# Preliminary evaluation of haemostatic and wound healing potential of Heliotropium europaeum

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# Abstract

*Heliotropium europaeum* has been traditionally used to stop bleeding and accelerate scarring. This study provides a scientific evaluation of *Heliotropium europaeum*'s hemostatic and healing potential. To evaluate the haemostatic effect of *Heliotropium europaeum*, the time of bleeding of fresh wounds induced experimentally in rats was studied. Excision wounds were induced upon four groups; each one contains six rats to estimate the healing properties of wounds. Group-1 was assigned as control (not treated), group-2 was daily treated with *Heliotropium europaeum* leaf powder, group-3 was treated with *Heliotropium europaeum* every 6 days and group-4 was treated with a reference drug, an emulsion containing 10% of *Mimosa tenuiflora* extract. All the parameters were significantly tested ($P < 0.05$) with comparison to a group control. The use of *Heliotropium europaeum* significantly shortened the bleeding time. The rats which were daily treated with *Heliotropium europaeum* healed in 12 days. This time period was significantly shorter than the control groups. Wound excision was uniformly induced randomly on the dorsum of rats in 4 groups (tested support and control). The post healing biopsies were histologically assessed, revealed a better healing quality, and continue complete tissue regeneration, abundant and well organized network of collagen fibers, and low numbers of inflammatory cells. The experimental data revealed that *Heliotropium europaeum* displayed remarkable haemostatic and wound healing activities.


# Introduction

Traditional medicines from ancient times to the present have always made extensive use of medicinal plants. Although most of these herbal remedies are about questionable effectiveness, the possibility of discovering molecules with novel pharmacological properties justifies the interest of the pharmaceutical industry for medicinal plants.

Despite the impressive progress of modern medicine, we do not yet have real and effective haemostatic and wounds healing treatments, diseases, however, quite common. Traditional medicines contain numerous remedies reputed healing [1] but few of them, such as *Centella asiatica* and *Aloe vera*, have passed the test of rigorous scientific study [2].

*Heliotropium europaeum* is a dicotyledonous plant, 10 to 30 cm tall and belongs to the *Boraginaceae* family. Its name is from Greek, helios for sun and trope for turning, an allusion to the turning of leaf and flowers to the sun [3]. This plant, that grows in summer [4], is native to the Middle East, West Asia and the region surrounding the Mediterranean Sea [5, 6].

It is a poisonous plant that causes, at high doses, death from hepatitis livestock, particularly sheep [7] and poultry [8]. Pyrrolizidiques alkaloids, found in the seeds of *Heliotropium europaeum* [9], are responsible for the hepato-toxicity of this plant [10].

In Iranian folk's medicine, it is used as antipyretic, emmenagogue, cholagogue, cardiotonic, anthelmintic and as a treatment for gout and headaches. It is also used in external applications for wound healing and treatment of warts [11].

Antimicrobial activities have been previously reported for *Heliotropium europaeum* [12]. However, the hemostatic and wound healing potentials of this plant have not been investigated yet. Therefore, the target of the present research was to evaluate the in vivo haemostatic and wound healing effects of *Heliotropium europaeum* leaves.

# Materials And Methods

1.1 Plant material:

*Heliotropium europaeum* leaves were gathered in Sfax., Tunisia, in October 2010. A baseline specimen has been identified by Pr Mohamed Chayeb, department of biology, Faculty of Sciences of Sfax. Fresh leaves were collected, dried in the shade and transformed in powder in a grinder; 50 mg of powder was topically administrated on wounds.

1.2 Animals:

**Animal Investigations**

The experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and have, therefore, been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments, and approved by the Committee of Animal Ethics (Protocol No. 94-1939).

Healthy Wistar male rats (180–200 gm) weighting were acclimated to the laboratory under sanitary conditions for 10 days. They were kept on the standard pellet supply and water a*dlibitum* throughout the experiment.

