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Original Article

Wound Healing Activity of an Essential Oil-Based Cream of *Origanum vulgare* L. on Mice

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ABSTRACT

Background and Aim: The objective was to evaluate the effect of a cream based on the essential oil of *Origanum vulgare* on skin lesions induced in *Mus musculus* Balb/c. **Materials and Methods:** The experimental animals were divided into four groups of 6 specimens each, in which skin lesions of approximately 1 cm in diameter were induced. Group I (Control) received no treatment, Group II (0.1% *Origanum vulgare* cream), Group III (0.5% *Origanum vulgare* cream), and Group IV (1% *Origanum vulgare* cream) received treatment for eight days. Skin lesions were measured on days 4 and 7. At the end of treatment, they were euthanized using sodium pentobarbital 60 m/kg v.ip. Skin samples were obtained and preserved in 10% formalin for histopathological analysis. **Results:** When measuring the size of the skin lesions induced in the study groups, it was observed that in the control group, the decrease in the length of the lesions occurred on the fifth day; in group II, III, and IV, the lesions length decrease occurs on the second day. At the end of the experience, the measurements of the lesions were much smaller for the III and IV groups (0.33 and 0.41, respectively) compared to the control group (0.40 cm). On day seven, statistically significant difference was found on skin lesion measure between Group IV and the Control, with a significance level of $p<0.05$. **Conclusion:** It is concluded that the 1% *Origanum vulgare* essential oil-based cream has a healing effect on dermal lesions induced in *Mus musculus* Balb/c.

Keywords: Wound Healing, *Origanum vulgare,* essential oil, cream, skin.

INTRODUCTION

Wound healing is a complex repair process in which the body must stop bleeding, repair, and close the wound. Reconstruction of damaged tissue consists of three phases: inflammatory, proliferative, and repair.[1-3] On different occasions, scar treatment tends to improve appearance and function. Some treatments use corticosteroids or collagen and antimicrobials; however, they have also caused various adverse effects; therefore, it has been necessary to resort to other therapies.[4,5]

Popular medicine widely uses medicinal plants, and multiple investigations have reported an improvement in the wound healing process by various plant extracts and isolated compounds, which has provided low-cost curative products with better safety against hypersensitivity reactions compared to synthetic pharmaceutical agents.[6-8] *Origanum vulgare L.* (*O. vulgare*), species known as "oregano", grows in Europe and was introduced in the 16th century from the Middle East. Within its botanical characteristics are the stems that are highly branched, reddish in color, and 40 cm high; its oregano leaves are arranged oppositely, oval, and hairy on the underside, small between 5 and 15 mm; its white, pink, or lilac flowers. These flowers are grouped in an apical inflorescence (at the tip of the stem). Its flowers have an overly sweet and aromatic nectar.[9,10]

O. vulgare has been shown to contain various chemical compounds such as thymol, carvacrol, terpinene, linalool, and cymene, having medicinal, culinary, and cosmetic uses, in addition to having analgesic, anti-inflammatory, antihypertensive, antimicrobial, repellent, antimalarial, antispasmodic and antirheumatic liniment activity used for sciatica and arthritis, and ointments against dermatitis. In addition, it is traditionally used as an antiseptic and healing.[11-13] The validation of the efficacy of treatments, such as that demonstrated by the essential oil of *O. vulgare*, is urgent and challenging, which is fundamental to developing new technologies and products potentially applied in wound care.[7] This study considers the formulation of healing creams based on *O. vulgare* on induced wounds in mice.

MATERIALS AND METHODS

Botanical Material

We collected 6 kg of *O. vulgare* "oregano" leaves from San Ignacio, District of Sinsicap, Province of Otuzco, Region of La Libertad. The plant species was identified by the Herbarium Truxillense of the Universidad Nacional de Trujillo with code N° 59403.

Lab Animal

Group of 24 specimens of *Mus musculus* Balb/c, adult males or females, randomly selected, with average weights of 30-35 g and 2 months of age, apparently healthy; acquired at the National Center of Biological Products of the National Institute of Health. The animal group were conditioned during

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the experimentation in the Biotherium of the Faculty of Pharmacy and Biochemistry of the Universidad Nacional de Trujillo and fed with standard diet and water *ad libitum*.

Essential oil extraction[14]

The essential oil was extracted from *O. vulgare* by the steam distillation method, a certain volume was obtained and measured by the gravimetric-volumetric method. Both the essential oil yield percentage (%EOP) and the physicochemical properties (relative density, refractive index, and pH) were determined.

Preparation of the creams[15]

The mixture of lanette wax (6g), liquid petroleum jelly (4 g), stearic acid (3 g), and cetyl alcohol (4 g) was prepared over low heat in a 150 mL beaker. It was shaken until the complete melting of the wax. The melted mixture was removed from the fire and was subjected to constant agitation. In another container, methylparaben (0.15 g), propylparaben (0.15 g), glycerin (5 g), distilled water (78 g), and *O. vulgare* essential oil were mixed until being dissolved, obtaining creams at concentrations of 0.1%, 0.5%, and 1%. The previous preparation was poured into the first container and continued stirring constantly until it cooled, and a homogeneous paste was formed. Finally, it was packaged, labeled, and stored at room temperature until its use.

