of collagen fibres significantly increased in the propolis and nanopropolis treated groups compared to the non-treated group. This was in line with other research by Jacob et al.[59], Nani et al.[60], and Ebadi & Fazeli[61], which showed that topical propolis extract promoted deposition of collagen and granulation tissue. It was obvious that immature collagen type III that was immature was replaced gradually by collagen type I that was mature in a time-dependent manner. It was worthy to mention that nanopropolis group showed collagen content and type nearly comparable to normal skin.

Propolis has anti-aging, healing of wounds, and tissue regeneration properties attributed to its inhibitory effect on MMP-3 activity, the enzyme involved in collagen and elastic fiber degradation[62,63]. It could possibly work by diminishing acute inflammatory exudation and stimulating macrophages and T lymphocytes, which would subsequently stimulate fibroblast activity[64]. Previous studies have shown that propolis has the capability to impede the rate of deposition of collagen type I only in the earliest phases of the process of healing (from Day 4 to 7 post wounding). Collagen deposition reached the normal level after two weeks[65,66]. Deposition of collagen type III can significantly quicken the process of healing as it plays a part in hemostasis, via binding of coagulation factors, inducing aggregation of platelets and signaling activities, or encouraging the formation of thrombi[67,68]. Furthermore, the regulation of collagen I fibrillogenesis and cell migration depends on the framework that collagen type III forms[69,70].

According to previous studies, accumulation of type I collagen inside the matrix of a propolis-treated wound may encourage the healing process. This is because collagen I, a portion of the skin's connective tissue, is necessary for the migration of keratinocytes and re-epithelialization[71,72]. So, propolis may hasten the proliferative stage of the process of healing, with consequent quick conversion of collagen type III into type I as well as modulation of the inflammatory response. The start of the maturation stage can differ according to wound size and whether it was primarily an incisional or excisional wound. It ranges from around three days to three weeks. A year or more may pass during the maturation stage. This could vary based on the type of wound[73,74].

Besides the aforementioned role of propolis in collagen formation, there was another important substance incorporated in the nano formula, copper particles. Two crucial characteristics of copper make it a superior component for use in formulas for the healing of wounds. First, copper is essential for angiogenesis, dermal fibroblast proliferation, the deposition of collagen and elastin fibers, as well as acting as a cofactor of Lysyl oxidase that is important for the effective cross-linking of extracellular matrix (ECM) proteins. Secondly, copper possesses strong, broad-spectrum antimicrobial capabilities. So, it can clear infection, induce granulation, and epithelialize necrotic wounds; reduce postsurgical swelling and inflammation; and reduce the formation of scars[51].

In the present study, endoglin (CD105) immunostained skin sections for the control group's normal skin showed a mild localized positive response within dermal cells. This was correlated to the existence of resident mesenchymal stem cells (MSCs) in specific regions known as niches close to the basement membrane. It became activated in response to stress, then migrated to repair tissues[75].

The present work revealed increased endoglin (CD105) immunoexpression in dermal stromal cells and the new blood vessels' endothelial lining in all experimental wounded groups at days 7 and 14 in a time-dependent manner. However, a significant increase in CD105 immunoexpression (represented by mean area %) in propolis and nanopropolis treated groups was observed as compared to non-treated group. This increase in immunoexpression indicated the activation of MSCs in the wound area. Activated MSCs have an important role in wound healing through different phases. MSCs have shown paracrine signaling effects, immunomodulatory effects that regulate inflammation, stimulating effects on the fibroblast, collagen production, stimulation of neovascularization as

well as re-epithelialization. MSCs also secrete numerous growth factors and cytokines[76,77].

Positive CD105 immunoreactivity in all experimental groups' vascular endothelium could be attributed to higher levels of ANG-1 angiopoietin (ANG-1) and VEGF-α in the wounds. By promoting endothelial cell proliferation, migration, and tubule organization, they play essential roles in angiogenesis[78]. In spite of this, Aging was associated with dysregulation in most of these factors, leading to delayed wound healing[79].

According to recent studies, propolis may influence the immune system via reducing inflammatory cytokine production and enhancing the impact on resident stem cells[80]. This may give a good explanation to the increased immunoexpression of CD105 at 7 and 14 days post wounding.

