



TOPICAL HEALING ACTIVITY OF THE HYDROETHANOLIC EXTRACT FROM THE SEEDS OF *VATAIREA GUIANENSIS* (AUBLET)

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ABSTRACT

Medicinal plants are culturally and broadly used empirically in the Amazon in the treatment of several diseases. The species selected in this study is *Vatairea guianensis*, which is used in traditional medicine for the treatment of skin infections, such as cutaneous mycoses. This work evaluated the healing activity of the hydroethanolic seed extract of *Vatairea guianensis* (HESE) on the open wounds of rats using topical administration. Rats were divided into the following treatment groups: G1 positive control (fibrinase); G2 negative control (saline solution); G3 dose 500 mg/kg; G4 250 mg/kg; and G5 100 mg/kg. Treatments were administered for seven days, and acute toxicity was measured orally in mice. Histological analyses of the wound healing process was performed using conventional methods, including hematoxylin and eosin (HE) and picosirius staining, to observe the histomorphological characteristics of the inflammatory reaction and a descriptive analysis of collagen deposition, respectively. The histological analysis showed that the HESE decreased the intensity of the inflammatory reaction in groups G3 and G1, stimulated the synthesis of collagen type III in G1, G3 and G4 and increased the synthesis of collagen in G2 and G5. The experiment with the HESE of *Vatairea guianensis* showed that it delayed the healing process in open wounds at doses of 500 and 250 mg/kg. These results may be a positive effect because these doses of HESE prevented the formation of hypertrophic scarring, which suggested a modulatory effect of the extract. The acute toxicity analysis in mice showed that the hydroethanolic extract had low oral toxicity.

Keywords: Healing activity; seed; hydroethanolic seed extract; *Vatairea guianensis*.

INTRODUCTION

An organism's ability to replace damaged or dead cells is a phenomenon of major importance for survival.^{1,2}

Healing includes a series of cellular, molecular and biochemical events that involve important tissue changes for maintaining the integrity of the organism. Healing by first intention occurs when the skin edges are adjacent to each other (i.e., re-approximated), which increases the probability that the healing process will occur naturally. The process of healing by second intention, which was used in this study, occurs when the wound is open and exposed, with a greater loss of cells and tissue. Healing by second intention differentiates itself from the process by first intention because it shows the physiological phenomenon of wound contraction.³

The family Leguminosae is one of the most important botanical families because of the number of plant species that are included. This family includes 642 genera that are distributed in 18,000 species. It comprises three subfamilies, Caesalpinioideae, Mimosoideae and Faboideae (Papillinoideae).^{4,5}

The genus *Vatairea*, which belongs to the Leguminosae – Faboideae family, is exclusive to the Neotropical zone and includes only 7 species of leguminous trees that are distributed from southern Mexico to southeastern Brazil, including Mexico, Guatemala, Honduras, Costa Rica,

Panama, Colombia, Venezuela, Guyana, French Guiana, Suriname, Peru and Brazil. The concentration center of the wider variety of this genus is located in the forest regions of central Amazonia. The genus name refers to a popular name that is used in Guyana.^{4,6,7}

Regarding the Amazonian forest species that belong to this genus, *Vatairea guianensis*, *Vatairea fusca*, *Vatairea erythrocarpa*, *Vatairea paraensis* and *Vatairea sericea* can be highlighted. Other species of the genus inhabit hillside forests, such as *Vatairea heteroptera*. In southern Mexico and Central America, the species *Vatairea lundelli* stands out.⁷

The species *Vatairea guianensis* is popularly known in Amazonia as *faveira*, *fava de empigem*, *faveira de empigem*, *fava bolacha*, *fava mutum*, *faveiro* and *Angelim do igapó*.⁸

