

Effect of a Cream Based on Hydroalcoholic Extract of *Sebastiana Obtusifolia* Leaves on Second-Degree Burns in Mice

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ABSTRACT

Introduction: *Sebastiana obtusifolia* (Müll. Arg.), a species belonging to the Euphorbiaceae family, has traditionally been used for medicinal purposes by rural communities due to its medicinal properties.

Objective: Therefore, the objective of this study was to evaluate the effect of a cream based on *Sebastiana obtusifolia* extract on burns induced in *Mus musculus* Balb/c. **Material and methods:** The experimental animals were divided into 6 groups with 5 specimens each. Forty-eight hours prior to the induction of second-degree burns, the hair was removed from the back area; Ketamine/xylazine 80 mg/kg/-10 mg/kg was then administered intravenously, and a round metal rod with a diameter of 1.0 cm was placed on the depilated skin at a temperature of 100°C for 5 seconds. Group A received no treatment; Group B was treated with a 1% silver sulfadiazine healing ointment; Group C was treated with a base cream; and the other two groups, D, E, and F, were treated with a cream formulated with *Sebastiana obtusifolia* at 0.5%, 1%, and 1.5%, respectively. The treatments were administered once a day for 21 days, measuring the burn area and taking the respective photographic controls throughout the experimental period. Euthanasia was then performed using sodium pentobarbital 185 mg/kg i.p. to obtain skin samples, which were preserved in 10% formalin and sent for histopathological analysis. **Results:** There was a statistically significant difference between the groups, with a significance level of $p < 0.05$ during the experiment. Histopathological sections showed that in groups D and F there was an abundance of collagen and fibroblasts in the direction of the dermis, attributable to the effect of the creams. **Conclusion:** Creams based on hydroalcoholic extract of *Sebastiana obtusifolia* "lloque blanco" have a healing effect on second-degree burns induced in *Mus musculus* Balb/c, with the greatest effect being observed with the 1.5% cream.

Keywords: Burn healing, cream, skin, *Sebastiana obtusifolia*

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INTRODUCTION

Burns represent a significant public health problem worldwide due to their high incidence, the potential risk of infectious complications, and the functional, aesthetic, and psychological impact they have on patients^{1,2}. A burn is defined as tissue damage caused by heat, chemicals, electricity, or radiation depending on their severity, burns are classified as first-degree (superficial), second-degree (partial thickness), and third-degree (full thickness)^{3,4}.

Burns are characterized by partial destruction of the epidermis and variable involvement of the dermis, causing intense pain, edema, blistering, and a high risk of infection and poor healing^{5,6}. Conventional burn therapy includes pain control, infection prevention, and adequate stimulation of the tissue regeneration process⁷. However, treatment can be limited, costly, or inaccessible, as its use has declined due to side effects, creating a need to develop safer, more economical, and more effective alternatives^{8,9}.

In recent decades, biomedical research has shown a growing interest in the use of natural products in the treatment of skin lesions. Medicinal plants contain secondary metabolites with anti-inflammatory, antioxidant, antimicrobial, and healing properties, which act by reducing oxidative stress, modulating the inflammatory response, stimulating fibroblasts, and promoting collagen synthesis¹⁰. In this

regard, species of the Euphorbiaceae family, widely distributed in Latin America, stand out for their richness in bioactive compounds such as flavonoids, terpenoids, tannins, and alkaloids, several of which have shown beneficial effects in tissue repair processes¹¹. These characteristics make various species of these family interesting candidates for the development of new bioproducts for healing.

Sebastiana obtusifolia (Müll. Arg.), a species belonging to the Euphorbiaceae family, it has traditionally been used for medicinal purposes by rural communities due to its medicinal properties; it is commonly known as "white loque" and is used to treat sunburn, liver conditions, and infections¹². However, scientific validation of its topical use in thermal injuries is still in its infancy, with little experimental literature evaluating its efficacy in animal burn models.

In this context, the present study evaluates the effect of a cream based on hydroalcoholic extract of *Sebastiana obtusifolia* leaves on second-degree burns in mice, with the aim of generating scientific evidence on its healing activity and its potential application as a low-cost natural therapeutic alternative. The experimental validation of this species could contribute to the development of new dermatological bioproducts based on native flora, particularly benefiting populations with limited access to conventional treatments.

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MATERIALS AND METHODS

Botanical Material

Five kilograms of *Sebastiania obtusifolia* leaves were collected from the village of Caserío de Vilcas, District of Sanagorán, Province of Sánchez Carrión, La Libertad, Perú.

Biological Material

30 *Mus musculus* Balb/c specimens, adults, males and females, selected at random, with average weights of 35 g and aged 3-4 months, apparently healthy. The specimens were housed in the animal facility of the Faculty of Pharmacy and Biochemistry of the National University of Trujillo, at a temperature between 20°C and 22°C with a 12/12 hour light/dark cycle, and received standard feed and water *ad libitum*.