1.3 Bleeding time test:

The effect of *Heliotropium europaeum* on experimentally induced new wound bleeding was evaluated using the rat bleeding time test on rats. 9 adult rats were assigned into 3 groups of 3 animals each one. The control animals were provided with the physiological serum. The second group was treated with the *Heliotropium europaeum* and the third group was just treated with distilled water. A tail tip from each animal was cut with a pair of scissors sharp previously sharped. Immediately, the tail was immerged, simultaneously with the start of the stopwatch, in the powder for 3 seconds for one group. Every 30 seconds, a filter paper is used to draw off the blood. The test is completed when the haemorrhage has stopped completely. Bleeding time was taken as the time for the first blood drop up to the time when the filter paper stopped showing bloodstain [13]. The bleeding time test was repeated for the three groups.

1.4 Excision wound:

Every animal in each group was anaesthetised with ether before wound creation. The dorsal fur of each animal was shaved, and then a full thickness excision (elliptic form 1, 5×1 cm) was created. The animals

were dispatched randomly into four groups each one contained 6 animals. The untreated Group-1used as a negative control; groups-2 was daily treated topically with an equal amount of *Heliotropium europaeum* leaves powder; group-3 was treated with the same powder each 6 days; Treatments spaced with six days were to imitate as far as possible the method of traditional use of the plant as single application. As against, daily applications were in accordance with the conventional use of healing therapies [14; 15], group 4 was daily treated with CICAFLORA®, an emulsion containing 10% of *Mimosa Tenuiflora*, as a reference drug. All the treatments were applied topically, starting from the wound induction until complete healing. Epithelization was considered to have occurred when the eschar fell off without leaving any residual raw wound [16]. The area of the wound was measured by tracing the edge of the wound using a transparent paper each day and the healed area was calculated by subtracting the area of the original wound. The percentage wound contraction was determined using the following formula of Mayrovitz: [17]

$$\mathrm{Percentwoundcontraction} = \frac{\mathrm{Healedarea}}{Totalwoundarea} \times 100$$

1.5 Histological evaluation

The histological exams were also carried out on the wound area in the tissue sections. Each sample was fixed in 10% buffered formalin, treated and blocked with paraffin preparation and then cuted into 3 µm sections and treated with hematoxylin-eosin (HE) stains and Van Gieson (VG) stains. The stained samples were all evaluated for the their following histological criteria; the re-epithelization extent, the epidermal squamous cells maturation and organization, the granular cell layer thickness and for the dermis collagenisation. For the changes on epidermis, the non-traumatized adjacent skin served as an internal control for the assessment of the epidermal maturation. For the dermis; the disorganization of the fibers of collagen, the prominent vascularity and the inflammation indicated the immaturities of the tissue of granulation. On the contrary, the presence of the new formed, horizontally-oriented fibers of collagen, as usually by Van Gieson stain, with cells scarce inflammatory were considered as an indication for the scar maturity.

1.6 Statistical analysis

The means of measurements of the wound area by the different groups was compared using a one-way ANOVA test, followed by student's tests.

The percentage of wound healing was statistically analyzed using one-way analysis of variance (ANOVA). All values of $p \leq 0.05$ were statistically considered significant. Histological data were considered nonparametric; and, no statistical tests were performed.

# Results

1.7 Bleeding time test:

In the present research, the effect of *Heliotropium europaeum* on bleeding was investigated. Results showed a significant reduction ($p < 0.05$) in time of bleeding in rats (Table 1). The significant reduction in bleeding time suggests that *Heliotropium europaeum* have positive effect on haemostatic phase of wound healing.

Table 1
Bleeding time test **(*)** $p<0.05$ compared to control (One Way ANOVA). N=6

| Solution | Bleeding time (s) |
|---|---|
| Control | 112 ,667 ± 2,517 |
| *Heliotropium europaeum* | 39 ± 3* |

1.8 Excision wounds

The wound size area and the contraction rates percentage were monitored during experimental period of 12-day (Fig. 3) to explore the potential of wound healing of *Heliotropium europaeum*.