Among the Palikur Indian population in northern Amazonia on the border of Brazil and French Guiana and extremely north of Amapá state, *Vatairea guianensis* is called *waru*. In Suriname, it is known as *gales habbes*, and in Peru, it is known as *anacapi* and *marupa del bajo*. In French Guiana, it is called *graine i dartres*, *bois dartre* and *maria congo*. In Venezuela, it is known as *guaboa*.⁹

Vatairea guianensis is a native species of Amazonia and is common in seasonally flooded forest areas, such as the *igapo* and *varzea* forests. It is rarely found on solid



ground but occurs throughout the regions that are bounded by the Amazon River and its tributaries, including the borders of French Guiana, Venezuela, Colombia, Peru and Suriname.¹⁰

Especially in the state of Amapá, Brazil, the occurrence of this species is higher in the city of Mazagão, but it is also found in other cities in the state, such as Porto Grande and the Archipelago Bailique.¹¹

Ethnopharmacological information from the literature reports that the juice of the fruit is used in Amazonian traditional medicine to heal ringworm and to treat certain skin diseases in Brazil, Venezuela, Colombia and French Guiana.⁷

In the middle and lower Brazilian Amazon, the population uses the seeds of *Vatairea guianensis* Aublet in the form of an alcoholic tincture or by a direct application of the soaked seeds to the skin against several kinds of superficial skin mycoses. The literature also reports that the population uses the barks from the stem and roots against fungi when this species is not in the fruiting phase.^{8,12}

Based on the aforementioned citations, this study aimed to evaluate the possible healing activity of the hydroethanolic seed extract (HESE) of *Vatairea guianensis*.

MATERIALS AND METHODS

Obtaining botanical material and the hydroethanolic seed extract (HESE)

The botanical material and the fruits of *Vatairea guianensis* were collected in the region of the municipality of Porto Grande, 100 km away from the capital of the Amapá state (Macapá), Brazil, in July of 2009. After obtaining the material, exsiccates of the plant material were deposited in the Herbarium at the Amapá Institute for Scientific and Technological Research (IEPA), identified by comparison by the botanist Benedito Rabelo, and registered with no. 1916. The HESE was obtained following the method described previously by Silva et al.¹³

Healing activity analysis of the HESE on the open cutaneous wounds of second intention on the backs of rats

Animals

This research project was approved by the Research Ethics committee of the Federal University of Amapá with the protocol approval no. 005A/2009. We used 25 female albino Wistar rats weighing 200 ± 90 g and aged from 110 to 160 days from the animal facility of the Central Laboratory of Public Health from the state of Amapá, Brazil.

Over the experimental stage, the adequately identified animals were kept in polyethylene cages and were maintained in controlled environmental conditions with

controlled temperature and humidity and light/dark cycles of 12 h. The animals had *ad libitum* access to water and food (Labina[®]) throughout the experiment.

The animals were distributed in equal numbers and were randomly assigned to 5 groups with 5 animals each ($n = 5/\text{group}$). Rats were identified, and immediately after surgery, the wounds were measured and photographed. Each animal received treatment strictly during standardized times (18:00 h – 19:00 h). Treatments were administered once daily for 7 days. The following treatment groups were used:

Group 1 – Positive control group – the animals were treated topically with fibrinase (desoxyribonuclease 666 U/g + fibrinolysin 1 U/g + chloramphenicol 0.01 g/g) at a dose of 36.88 mg/kg.

Group 2 – Negative control group – the animals were treated topically with an aqueous sodium chloride solution (0.9% saline solution).

Group 3 – Experimental group - 500 mg/kg - the animals were treated topically with HESE in saline solution, at a dose of 500 mg/kg once daily for 7 days.

Group 4 - Experimental group - 250 mg/kg - the animals were treated topically with HESE in saline solution, at a dose of 250 mg/kg once daily for 7 days.

Group 5 - Experimental group - 100 mg/kg - the animals were treated topically with HESE in saline solution, at a dose of 100 mg/kg once daily for 7 days.