It is worthy to mention that CD105 immunoexpression was elevated in non-treated group after 21 days post wounding while propolis and nanopropolis groups revealed significant decrease in dermal immunoexpression. It was nearly comparable to the normal control. This reduction may be due to entry in the maturation and remodeling phases of wound healing. Keratinocytes of the epidermis were also still showing increased immunoexpression versus normal control. This could be attributed to partially incomplete re-epithelialization.

CONCLUSION

In conclusion, the current study results confirmed the importance of CD105 in the process of wound healing. Additionally, it demonstrated how effectively propolis and nanopropolis formulas treated aged wounds. This technique might be effective for treating chronic wounds safely and efficiently.

ABBREVIATIONS

VEGF: Vascular endothelial growth factor, **HIF-1α:** Hypoxia-induced factor-1-alpha, **CD105:** Endoglin, **PSR:** Picro Sirius red, **H & E:** hematoxylin and eosin, **MMP 2,3,9:** Matrix metalloproteinase 2, 3, 9, **TGF-1:** Transforming growth factor-1, **MSCs:** Mesenchymal stem cells, **ANG-1:** Angiopoietin, **ECM:** Extracellular matrix.

ETHICAL APPROVAL

The National Organization for Drug Control and Research's (NODCAR) Animal Ethics Committee supervised the care and usage of the animals, which has been done in accordance with international standards for the usage of animals in research purposes and with respect to ethical principles.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة مقارنة حول تأثير تركيبتين موضعتين لكريم عكبر النحل في الشكل العادي والنانو على التئام الجروح في الجرذان المسنة (دراسة هستولوجية وهستوكيميائية مناعية)

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الخلفية: يمكن أن يؤدي التقدم في السن إلى تأخر التئام الجروح. البروبوليس (عكبر النحل) له تأثير جيد على الشفاء.
الأهداف: تم إجراء هذا الفحص لتقييم ما إذا كانت كريمات البروبوليس والنانوبروبوليس يمكن أن تساعد في التئام الجروح في الجرذان المسنة.
المواد والطرق: تم فصل خمسين من الجرذان البيضاء المسنه إلى أربع مجموعات: المجموعة الأولى (المجموعه الضابطة للجلد السليم) (٥ جرذان) ؛ المجموعات الجريحة: المجموعة الثانية (المجموعة الضابطة الجريحة) (٣ مجموعات فرعية ، ٥ جرذان لكل منها) ؛ المجموعة الفرعية ٢أ: تم تطبيق الكريم الحامل موضعيا مرة واحدة يوميًا لمدة ٧ أيام ؛ المجموعة الفرعية ٢ب: مرة واحدة يوميًا لمدة ١٤ يومًا ؛ والمجموعة الفرعية ٢ج: مرة واحدة يوميًا لمدة ٢١ يومًا ؛ المجموعة الثالثة (مجموعة البروبوليس) ، التي تم تقسيمها إلى ٣ مجموعات فرعية كل منها ٥ جرذان ،(المجموعة الفرعية ٣ أ) تم تطبيق كريم البروبوليس موضعيا مرة واحدة يوميًا لمدة ٧ أيام ؛ (المجموعة الفرعية ٣ ب) مرة واحدة يوميًا لمدة ١٤ يومًا ؛ و(المجموعة الفرعية ٣ج) مرة واحدة يوميًا لمدة ٢١ يومًا ، المجموعة الرابعة (مجموعة النانوبروبوليس) ، والتي تم تقسيمها إلى ٣ مجموعات فرعية كل منها ٥ جرذان ، وتم تطبيق كريم النانوبروبوليس موضعياً لنفس الفترات المذكورة أعلاه في المجموعة الثالثة. تم استخدام صبغة الهيماتوكسيلين والأيوسين ، والتفاعل الكيميائي النسيجي بيكرو سيريوس الأحمر (picro Sirius red)، و CD ١٠٥ المناعي في هذه الدراسة.
النتائج: تحسن التئام الجروح بشكل ملحوظ في مجموعتي البروبوليس والنانو بروبوليس ، مع زيادة ملحوظة في معدل إغلاق الجرح وترسب الكولاجين.
الخلاصة: أكدت نتائج الدراسة الحالية أهمية CD١٠٥ في عملية التئام الجروح. بالإضافة إلى ذلك ، فقد أظهر مدى فعالية تركيبات البروبوليس والنانوبروبوليس في علاج الجروح المسنة. قد تكون هذه التقنية فعالة في علاج الجروح المزمنة بأمان وفعالية