The excision wound model results of each groups are shown in Table 2 and Fig. 1. In group-2, the significant healing effect was observed (Fig. 4) from day 3 with 43.24% ($p < 0.05$) and it reached 100% ($p < 0.001$) contraction on day 12 of the period of treatment. Whereas, group-3 and 4 showed comparable wound contractions, presenting significant healing effect ($p > 0,05$) but slower than contractions of group-2. The delayed wound healing processes is observed in the control group (group-1) compared to the other groups.

Table 2
Effect of *Heliotropium europaeum* leaf and CICAFLORA® on wound contraction in excision wound model. Values are expressed as mean ± SEM ($N = 6$). **(*)** $p < 0.05$ when compared to control. **(**)** $p < 0,001$ when compared to control.

| Day | Wound area ($cm^2$) (mean ± SEM) | | | |
|---|---|---|---|---|
| | Control | Group-2 | Group-3 | Group-4 |
| 1 | 1,298 ± 0,079 | 1,295 ± 0,061 | 1,291 ± 0,071 | 1,293 ± 0,117 |
| 3 | 1,065 ± 0,130 | 0,735 ± 0,084* | 1,091 ± 0,085* | 1,034 ± 0,059* |
| 6 | 0,637 ± 0,069 | 0,421 ± 0,093** | 0,625 ± 0,107* | 0,624 ± 0,100* |
| 9 | 0,315 ± 0,092 | 0,140 ± 0,083** | 0,228 ± 0,095** | 0,249 ± 0,103** |
| 12 | 0,166 ± 0,022 | 0,000 ± 0,000** | 0,039 ± 0,027** | 0,042 ± 0,041** |

*Heliotropium europaeum* exhibits better wound healing activity compared to the other treated groups. Results show a significant effect of *Heliotropium europaeum* on wound healing comparing to CICAFLORA® (*Mimosa Tenuiflora*).

1.9 Histological evaluation

The results of the different biopsies (Fig. 2) revealed a complete epithelial regeneration in groups-2 and 4 (figures A: I.2, II.2, and I.4, II.4). The skin was thick, well covered and structured of the totality of wounded zone. It had a normal appearance, similar to the adjacent healthy skin used as internal control. In group-3, there was a thin and poorly organized epithelium, indicating a lower quality epithelial regeneration (figures A: I.3 and II.3). For the control group an incomplete epithelial regeneration was mentioned (Figures A: I.1 and II.1).

Staining by Van Gieson in the three groups (2.3 and 4) showed horizontal and well organized collagen fibres (Figures B: I.2, I.3 and I.4). However, the wound healing in the control group was retarded (Figure B: I.1) and the tissue of granulation contained many vessels and polymorphous inflammatory cells showing a persistent chronic inflammation (Figure A: II.1). For the daily treated group with *Heliotropium europaeum*, we noticed that the healing was well advanced; collagen density was significantly higher than the reference drug. On the other hand, there were fewer inflammatory cells (Figure A: II.2).

# Discussion

In this comparative study, we demonstrated the healing properties of *Heliotropium europaeum*. In daily treated group with this plant, we observed a complete closure of the scar after 12 days with a complete tissue regeneration of the different layers of the skin, whereas in the other groups, healing was incomplete and delayed. On the other hand, the analysis of bleeding time highlighted a haemostatic effect of *Heliotropium europaeum*.

Haemostasis, the arrest of bleeding from an injured blood vessel, is a defence mechanism that allows the protection of the vascular system integrity [18], it includes the changing of blood from a fluid to a solid state. The time of bleeding is a crude haemostasis test, that indicates the process that platelets interact with blood vessel walls to create blood clots. According to this test, *Heliotropium europaeum* has significantly shortened the bleeding time.

Excision wound and histological results are in support that the wound healing and repair are accelerated by the daily application of *Heliotropium europaeum* which was high-lighted by the fast cluster (12 days), the complete cover of the wound with an organised epidermis, the presence of mature scar tissue in the dermis and some inflammatory cells. The enhanced healing ability of *Heliotropium europaeum* could be explained on the basis of the anti-inflammatory effects of the plant that are well documented in the literature. [12]. Dash and Murthy [19] mentioned that the aqueous and methanol extract of leaves of *Heliotropium indicum* possess better wound care proprety. The authors reveled a complete scare closure

in 16th days with methanol extract which contain phytoconstituents known to promote the process of wound healing.