Healing evaluation[16]

The dorsal area of the specimens was depilated 24 hours before the induction of the wounds; after, the anesthetic Lidocaine 2% was applied topically, proceeding to cut perpendicularly to the longitudinal axis of the mouse with a scalpel, approximately 1 cm long by 2 mm deep.

The distribution of the experimental animals was carried out in 4 groups of 6 animals each, following the random criterion: Group I (Control) lesion was induced and did not receive any treatment, Group II (*O. vulgare* cream at 0.1%), Group III (*O. vulgare* cream at 0.5%) and Group IV (*O. vulgare* cream at 1%), the treatments were administered twice daily (8 a.m./8 p.m.) for 8 days. The treatments were applied with a sterile swab in sufficient quantity to cover the dermal lesion, and the healing time of the wounds was observed. The length measurements of the lesions were taken daily, using a vernier, obtaining a length average of the lesions each day during the experimentation.

Histopathological study[15]

Mice were euthanized using sodium pentobarbital 60 mL/kg v.ip. Skin samples were obtained by making a 1.5 cm long and 1 cm wide cut around the scar. These samples were preserved in sterile vials with 10% diluted formalin for histopathological analysis.

Statistical analysis

The figures were prepared using R-4.3.0 for Windows®, and the data were subjected to analysis of variance followed by Tukey's HSD test. A difference with $p<0.05$ was considered statistically significant.

Ethical aspects

The Faculty of Pharmacy and Biochemistry of the Universidad Nacional de Trujillo ethics committee approved the study protocol (Certificate N°002-018/CEIFYB).

RESULT

The physicochemical characteristics of *Origanum vulgare* L. oil were determined as shown in Table 1.

The measurement of skin lesion was evaluated for seven days, finding that groups II, III and IV have a statically significant difference with the Control Group (Group I) on the fourth day, but group IV maintains it until the seventh day (Figure 1).

Table 1. Physical and chemical characteristics of *Origanum vulgare* L. essential oil.

% EOP (percentage)	0.8%
Density (mg/mL)	0.92
pH (pH scale)	5
Refractive Index Units (RIU)	1.47

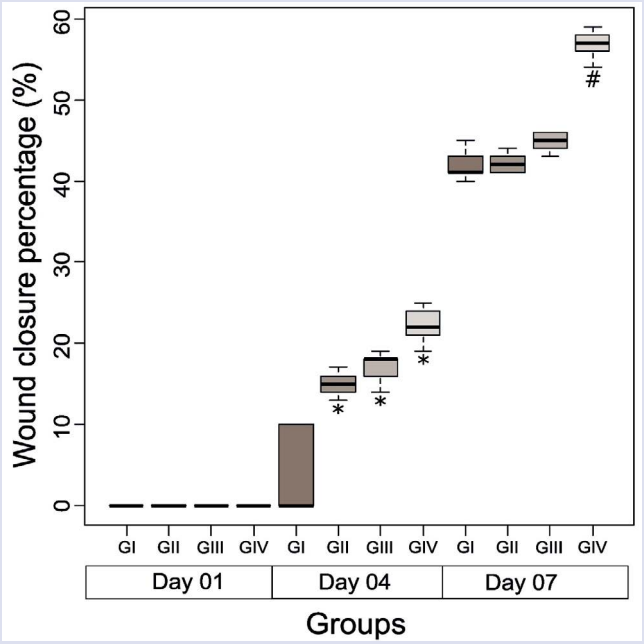

Figure 1. Evaluation of the average size of the dermal lesions induced in mice: **GI:** Control Group, **GII:** 0.1% *O. vulgare* cream, **GIII:** 0.5% *O. vulgare* cream, and **GIV:** 1% O. *vulgare* cream. (*): Group with a significant difference about the IG (Control Group); (#): Group with a significant difference about IVG (1% *O. vulgare* cream). ANOVA and Tukey's HSD test at $p < 0.05$.