Preparation of the hydroalcoholic extract

The hydroalcoholic extract was prepared using dried and pulverized leaves, which were left to macerate in 70° ethyl alcohol for 72 hours with magnetic stirring. It was then filtered with cotton and placed in an oven at 40°C for 48 hours to obtain the dry extract, which was stored in an amber bottle and kept refrigerated at -10°C until use.

Preparation of creams based on *Sebastiania obtusifolia*^{13,14}

To formulate the cream, the following ingredients were mixed: Lanette wax, liquid petroleum jelly, and cetyl alcohol over low heat, stirring until the wax melted completely. Glycerin was then added until the base cream was obtained. Dry extract of *Sebastiania obtusifolia* was added until concentrations of 0.5, 1, and 1.5% were obtained. The creams were then packaged, labeled, and stored at room temperature until use.

Distribution of experimental groups¹⁵

The 30 *Mus musculus* Balb/c specimens were randomly divided into 6 groups: Group A (Control): 5 *Mus musculus* Balb/c specimens that were

burned to the second degree and received no treatment; Group B (Silver Sulfadiazine): 5 *Mus musculus* Balb/c specimens that suffered second-degree burns and were treated with 1% silver sulfadiazine once a day for 21 days; Group C (Base cream): 5 *Mus musculus* Balb/c specimens that suffered second-degree burns and were treated with the base cream formulation; Group D (0.5% cream): 5 *Mus musculus* Balb/c specimens that were burned to the second degree and treated with 0.5% *Sebastiania obtusifolia* cream once a day for 21 days; Group E (1% cream): 5 *Mus musculus* Balb/c specimens that were given second-degree burns and treated with 1% *Sebastiania obtusifolia* cream once a day for 21 days; and Group F (1.5% cream): 5 *Mus musculus* Balb/c specimens that were given second-degree burns and applied 1.5% *Sebastiania obtusifolia* cream once a day for 21 days.

Induction of second-degree burns¹⁵

Prior to inducing second-degree burns, 48 hours beforehand, the hair was removed from the mouse's back area. To induce second-degree burns, the mice were first anesthetized with ketamine/xylazine 80mg/kg/-10mg/kg intraperitoneally, then a 1.0 cm diameter metal rod was placed on the interscapular area of the mouse for 5 seconds at a temperature of 100°C. After the burn induction process was completed, each animal was placed in a separate cage.

Evaluation of the healing effect¹⁵

The healing time of second-degree burns was observed, and the respective photographic controls were performed throughout the experimental period. The area of the second-degree burn was measured using a vernier caliper, and the percentage of healing was calculated using the following formula: $\left(\frac{\text{area on the first day} - \text{area on the second day}}{\text{area on the first day}} \right) \times 100$.

Histopathological Analysis

The animals were euthanized using 185 mg/kg sodium pentobarbital administered intraperitoneally. Skin samples were obtained by making