Although group 3 (*Heliotropium europaeum* treatment every six days) and group 4 (treated with CICAFLORA®) had a similar cure rate, histological analysis has found a better epithelial repair in group-4, with a more structured epithelium. However, Re-epithelization in group-3 was better than control group, suggesting that *Heliotropium europaeum* remains effective even in spaced applications. We can therefore conclude that if the final quality of epithelial regeneration was comparable with *Heliotropium europaeum* and CICAFLORA®, the first possible to obtain a faster wound closure, increased collagen synthesis and a larger decline of inflammation [20].

Mechanisms of action of healing plants are, still, poorly understood. The essential oil of *Centella asiatica* appears to act through the stimulation of collagen type I by fibroblasts synthesis and the anti-inflammatory properties [21; 22]. Several mechanisms could explain the healing properties of *Heliotropium europaeum*. In fact, the histological analyses, revealing a high production of collagen fibers with no inflammatory cells in group-2, suggest fibroblasts stimulation by *Heliotropium europaeum* associated with an anti-inflammatory effect. According to Dash and Murthy [19], high collagen turnover induced by *Heliotropium indicum* may be related to the synergy of phytoconstituents like the flavonoids which reduce the lipid peroxidation by preventing or slowing the onset of the necrosis of cells and next improving the vascularity [23].

At list, Getie et al. [23] evocate that the antioxidant effect of any drug prevent the damage of cell and promote the synthesis of DNA, increase vascularity, increase the strength of the fibers of collagen and improve the viability of fibrils of collagen.

An antimicrobial effect of *Heliotropium europaeum* plant, reported by Semnani and Saeedi [12] associated with the antioxidant effect, which can be due to additive or individual effect of the phytoconstituents, that could contribute to the wound healing process. According to Dash and Murthy [19], the phytochemical studies of the *Heliotropium indicum* oil isolated active compounds like tannins, alkaloids, phenolic substances, triterpenoids and flavonoids which are famous for their antimicrobial and antioxidant activities that promote wound healing process [24].

Finally, the haemostatic effect of Heliotropium europaeum reported in this study could also provide to explain the healing process. In fact, stabilized fibrin, is a key in the initial process to heal the skin. It allows to recruit fibroblasts by chemotactic effect and to stimulate the production of collagen by these cells [25].

During this study, skin healing took place in dry conditions. However, it is well established that moist wound care is faster and better than under dry crust [26]. Indeed, a wetland can provide optimal conditions of healing for nutrient and vitamins supply and for cell growth and metabolism. The use of *Heliotropium europaeum* extracts would be possible to obtain better results especially on wound healing activity.

Haemostatic and healing effects of *Heliotropium europaeum* we have recorded in this experimental study, have not been described above in the literature. However, further work must be done to authenticate the healing effect and identify the active components of *Heliotropium europaeum.*

To conclude, herbal medicine is a therapeutic method that uses the action of medicinal plants. This method, based originally on old traditional practices, is now based on scientific studies and clinical tests that accurately assess the effectiveness of the treatment options used. The aim of this research was to evaluate experimentally the haemostatic and healing properties of *Heliotropium europaeum*. The results concord with other scientific pharmacological evidence listed on folk's medicine using this plant for wound healing. However, it is essential to conduct further studies to authenticate these findings, to isolate and identify the active ingredients in the Tunisian plant that are responsible for its biological activities and to consider quantitative histological evaluation and biochemical studies of the scar tissue.