The topical application occurred immediately after the surgical procedure in all of the groups. The wounds did not receive any type of bandage, but they were cleaned daily with saline solution before topical application. Wounds remained exposed to the environment throughout the period of experimentation.

All groups underwent the same surgical procedure and were monitored for a period of 7 days during treatment. After this time, the animals were sacrificed in a CO₂ chamber.

Surgical procedure and measurement of cutaneous wounds and biopsies

The surgical procedure was performed after anesthesia with sodium thiopental 45 mg/kg, i.p. (Cristalia Laboratory, S. Paulo, Brazil) and followed the method described previously by Albuquerque Junior et al.¹⁴ to produce a lesion of approximately 6 mm in diameter.

Immediately after the surgical procedure, the wounds were measured using a digital caliper (ZAAS Precision-Digital Caliper 0-150 mm) and photographed with a digital camera ("Cyber-shot", Sony, 12.1 megapixels). The digital images were stored in Windows Bitmap (BMP) format and analyzed using ImageLab software (version 2.4, Borra, SP, 2000). These procedures were standardized and performed daily immediately after surgery until the last day of the experiment when the measurements were obtained both in the craniocaudal and left-right axes.



The crust that formed on the 2nd postoperative day was considered in the measurement of the wounds and in the wound area using the method described previously by Prata et al.¹⁵ The degree of wound contraction, which was expressed as a percentage, was determined by the method described previously by Ramsey et al.¹⁶

At the end of 7 days of treatment, the animals were sacrificed. The wound was excised with a safety margin of healthy skin around the lesion to the depth of the muscular fascia under aseptic conditions, and a median section was performed. Wound pieces were fixed in a 10% formaldehyde solution for histological analysis.

Histomorphological analysis

The slides were stained with hematoxylin and eosin (HE) and picosirius, and the histomorphological characteristics that are associated with the inflammatory/reparative process were assessed in histological sections that were stained with HE. The following criteria were considered in the analyses: the intensity and type of inflammatory infiltrates.

The intensity of the inflammatory process was analyzed and determined according to the following criteria: (1) inflammatory cells, regardless of their phenotype, were less than 10% of the cell population that was observed in the area of the surgical wound, (2) the inflammatory cells, regardless of their phenotype, constituted 10% to 50% of the cell population that was observed in the area of surgical wound, and (3) the inflammatory cells, regardless of their phenotype, constituted more than 50% of the cell population in the area of the surgical wound.

The type of inflammatory reaction was determined by the quantification of the different individual inflammatory cells, including neutrophils, eosinophils, lymphocytes, plasma cells and macrophages. Inflammatory cells were identified by their specific morphology. Therefore, for this analysis, the following criteria were applied: (1) the cell phenotype under study corresponded to less than 10% of the total inflammatory cells that infiltrated the wound area; (2) the cell phenotype under study corresponded to more than 10% and less than 50% of the total inflammatory cells in the wound area; and (3) the cell phenotype under study corresponded to more than 50% of the total inflammatory cells in the wound area. After this semiquantification, the inflammatory reaction was categorized based on the predominant inflammatory phenotype of acute inflammation (i.e., a predominance of neutrophils and/or eosinophils), subacute inflammation (i.e., a balance between neutrophils and/or eosinophils and lymphocytes and plasma cells), nonspecific chronic inflammation (i.e., lymphocytes and/or plasma cells predominate) and chronic granulomatous inflammation (i.e., macrophages and/or giant cells predominate). The reaction of granulation was categorized according to the following criteria: (1) the proliferative phase was predominantly endothelial, (2) the proliferative phase showed vascular characteristic, and (3) the proliferative phase showed fibrovascular characteristics.

Assessment of the deposition pattern of collagen fibers

The slides were stained with picosirius (Sirius Red), which specifically labels collagen deposits, and analyzed using a polarized light microscope.

