

**EFFECT OF THE CUBAN PROPOLIS COLLECTED IN MANZANILLO
AREA ON THE WOUNDS HEALING IN RATS**

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Summary

The necessity to evaluate the effect on the wounds healing of the Cuban propolis collected in Manzanillo area was the aim of this work. Forty Sprague Dawley rats, (CENPALAB; CUBA), twenty female and twenty male were used in the study. Nine millimetres-diameter, skin wounds were practiced on the external side of the right upper hind limb with a skin biotome (Acu, Acuderm Inc, USA) in aseptic conditions. Rats were distributed among 4 experimental groups of 10 animals each one. In every animal two wounds were medicated with 10% propolis cream and 1% silver sulfadiazine respectively, one with the base of the formulation and another was the non-medicated control. Wounded tissue was harvested on day 5 and day 8, and used for histological examination and morphometric studies with computerized-assisted using DIGIPAT image processing package. Propolis cream treated wounds had a total re-epithelization of 60%. Statistically the stimulation of the total re-epithelization was more significant ($p < 0.05$) in comparison to the others. Similarly, these wounds had the largest rate of epithelial linear ingrowth and the lowest values of wound perimeter ($p < 0.05$). Dermis reconstitution was stimulated in the propolis cream treated wounds but it wasn't significantly improved with respect to the non-medicated control. Inflammatory infiltrate was reduced in propolis cream treated wounds, and showed a quickly highest fibrovascular reaction compared to the others. The propolis cream help to the wound healing but did not accelerate this process.

Key words: propolis, wounds healing, rats, skin wounds

Methods

Propolis cream was elaborated with propolis extract collected from beehives belonging to the Apiculture Station of the Manzanillo area, Granma province. In the formulation an emulsion base of oil in water was used.

Forty Sprage Dawley rats (National Center for Laboratory Animal Production, Havana, Cuba), twenty female and twenty male with 250g average weight were kept under controlled environmental conditions (constant air humidity with a 12h light dark cycle) with free access to water and food, the procedures were approved by the ethical committee for animal experimentation of Research Center and Biological Evaluations.

Nine millimeters-diameter, skin wounds were practiced on the external side of the right upper hind limb with a skin biotome (Acu Punch, Acuderm Inc, USA) in aseptic conditions and under sufficient anesthetic conditions induced with diethylether mask.

Rats were distributed among four experimental groups of 10 animals each:

Group A: Male rats sacrificed on day 5 of treatment

Group B: Male rats sacrificed on day 7 of treatment

Group C: Female rats sacrificed on day 5 of treatment

Group D: Female rats sacrificed on day 7 of treatment

In each animal were practiced four wounds that were:

I: Untreated

II: Treated with base of the formulation

III: Treated with propolis cream of 10%

IV: Treated with 1% silver sulfadiazine

Treatment was initiated immediately after creating the wounds and continued daily up to the 7th day, when the experiment was stopped according to previous studies on the model. ⁽¹⁾⁽²⁾

Ulcer area and a portion of surrounding tissue were excised using surgical scissors and fixed in 10% buffered formalin. Tissue samples were paraffin-embedded and sectioned at 5 µm, before hematoxilin-eosin, PAS/Alcian Blue, and van Giesson stainings.

Scanned images were processed using DIGIPAT image processing package and the non-epithelized area was determined.

The following calculations were further made: percentage of total re-epithelized area, wound perimeter and epithelial linear ingrowth. This parameter was calculated by dividing the healed area of each wound by the average value of the perimeter per experimental group. It represents the linear growth of the epithelial layer onto repaired area. ⁽³⁾

Histologic determinations

Dermal reconstitution was qualitatively graded as: ⁽⁴⁾

0: an immature granulation tissue with a null or incipient formation of collagen bundle fibrils, focally distributed in the wound bed, neither aligned, nor giving rise to an organized meshwork. Severe inflammatory infiltrate of mononuclear cells and poor neovascularization should be found. Negligible or faint affinity for van Giesson`s stain is considered.

1: some collagen fibrils should be present, resembling a primitive degree of organization, focally distributed, without horizontal alignment along the wound bed. Neovascularization should be noted as an immature event, with a limited number of primitive vessels lacking blood. A moderate inflammatory infiltrate is observed as well as focal positivity to van Giesson`s stain.

2: a general image extracellular matrix reconstitution, with mature and organized granulation tissue containing horizontally deposited collagen fibrils. Affinity to van Giesson`s staining is observed with varied intensity across the area. Some active, dilated and blood-containing vessels should be noted. Swelling is minimal.

3: complete extracellular matrix reconstitution, with mature and organized collagen fibrils, horizontally deposited in the neodermis. Even affinity to van Giesson`s staining should appear. Some vessels might contain blood or might be found collapsed by surrounding collagen fibrils.