# Declarations


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# Figures

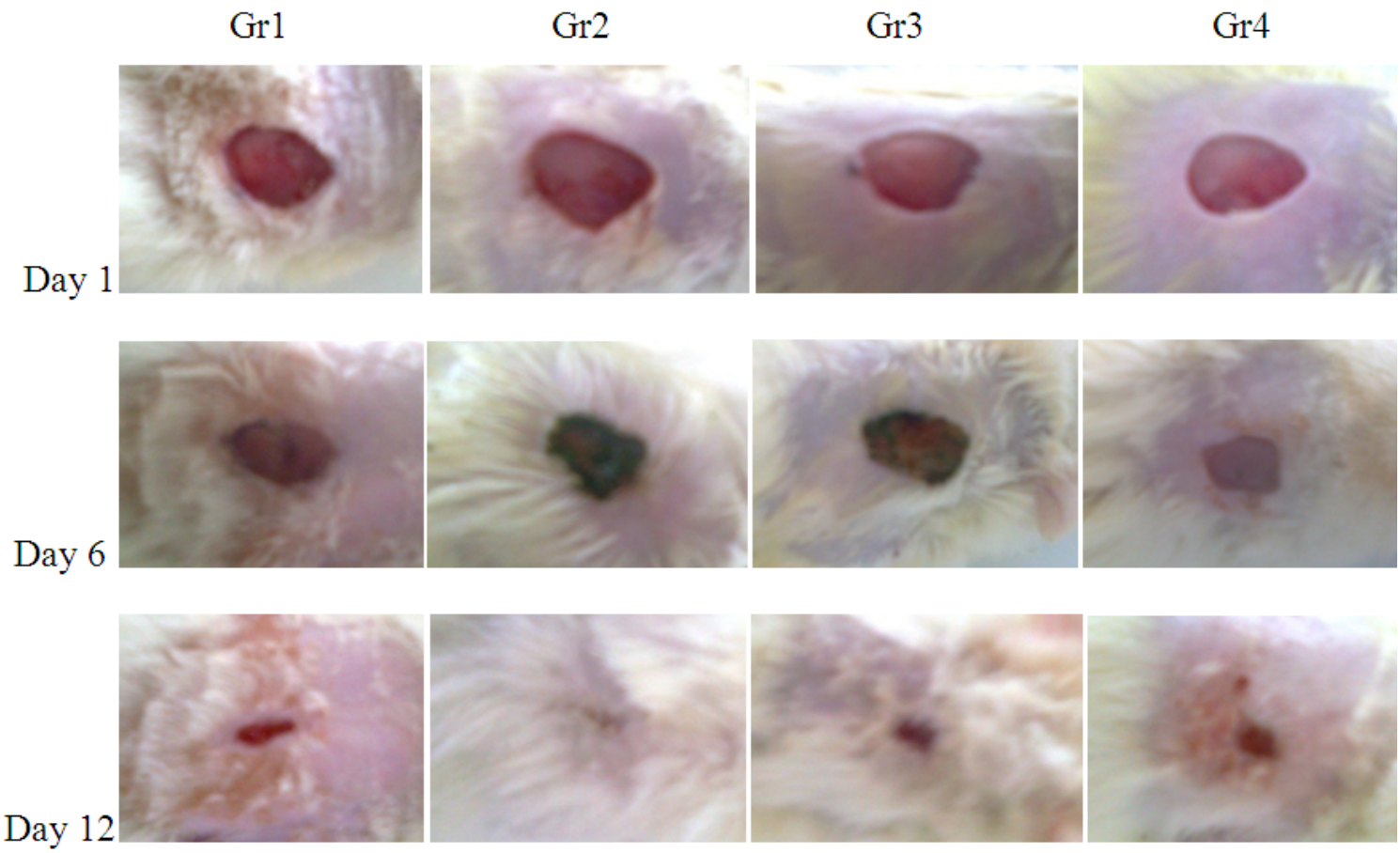
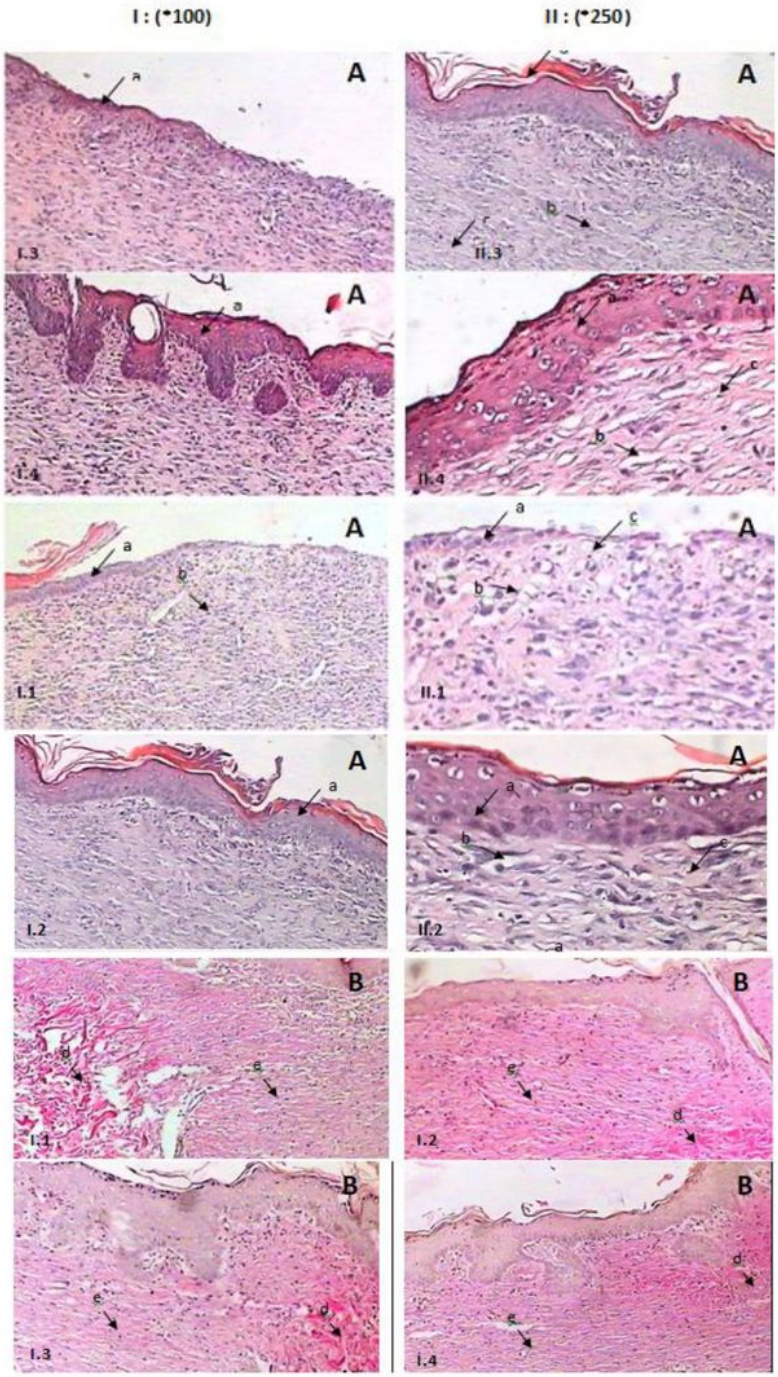