The analysis of all of the groups was descriptive, and emphasized the identification of collagen, which was classified as type I or III according to the birefringence that was shown (e.g., green, greenish yellow, golden yellow, orange and red), the appearance of fiber density (e.g., loose, moderate, dense), the arrangement of bundles (e.g., reticular, parallel, interlaced) and thickness (e.g., thin-soft, thick-soft, coarse-soft).

Analysis of the acute toxicity of HESE in mice

A total of 40 male mice (Swiss, albino) weighing 20 to 30 g were divided into groups of 10. One group received a single dose of 2,000 mg/kg HESE, and another group received a dose of 5,000 mg/kg HESE. HESE was diluted in a solution of 1% Tween® 80 and was administered orally by gavage. Each group was compared to the negative control group of 10 animals that received only distilled water. The animals were observed for an uninterrupted period of 4 hours after administration to assess possible behavioral changes and deaths according to the method described previously by Malone & Robichaud¹⁷. The animals were evaluated for an additional period of 14 days with access to food and water *ad libitum*. This test followed the guidelines of the OECD (Organization for Economic Cooperation and Development, Guideline 425).

Statistical analysis

The results from the macroscopic evaluations are shown as the means \pm SE (standard error) and were considered statistically significant when the results showed the probability of the null hypothesis was lower than 5% ($p < 0.05$). To compare means, an ANOVA was used followed by the Student-Newman-Keuls test for multiple comparisons. Statistical analysis was performed using the statistical software Instat and SigmaStat.

RESULTS

Assessment of the healing activity of the HESE on open cutaneous wounds of second intention in the backs of rats

The daily mean values of the wound areas of each group showed that there was a reduction of injury in all of the groups throughout the experiment for the evolution of healing and wound contraction. However, on the 3rd, 5th and 7th days of treatment ($p < 0.05$ - Student-Newman-Keuls test), the group G5 that was treated with a dose of 100 mg/kg HESE showed a smaller area compared to the group G2 (negative control). This dose also showed a statistically significant difference regarding the wound area compared to the group G1 (positive control, fibrinase) on the 5th day ($p < 0.01$, Student-Newman-Keuls test) (Table 1 and figure 1).



The results in the experimental conditions of this study showed that the positive control group (fibrinase) had a mean contraction of 79.37%, and the mean of the negative control group (saline solution) was 77.69%. In the G3 group (500 mg/kg HESE), the mean contraction was 74.79%. The mean contraction in the G4 group (250 mg/kg) and the G5 group (100 mg/kg) was 64.07% and 77.89%, respectively. Notably, there was variation in the percentage of the different groups on the 7th day, however, there was no statistically significant difference compared to the G2 group (negative control) (Figure 1 and table 1).

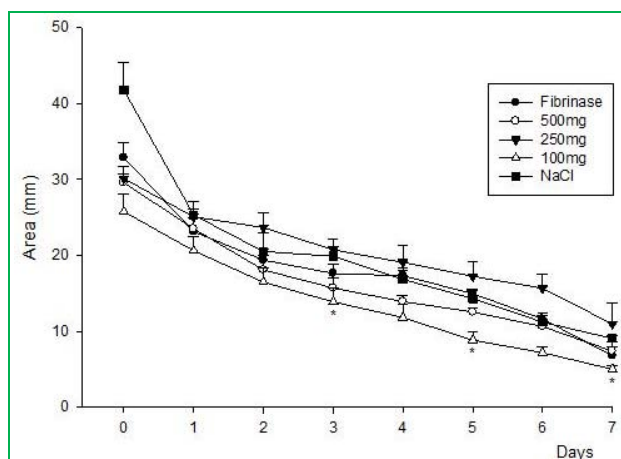


Figure 1: The effect of topical treatment with the hydroethanolic extract of *Vatairea guianensis* (500 mg/kg, 250 mg/kg, 100 mg/kg), fibrinase (36.88 mg/kg), and saline solution (0.5 ml /animal - NaCl), at the wounds of Wistar rats following 7 days of treatment (n = 5/ group). Student-Newman-Keuls test for multiple comparisons * p < 0.05.

