In vivo wound healing potential of *Olea europaea* L. leaves' polyphenols

Halima Saiah1, Wassila Saiah2

1 Faculty of SNV, Department of Biology, University of Hassiba Ben Bouali, BP 151, 02000, Chlef, Algeria,

2 Department of biological sciences and physiology, Laboratory of bioenergetics and intermediary metabolism, University of Sciences and technology Houari Boumediene, Algiers, Algeria.

ABSTRACT

The present study was carried out to explore the in vivo wound healing potential of Algerian Olea europaea leaves. Polyphenols were extracted with 80% methanol. A 10% ointment was made up from methanol extract of O. europaea leaves. Studies were performed on excision wound model using albino Wistar rats. The test ointment (50 mg) was topically applicated over the wound surface. The healing potential of the test ointment was assessed by measuring biophysical parameters including wound contraction rate and epithelialization period. Hydroxyproline content represented a biochemical parameter and was evaluated on 4th, 8th, 12th and 16th post-wounding days. Moreover, histological assessments were performed to evaluate fibroblasts proliferation, collagen deposition and neovascularization. The test ointment-treated groups healed significantly faster, which was indicated by improved contraction rate (90.61±2.59) % in comparison to control group (73.12±3.51 %). Period of epithelialization decreased by 21.33 ± 1.28 days compared to the control treated with Vaseline (25.66 ± 1.26 days). Moreover, biochemical analyses revealed a significant increase in hydroxyproline contents on day 16 (0.96 ± 0.03 mg/100 mg of granulation tissue) of the ointment-treated wounds in comparison to control group (0.89 ± 0.09 mg/100 mg of granulation tissue). Histological findings indicate that O. europaea promotes the skin healing process by promoting fibroblasts proliferation, collagen fibers deposition and neovascularization. This study had demonstrated that the methanol extract of O. europaea leaves promoted the acceleration of the healing process when compared to the control group. This might be due to the combined effect of the extract's.constituents.

METHODS

Plant material

The freshly harvested leaves,, were washed and dried in the shade 15 days, When dried, they were crushed in an electric mill and recovered in clean paper bags.

Animals

Male albino rats (*Rattus norvegicus)* weighing 150 ± 20g were used. They were kept at conventional conditions of humidity, temperature and light. Food and water were provided *ad libitum*.

Extraction procedure

The methanol extract was prepared by repeated extraction for the dried powder using Soxhlet extraction apparatus with 80% methanol. The ethanol was evaporated under reduced pressure at 45 °C.

Wound model

Full-thickness wound (2cm×2cm) was created on the dorsolumbar region on each rat. A 10 % (w/w) ointment was prepared by mixing the methanol plant extract with white petroleum jelly.

Group 1 was left untreated (negative control); Group 2 received topical application of petroleum jelly (vehicle); Group 3 received topical application of 300 µL of MOE-ointment once daily until day of sacrifice; Group 4 topically treated with silver sulfadiazine 1% (positive control).

Wound contraction rate

Wound area of each rat was assessed on days 0, 4, 8, 12, and 16 post-wounding by tracing the wound limits on transparent paper using a permanent marker. The wound areas recorded were measured using a graph paper (El-Ferjani et al., 2016). Contraction rate was assessed according to the formula below (Nagesh et al., 2015):

(Wound area on day 0 - wound area on Nth day)/(Wound area on day 0) X 100

Nth day=4, 8, 12, and 16th post wounding days.

Period of re-epithelialization

Re-epithelialization period was estimated as the time required for falling of scab and until normal skin replaces the wound (Shailajan & Gurjar, 2016).

Estimation of hydroxyproline

The granulated tissue without surrounding tissue was excised and hydrolyzed in 6 N HCl at 130 ℃ for 4 hr in sealed tubes. Estimation of hydroxyproline was done as per Woessner (1961).

Histopathology

Wound granulation tissues samples from controls, treatment, and standard therapy groups were excised without surrounding tissues on the 16th post-wounding day, processed and stained with Haematoxylin-Eosin (HE). Under light microscopy, sections were assessed for the amount of inflammatory cells infiltration, fibroblast proliferation, collagen deposition, and neovascularization (Faisal et al., 2014).

Statistical analysis

The statistical significance of the results was evaluated using one-way ANOVA followed by Duncan's comparison test. IBM SPSS Statistics 21.0 software was used. Values of p<.05 were considered to be statistically significant.