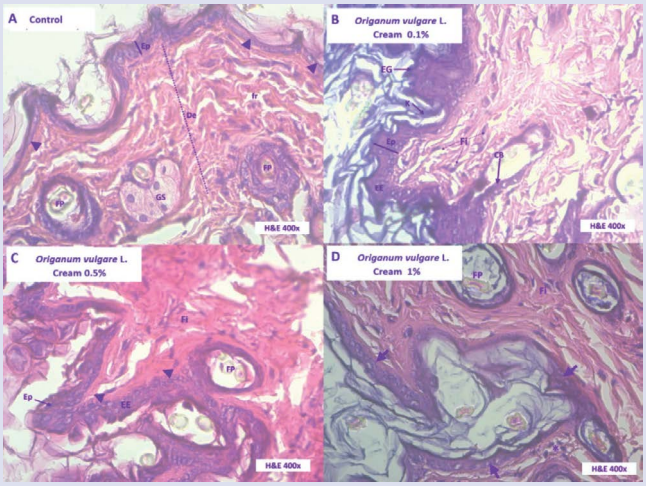

Figure 2. Histopathological sections of the skin of *Mus musculus* Balb/c. A: Group I (Negative Control). B: Group II (0.1% *O. vulgare* cream), C: Group III (0.5% *O. vulgare* cream) and D: Group IV (1% *O. vulgare* cream). Keratinous stratum corneum (K), stratum granulosum (EG), few Langerhans cells in the stratum spinosum (EE), undifferentiated pigmented basal cells (BC), connective tissue (Fi), fibroblasts (*), hair follicles (FP) and intense activity of the sebaceous glands (GS). (Hematoxylin and Eosin, 400X).

The histopathological analysis of *Mus musculus* balb/c skin was performed and is observed in Figure 2. In Group II, the keratinous stratum corneum (K) was observed, unlike groups III and IV; in addition, in Group II undifferentiated pigmented basal cells (CB) and stratum granulosum (EG) were observed. On the other hand, in Group II and III few Langerhans cells are observed in the stratum spinosum (SE), and in Group IV fibroblasts are observed (*). In Groups II, III and IV, connective tissue (Fi) is observed. In the Control Group (Group I) intense activity of the sebaceous glands (GS) is observed.

DISCUSSION

Medicinal plants are used as a new alternative in pharmacological treatments since they contain substances that will enhance each other to obtain the desired effect.[17] Studies have been carried out on various medicinal plants that seek to explain and publicize their antibacterial and antifungal properties against various pathological microorganisms.[18] Before, the beneficial effects of oregano on health were unknown, but in recent years, there have been numerous investigations of oregano and its properties.[19-21]

Table 1 shows the *O. vulgare* essential oil yield, which was 0.8%. This percentage is attributed to a set of climatic factors such as the altitude of the place of cultivation, type of soil, collection time, and extraction method. In addition, the percentage may vary due to the time elapsed between the collection and the extraction process of the essential oil.[14] In the physicochemical characteristics of the *O. vulgare* essential oil, a density of 0.92 g/ml, a refractive index of 1.47, and a pH of 5 were obtained. The value of the refractive index and density is related to the presence and quantity of aromatic oxygenated compounds (phenols) that the plant presents. Regarding the determination of pH, high-quality essential oils have a pH close to 5 (maximum 5.8), which are characterized as acid essential oils.[19]

When measuring the size of the skin lesions induced in the study groups, it was observed that in the control group, the decrease in the length of the lesions occurred on the fifth day (0.95 cm); in group II, III, and IV, the lesions length decrease occurs on the second day (from 0.85 to 0.95 cm). At the end of the experience (eighth day), the measurements of the lesions were much smaller for the III and IV groups (0.33 and 0.41, respectively) compared to the control group (0.40 cm) (Figure 1).

The Analysis of Variance (ANOVA) shows that there is a statistically significant difference on days 4 and 7 in at least one study group, with 95% confidence ($p<0.05$). To determine in which study groups, from the second day, there is a more significant difference, the Tukey HSD test was used, where groups II, III, and IV were shown to have an honestly significant difference from the second to the fourth day, with group IV maintaining the difference until the treatment ended.

Reparative re-epithelialization, hair follicles, and fibroblasts that allow progressive dermal restitution during the scarring process (wound healing) were observed in the histopathological cut of skin in groups II and III by the microscope. The effectiveness was notorious due to the presence of dense swirled collagen and fibroblasts (Fi) in the horizontal direction to the dermis. Those findings correspond to the maturation for dermal reconstitution[22], an effect attributable to a higher concentration of oregano essential oil.

One of the main components of *O. vulgare* is carvacrol, which possesses antimicrobial properties. This could be related to promoting the rapid wound-healing process.[11]

Based on the results, the creams formulated from *O. vulgare* essential oil have a healing effect, promoting the rapid wound healing process; the cream from 1% *O. vulgare* essential oil presented better effectiveness.

CONCLUSION

The Essential Oil-Based Cream of *Origanum vulgare* demonstrated to accelerate the healing process of the induced wounds in mice, the most effective treatment being the 1% cream. It is postulated that the healing mechanism of *O. vulgare* is related to phytoconstituents such as carvacrol that exert antimicrobial effects, which contribute to the optimal healing process.

AUTHORS' CONTRIBUTIONS

VEV, JFC, CGS: Conceptualization, drafted the manuscript, and preparation of extract. LPK, WSG: Collected the plant species and entered them in the herbarium. LPK, CRS: Prepared cream formulations. VEV: Validation, supervision, and formal analysis. JAC, DYD, and RMG: Performed the statistical analysis and the preparation of images. AAC, JLC, and CLA: Performed organ harvesting for histopathological analysis, kept the animals during the investigation, and administered treatments. All authors have read, reviewed, and approved the final manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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