Histomorphological analysis

On the 7th day, the G1 group, (positive control) exhibited an inflammatory reaction of moderate intensity with an

infiltration of inflammatory cells that was categorized as chronic due to the observation of a significant increase in the numbers of mononuclear leukocytes (lymphocytes) in the superficial portion of the wound (Table 2). Moreover, a large amount of blood vessels were filled with erythrocytes and featured a predominantly vascular granulation reaction.

The G2 group (negative control) showed a significantly intense inflammatory reaction with an inflammatory infiltration pattern that was categorized as subacute on the 7th day due to the predominance of polymorphonuclear cells (neutrophils) in the surface in addition to the mononuclear lymphocytes in the deeper region and the remarkable presence of edema (Table 2).

The G3 group (500 mg/kg HESE), showed moderate inflammation with an inflammatory infiltration pattern that was categorized as chronic on the 7th day due to the observation of a more extensive amount of mononuclear leukocytes (lymphocytes) (Table 2).

However, the G4 group (250 mg/kg HESE) showed inflammation of significant intensity but with an inflammatory infiltration pattern that was categorized as subacute on the 7th day due to the balanced numbers of neutrophils and lymphocytes in addition to exhibiting a proliferation of cells with ovoid nuclei and dispersed chromatin, which is consistent with endothelial cells (Table 2). Finally, the G5 group (100 mg/kg HESE) showed inflammation of considerable intensity with an inflammatory infiltration pattern that was categorized as subacute on day 7 due to the observation of a significant and balanced number of neutrophils and lymphocytes with a predominantly endothelial granulation reaction (Table 2).

Table 1: Wound area (mm²) during the immediate post-surgical time point and on the 7th day of treatment and the percentage of contraction in the different experimental groups.

Group	Immediate post-operative day (mm ²)	7 th day (mm ²)	Contraction (%)
G1 - Fibrinase 36.88 mg/kg	32.90±2.02	6.79±1.18	79.37
G2 - saline solution 0.9%	41.80±3.65	9.08±0.45	77.69
G3 – HESE 500 mg/kg	29.59±1.80	7.42±1.30	74.79
G4 – HESE 250 mg/kg	30.39±1.66	10.97±2.71	64.07
G5 – HESE 100 mg/kg	25.77±2.39	5.52±0.40	77.89

The numbers show the mean ± SE of 5 animals/group

Table 2: Summary of the morphological differences in the pattern of inflammation, cell recruitment and granulation reaction of different experimental groups.

Groups	Inflammation pattern	Cell recruitment	Granulation reaction
G1 – Fibrinase 36.88 mg/kg	Chronic	Lymphocytes	Vascular
G2 - saline solution 0.9%	Subacute	Neutrophils / Lymphocytes	Endothelial
G3 - HESE 500 mg/kg	Chronic	Lymphocytes	Vascular
G4 - HESE 250 mg/kg	Subacute	Neutrophils / Lymphocytes	Endothelial
G5 - HESE 100 mg/kg	Subacute	Neutrophils / Lymphocytes	Endothelial