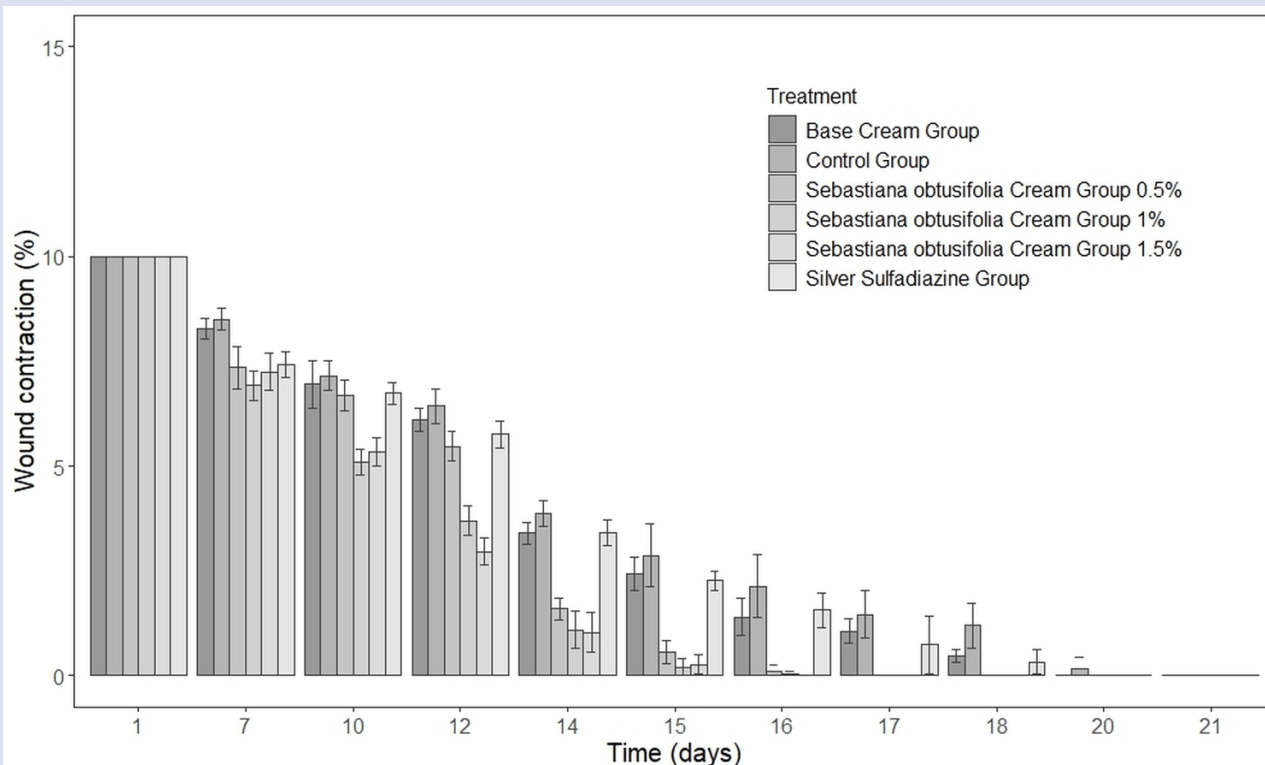


Figure 1. Percentage of healing of second-degree burns induced in *Mus musculus* Balb/c in the experimental groups. $p < 0.05$, $n = 5$