KEY FINDINGS

Wound contraction rate

Figure 1 represents the rates of wound closure calculated on day 4, day 8, day 12, and day 16. It reveals that the topical application of MOE ointment significantly increased (p<.05) wound contraction rate in comparison with controls groups.

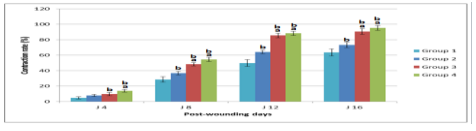

FIGURE 1 Effect of MOE ointment on wound contraction rate (%)

Period of re-epithelialization

Figure 1 depicts the time necessary for entire re-epithelialization of the wound. The MOE-ointment treated animals showed a significant reduction in the epithelialization period (21.33±1.28) % indicating a faster epithelialization process when compared to the vehicle-treated group (25.66±1.66) %. In the negative control group, wound persisted over 29 days,

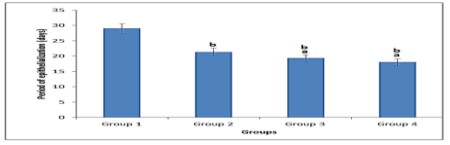

FIGURE 1 Period of epithelialization in days by group. Data were expressed as mean 6 SD (n=6). A Significantly different from vehicle treated group at p<.05. b Significantly different from negative control group at p<.05

Hydroxyproline content of granulation tissue

FIGURE 3 describes the results of the biochemical parameters of the granulation tissues. These results displayed an increase in the proteins and hydroxyproline contents of all experimental groups between the 4th and 16th days.

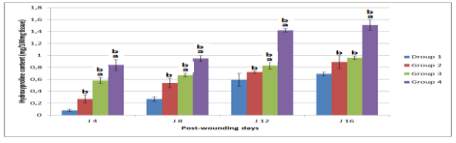

FIGURE 3 Effect of MOE ointment on hydroxyproline content of granulation tissue

Histopathological analysis

Histological evaluation was performed for controls, treatment, and standard therapy groups samples. Treatment with MOE-ointment promoted remarkable inflammatory cells infiltration, increased neovascularization, enhanced cells proliferation, and deposition of horizontally arranged collagen fibers,

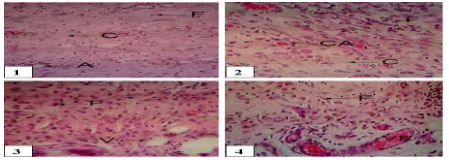

FIGURE 4 Photomicrographs of 16-day-old healing wound sections stained with H&E stain, **1**:-ve control; **2:** +ve control; **3:** vehicle; **4:** MOE-treated. Dose: 50 mg\excision wound given for 6 successive post wounding days. **A:** arteriole, **CA:** capillary **C:** collagen, **F:** fibroblast, **V:** venule. **VE**: vein. (Mgnification: 900X).

CONCLUSIONS

Results obtained in this study can establish the claimed wound healing activity for *O, europaea* that is used in folk medicine. Although further studies are highly recommended to investigate the phenolic composition of *O, europaea* leaves and determine the specific mechanism of action of main phenolic compounds in cellular and macromolecular levels.

REFERENCES

El-Ferjani, R. M., Ahmad, M., Dhiyaaldeen, S. M., Harun, F. W., Ibrahim, M. Y., Adam, H., . . . Batran, R. A. (2016). In vivo assessment of antioxidant and wound healing improvement of a new schiff base derived Co (II) complex in rats. Scientific Reports, 6(1), 1–12. .1

Nagesh, H., Basavanna, P., & Kishore, M. (2015). Evaluation of wound healing activity of ethanolic extract of Azadirachta indica leaves on incision and excision wound models in Wistar albino rats. International Journal of Basic and Clinical Pharmacology, 4, 1178–1182. .2

Shailajan, S., & Gurjar, D. (2016). Wound healing efficacy of Mimusops Elengi L. flowers in albino wistar rats using excision wound model. Pharmacognosy Communications, 6(2), 72–79. .3

Woessner, J. (1961). The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Archives of Biochemistry and Biophysics, 93(2), 440–447. .4

Faisal, AK., Allah, B. K., Muhammad, G. S., Muhammad, S. K., Ghulam, M. L., Abdul, M., . . . Faiz, M. K. (2014). Histological characterization of wound healing of flank versesmidline ovariohysterectomy in different age groups of cats. Journal of Clinical Pathology and ForensicMedicine, 5(2), 6–16. .5