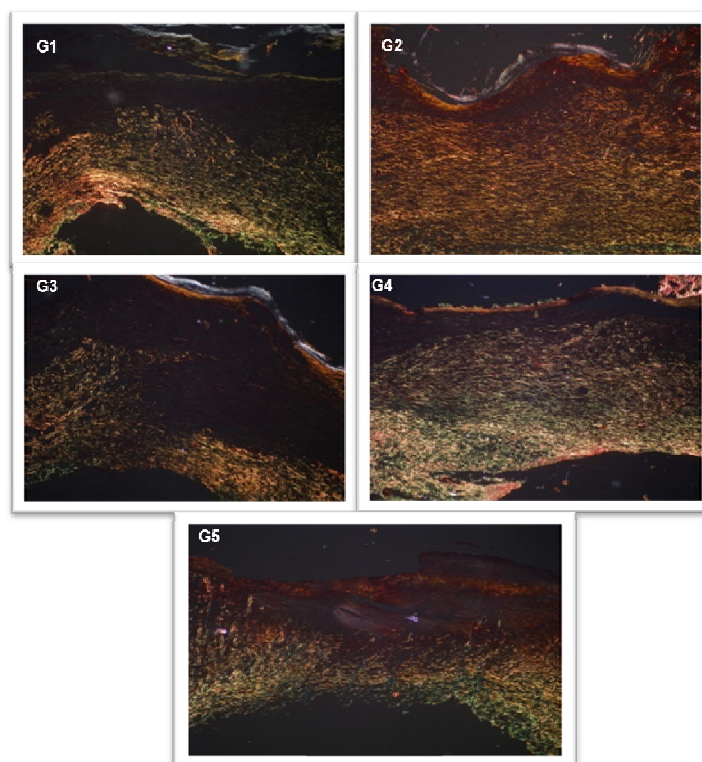


Figure 2: Groups G1 (Fibinase 36.88 mg/kg), G3 (HESE 500 mg/kg) and G4 (HESE 250 mg/kg) exhibited a predominance of mainly collagen type III fibers (greenish yellow), loosely arranged with a reticular pattern. In the G2 (saline solution 0.9%) and G5 (HESE 100 mg/kg) groups, the observed fibers were mainly of type III but with a clear deposition of fibers of collagen type I (gold) with a parallel arrangement of collagen fibers.

It is noteworthy that the presence of interstitial edema was more prominent in the G2 group (negative control) compared to the other groups. In the G3 (500 mg/kg HESE), G4 (250 mg/kg HESE) and G5 (100 mg/kg HESE) groups, the edema was less evident. Moreover, the G3 group (500 mg/kg HESE) showed behavior that was similar to the G1 group (positive control) in the intensity of the inflammatory process, which showed moderate inflammation with an infiltration pattern that was categorized in both groups as chronic nonspecific. The G4 and G5 groups showed results similar to the G2 group (negative control) with an infiltration pattern that was categorized as subacute.

Furthermore, the morphological expression of the granulation reaction in the G1 group was categorized as vascular. In the G2 group, the granulation reaction was endothelial with 1 specimen that showed a fibrovascular reaction. The G3 group expressed a morphologically vascular granulation reaction in most specimens. The other experimental groups (G4 and G5) exhibited a predominantly endothelial granulation reaction in most specimens. A summary of these results is shown in Table 2.

Assessment of the deposition pattern of collagen fibers

The sections that were stained with picosirius were examined under polarized light on the 7th day. In the G1 group, a predominance of collagen type III with a greenish-yellow birefringence and loosely-arranged fibers

with reticular disposition fibers was observed. Individually, the fibers were thin and delicate (Figure 2).

The G2 group presented a particular variety of birefringence that showed a predominance of yellowish-gold fibers (type I) and greenish-yellow (type III) fibers of loose density that were arranged in a reticular pattern with an increased amount of clearly wider interfibrillar spaces (i.e., looser tissue) and thicknesses with a thin and delicate aspect. Of particular interest was the observation of the clear presence of a larger and better quantity of collagen type I and a tendency for parallelism compared to the G1 group (Figure 2).

In the G3 group, the histological sections revealed the general predominant presence of collagen type III with greenish-yellow birefringence and loosely arranged fibers with a reticular disposition and a thin and delicate appearance (Figure 2).

In the G4 group, the sections revealed the prevalence of collagen fibers of type III with a greenish-yellow birefringence of moderate density that were arranged in a reticular pattern with the appearance of thin and delicate fibers (Figure 2).

Finally, the G5 group exhibited predominantly type III collagen fibers with greenish-yellow birefringence that was loosely and reticularly arranged with a thin and delicate appearance. In this group, a tendency for fiber

parallelism, which indicates the presence of type I fibers, was observed (Figure 2).