**Figure 1**

Excision wound on day 1, 6 and 12. (Gr1) control; (Gr2) daily treated with HE; (GR3) treated every 6 days with HE; (GR4) CICAFLORA®



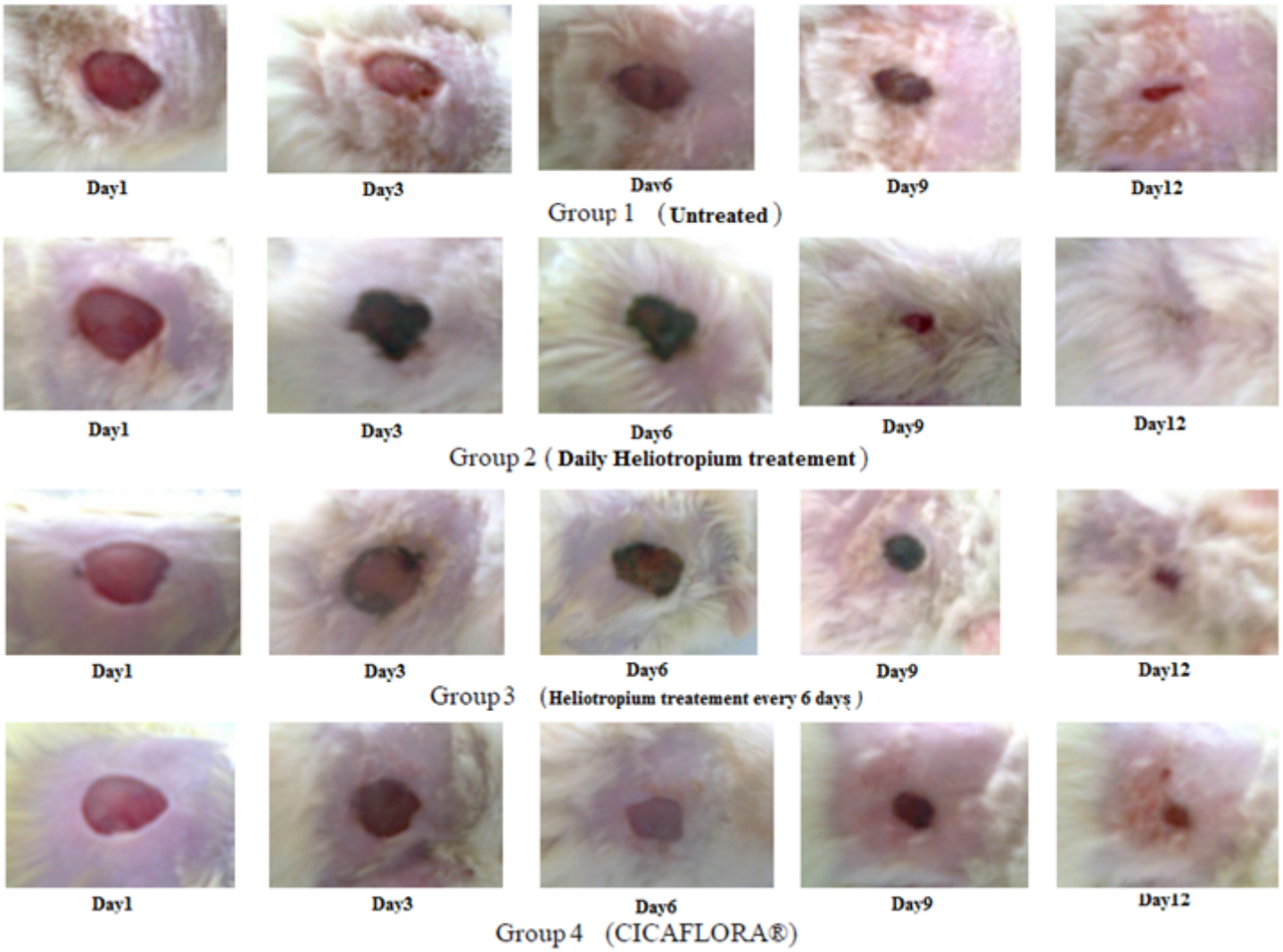

## Figure 2

Histopathological view of wound healing and epidermal/dermal re-modeling. Hematoxylin & eosin (HE) stained epidermis and dermis in rats (A), and the dermis stained with Van Gieson (VG) in rats (B).

1) The negative control group (untreated), 14 day old wound tissue. 2) Daily treated with HE group, 14 day old wound tissue. 3) Every 6 days treated with HE group, 14 day old wound tissue. 4) CICAFLORA®