All measurements are expressed as means \pm standard errors. Percentage of total re-epithelized area, wound perimeter and epithelial linear ingrowth were compared using the Mann-Whitney U test. A significance value of $p < 0.05$ was used. All tests were 2-tailed.

Results

Wound treated with propolis cream of 10% had a total re-epithelized area greater of the 60%. This was significantly larger than the percent of re-epithelization calculated for the other experimental groups. ($p < 0.05$).

Similarly, these wounds had the largest rate of epithelial linear ingrowth and the lowest values of wound perimeter. ($p < 0.05$).

Changes in the macroscopic features of wounds from group III were observed at day 5 after injury. Wounds showed a visible reddening and more abundant leakage, possibly associated with neovascularization. An unexpected finding was the evolution of the wounds treated with 1% silver sulfadiazine.

Histological evaluation of the wounds showed that dermal reconstitution was not importantly stimulated in groups receiving propolis cream in relation with control group (spontaneous healing). Inflammatory infiltrate was reduced in propolis cream treated wounds, and showed a quickly highest fibrovascular reaction compared to the others. The propolis cream helps the wound healing but did not accelerate this process.

Table 1 Morphometric evaluation of the group A

Wounds	Percentage of total Wound re-epithelization (%)	Wound perimeter (mm)	Epithelial linear ingrowth (mm)
I	55.5±1.9 ^a	17.2±5.1 ^a	3.3±0.4 ^a
II	53.7±1.2 ^a	18.4±3.3 ^a	3.6±0.3 ^a
III	68.6±2.4 ^b	11.7±2.8 ^b	5.4±0.1 ^b
IV	50.4±0.8 ^a	18.7±3.1 ^a	3.1±0.1 ^a

Values are the mean and standard error of each epithelial-related parameter. Test of Mann Whitney U. Difference letters indicated significant difference between wounds ($p < 0.05$)

Table 2 Morphometric evaluation of the group B

Wounds	Percentage of total wound re-epithelization (%)	Wound perimeter (mm)	Epithelial linear ingrowth (mm)
I	60.8±2.1 ^a	12.7±5.1 ^a	6.8±0.1 ^a
II	59.5±1.7 ^a	13.3±3.7 ^a	5.9±0.1 ^a
III	77.3±2.5 ^b	8.98±2.7 ^b	8.3±0.1 ^b
IV	58.1±2.1 ^a	14.4±4.3 ^a	5.4±0.1 ^a

Values are the mean and standard error of each epithelial-related parameter. Test of Mann Whitney U. Difference letters indicated significant difference between wounds ($p < 0.05$)

Table 3 Morphometric evaluation of the group C

Wounds	Percentage of total Wound re-epithelization (%)	Wound perimeter (mm)	Epithelial linear ingrowth (mm)
I	53.5±1.7 ^a	17.1±2.7 ^a	3.6±0.3 ^a
II	51.8±1.1 ^a	18.4±3.1 ^a	3.1±0.3 ^a
III	64.9±2.2 ^b	14.3±2.1 ^b	5.6±0.4 ^b
IV	49.3±1.8 ^a	19.5±3.3 ^a	2.7±0.3 ^a

Values are the mean and standard error of each epithelial-related parameter. Test of Mann Whitney U. Difference letters indicated significant difference between wounds ($p < 0.05$)

Table 4 Morphometric evaluation of the group D

Wounds	Percentage of total Wound re-epithelization (%)	Wound perimeter (mm)	Epithelial linear ingrowth (mm)
I	61.8±1.9 ^a	16.8±2.3 ^a	4.5±0.3 ^a
II	57.2±1.8 ^a	15.2±2.5 ^a	4.2±0.3 ^a
III	79.1±2.1 ^b	12.7±2.2 ^b	7.8±0.1 ^b
IV	55.4±1.4 ^a	17.5±3.1 ^a	3.9±0.3 ^a

Values are the mean and standard error of each epithelial-related parameter. Test of Mann Whitney U. Difference letters indicated significant difference between wounds ($p < 0.05$)

Discussion

The use of propolis for the treatment of wounds has been reported by several European authors.^{(5) (6) (7)} Recent evidence suggests that propolis induce epidermal cell maturation and the growth of the granulation tissue.⁽⁸⁾ Besides this substance form a thin skin that protects the surface of the wound.⁽⁹⁾ However the wound model used demonstrated that propolis did not stimulate wound healing.

Wounds receiving the 1% silver sulfadiazine treatment showed a torpid evolution, and exhibited a poorly organized granulation tissue in relation to untreated wounds. These results demonstrated that 1% silver sulfadiazine is not useful in experimental healing model as positive control.

The sex did not influence the wound healing of the animals. The comparison between two sacrificing days (5 and 7) show that the propolis did not accelerate the wound healing process.

An adequate healing of the wounds was not obtained by silver sulfadiazine, probably because this drug has bactericide and antiseptic effects, properties that favor indirectly healing but do not accelerate the process.

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