Analysis of acute toxicity of HESE in mice

The analysis of the acute toxicity of orally administered HESE in animals that were treated with 2000 mg/kg showed that there were no behavioral changes, such as locomotor activity changes, piloerection, analgesia, hypoactivity, hyperactivity, lachrymation, anesthesia, the loss of paw withdrawal reflex, alertness, apathy, stereotypy, aggressiveness, sweating, urination, diarrhea or convulsions. At a dose of 5000 mg/kg HESE, there were also no signs of the aforementioned symptoms. Moreover, neither of these doses led to the death of the animals.

DISCUSSION

Wound healing is a biological event of intense complexity that involves the fundamental process of inflammation and subsequent chemotaxis, cell proliferation, collagen deposition and.³

The process of healing by secondary intention has the characteristic induction of wound repair with a greater loss of cells and tissues without the attachment of the edges but with the formation of fibrin clot that fills the lesion. The reaction of granulation grows from the edges to complete the repair.¹⁸

The stimulation of medicinal substances for the study of the healing process is particularly interesting, especially because it represents the possibility for improving the process of wound healing. It also may be useful for the treatment of superficial injuries, even injuries that require the filling of a lost tissue area (open wound)^{1,19}. Therefore, the stimulus that is provided by alternative therapies, such as natural products and medicinal plants, has been widely discussed by several authors^{20,21}

Several plant species of popular use that stimulate healing have been broadly investigated. Therefore, the HESE of *Vatairea guianensis* Aublet, which is leguminous species native to the Amazon of the Amapá state, Brazil, was submitted for the study of the wound healing process. Previous chemical studies of the species *V. guianensis* and the genus *Vatairea* have shown the remarkable presence of anthraquinones, which are responsible for several biological activities, including antimicrobial, antifungal and healing.²²

The search for strategies that render products and natural or synthetic substances that promote the improvement of the healing process has been discussed over the years, especially the use of alternative therapies, including medicinal plants and their derivatives, extracts and tinctures.²⁰

The macroscopic analysis of the repair areas in the experimental animals showed that the topical use of HESE of *Vatairea guianensis* in the studied doses promoted wound contraction over time. The wounds of second

intention that were performed in this study produced wound contraction that was promoted by the action of myofibroblasts, which contain actin filaments. These cells are responsible for this phenomenon²³. By visual inspection of the experimental groups, G5 showed a better clinical response. However, there was no statistically significant difference in the percentage of wound contraction in the experimental groups compared to the negative control.

In the microscopic histological analyses, the inflammatory reaction of the groups oscillated from intense to moderate on the 7th day, although the pattern of leukocyte infiltration exhibited differences between the five studied groups. Therefore, these results suggested that treatment with the topical administration of the HESE of *Vatairea guianensis* exhibited an anti-inflammatory property. Comparing the HE staining of the morphological findings of the positive control G1 group with the experimental G3 group, the inflammatory reaction was moderate with a leukocyte profile that showed a considerable infiltration of lymphocytes.

These results may indicate the beginning of the chronic nature of the healing process, and may therefore indicate an acceleration of the events that are characteristic of the inflammatory response because this cell is typical of chronic inflammation. The G2 (negative control), G4 (250 mg/kg) and G5 (100 mg/kg) groups showed an inflammatory reaction that was intense but with a balanced infiltration of neutrophils and lymphocytes, which is a response that is characteristic of a subacute inflammatory reaction.

Notably, in the early stages of host response to injury, the influx of neutrophils is determined by the action of chemical mediators that are released during tissue injury, such as prostaglandins and leukotrienes²⁴. However, it is emphasized that in the repair process, the duration of the neutrophil inflammatory response is proportional to the degree of chemokines that are released, which, in turn, is directly dependent on the intensity of the injury and the degree of secondary bacterial contamination²⁵. This explains the fact that in the G2, G4 and G5 groups, the permanent acute reaction remained evident even after 7 days. This result could be related to bacterial contamination because the wound remained exposed throughout the experimental period.