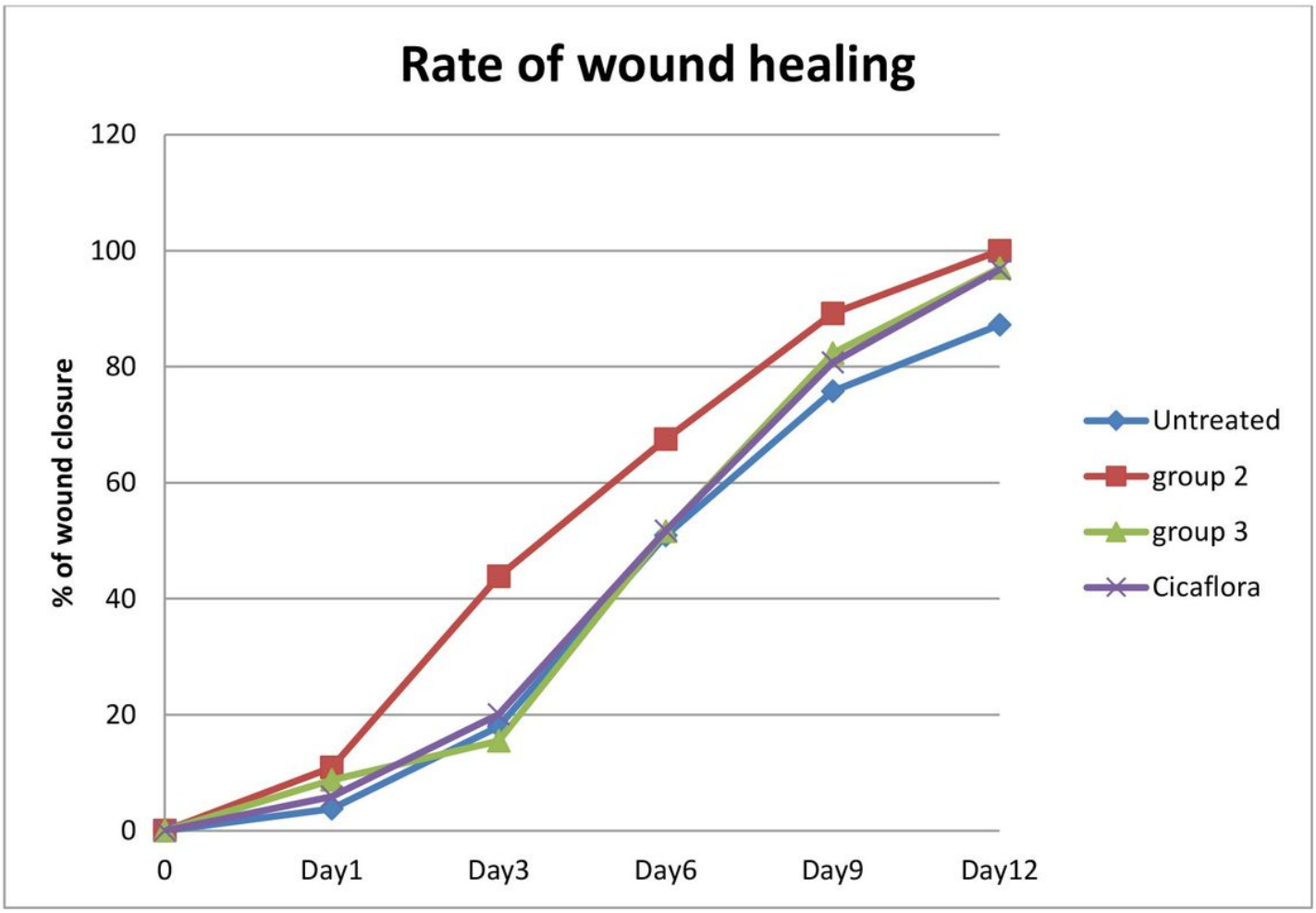
group, 14 day old wound tissue. a) Epidermis

b) Dermis. c) Inflammatory cell. d) Adjacent non-traumatized skin (normal skin). e) Scar tissue.




## Figure 3

Visual observation of the wound healing experiment on days 1, 3, 6, 9 and 12. (Group 1) Untreated group, (Group 2) group daily treated with Heliotropium , (Group 3) group treated with Heliotropium every 6 days, (Group 4) group treated Cicaflora.




## Figure 4

Rate of wound healing during 14 days.