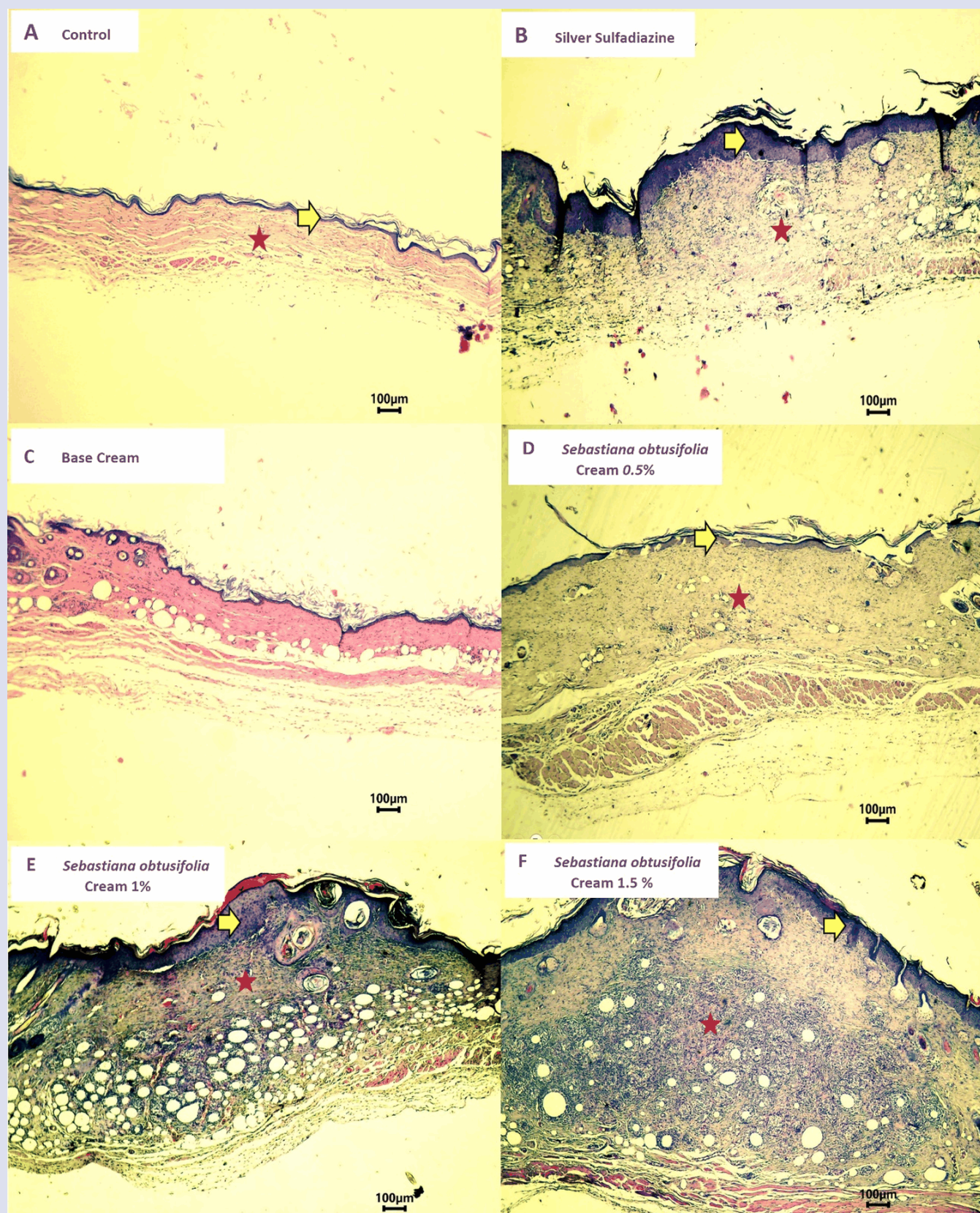


Figure 2. Histopathological sections of *Mus musculus* Bal/c skin. A: Control group. B: Silver sulfadiazine group. C: Base cream group. D: *Sebastiana obtusifolia* 0.5% cream group. E: *Sebastiana obtusifolia* 1% cream group. F: *Sebastiana obtusifolia* 1.5% cream. (Hematoxylin and Eosin, 400X)

a 1.5 x 1.5 cm incision around the scar. These samples were preserved in sterile jars with 10% diluted formalin and sent for histopathological analysis.

Statistical analysis

The results obtained in this study were analyzed using the R-4.1.1 program for Windows®, analysis of variance (ANOVA), and the *post-hoc* test (Tukey's test), considering a statistical significance of $p < 0.05$.

RESULTS

Evaluation of the healing effect

The percentage of burn healing was evaluated using the wound closure parameter to determine the progress of the healing process. The wound closure percentages on day 21 for Groups D, E, and F (*Sebastiana obtusifolia* creams at 0.5, 1, and 1.5%) showed a significant difference compared to Groups A (Control), Group B (Silver Sulfadiazine), and Group C (Base Cream) ($p < 0.05$). Comparing all groups, the greatest effect was observed in those who received *Sebastiana obtusifolia* creams (Figure 1).

Histopathological Analysis

Histopathological changes in mouse skin in Group A (Control) showed the presence of scabs or eschar with connective tissue containing fibroblasts, low activity of the spinous layer and basal cells to restore the dermal papillae, and dense areas of collagen in the lower right area, findings that correspond to the progressive physiological healing process (Figure 2A). In Group B (Silver Sulfadiazine), there was evidence of slight repair of the epidermis to restore intradermal papillae, indicative of the onset of re-epithelialization, and sweat gland activity was observed (Figure 2B).

Groups D, E, and F, which received *Sebastiana obtusifolia* creams, showed healing activity at 21 days of evolution, with recovery of the corneum, granular, spinous, and basal cell layers of the epidermis. In the dermis, abundant collagen and fibroblast activity arranged horizontally were observed, with this effect being greater in group IV (Figure 2D, Figure 2E, & Figure 2F).

DISCUSSION

The results of this study show that the cream made from the hydroalcoholic extract of *Sebastiana obtusifolia* leaves had a significant healing effect on second-degree burns in mice, outperforming both the group treated with silver sulfadiazine and the base cream group. These findings are particularly relevant considering that silver sulfadiazine continues to be used as one of the standard topical treatments for burn management, mainly due to its antimicrobial properties and its ability to reduce bacterial colonization in lesions^{16,17,18}. However, various studies have indicated that this drug can delay re-epithelialization in the healing process and cause local adverse effects, prompting the search for safer and more effective phytotherapeutic alternatives^{19,20,21}.

In this context, *Sebastiana obtusifolia* extract showed a remarkable ability to accelerate wound contraction, promote re-epithelialization, and favor the orderly formation of granulation tissue. The faster reduction in lesion area observed compared to controls indicates a direct action of the extract on regenerative processes. This effect may be related to the presence of secondary metabolites such as flavonoids, tannins, and phenolic compounds, previously identified in species of the genus *Sebastiana*²². These molecules have been widely associated with inflammatory modulation, angiogenesis, and fibroblast proliferation, which are essential elements in physiological healing^{23,24,25}.

Furthermore, histological analyses support the macroscopic data, as animals treated with *S. obtusifolia* cream showed less inflammatory infiltrate, better organized collagen, and regenerating epithelium,

in contrast to what was observed in the control groups. Overall, the results show that the cream with hydroalcoholic extract of *S. obtusifolia* not only equals but exceeds the efficacy of silver sulfadiazine in the regeneration of second-degree burns in a mouse model, positioning this species as a promising phytotherapeutic alternative. Future studies should characterize the metabolites responsible for the activity, evaluate long-term skin safety, and explore more stable formulations for eventual clinical use.

CONCLUSIONS

It is concluded that creams based on the hydroalcoholic extract of *Sebastiana obtusifolia* have a healing effect on second-degree burns induced in mice, with the greatest effect being observed with the 1.5% concentration ointment.

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