Evidence of an increased infiltration of lymphocytes in the G1 and G3 groups compared to the others groups suggested a low release of chemokines at the site of injury and, consequently, a faster transition from polymorphonuclear to mononuclear cells.

In this context, it is interesting to note that inflammation is absolutely necessary to the repair process, as seen by its role in the removal of the pathogenic agent and, in the last instance, the reparation of tissue injuries²⁶. However, when this phenomenon is exacerbated or is present for too long, it can act to delay healing. Therefore, one can infer that the intensity of the inflammatory response that



was observed in the G2, G4 and G5 groups was not exacerbated, and consequently, it did not appear to negatively influence the installation of the phenomena that characterize the repair process. Furthermore, in these groups, the reaction of granulation during the early stages with the predominance of cells with loose connective tissue and few fibrils was observed.

In wound healing by second intention, which is characterized by an excessive loss of tissue, collagen deposition represents a key factor in skin recovery after an injury¹⁸. In this condition, the healing process occurs slowly and requires the formation of the granulation reaction, which is composed of a great amount of blood vessels and cells that fill the void left by the loss of tissues. Therefore, when new layers of granulation reaction are formed, the older layers that are located deeper lose blood vessels, which provides space for fibroblasts and collagen bundles that predominate.²⁴

Collagen synthesis begins on the 3rd day and reaches its peak in 3 to 6 weeks. After this period, the healing process enters the remodeling phase. Therefore, the maturation of scar tissue involves the replacement of collagen type III, which is strongly present in connective tissue, to collagen type I, which is abundant in many tissues and confers a high resistance to skin, tendons and ligaments²⁷. In this context, the use of specific staining for collagen, such as picrosirius, is of great importance because it allows for the specific analysis of the types of collagen using polarized light.²⁵

In the present study, the picrosirius staining was used to examine the pattern of collagen deposition analysis because it has a strong sensitivity and specificity for collagen fibers.²⁷

The sections that were stained with picrosirius showed the G2 (negative control) and G5 (100 mg/kg HESE) groups showed a more frequent appearance of fibers that were arranged in parallel, which indicated the early formation of collagen type I. Of particular interest was the observation that the G1 (fibrinase) G3 (500 mg/kg) and G4 (250 mg/kg) groups showed a higher frequency of fibers with a reticulate pattern, a thin and delicate thickness and a greenish-yellow birefringence, which is characteristic of the deposition of collagen type III. The verification of the appearance of collagen type I in the G2 and G5 groups suggested the replacement of collagen fibers type III by type I, which represents a characteristic step in the evolution of the healing process as previously reported.²⁸

The G3 (500 mg/kg) and G4 (250 mg/kg) played a role similar to the positive control that was represented by the drug fibrinase, which contains a proteolytic enzyme that stimulates the granulation reaction by modulating the synthesis of collagen. However, the G5 (100 mg/kg) and G2 (negative control) groups showed similar effects regarding collagen evolution. These findings suggested a modulation of the healing process by the tested extract. In both of these groups, parallel fibers were displayed,

which indicated the onset of type I collagen fibers replacement of type III collagen. Therefore, these findings suggested that a faster evolution of the healing process occurred with the treatment of the HESE of *V. guianensis* in the G2 and G5 (100 mg/kg) groups. Moreover, in the G1 (positive control), G3 (500 mg/kg) and G4 (250 mg/kg) groups, HESE caused a delay in the repair process.

Assessing the relationship of the control groups with the experimental groups, our findings implied that the HESE of *V. guianensis*, in addition to stimulating the reaction of granulation similarly to fibrinase, also plays a role in the modulation of the speed of collagen synthesis to avoid a hypertrophic scar, which is characterized by excessive collagen. Therefore, the extract also stimulated the synthesis of collagen type III in the G1, G3 and G4 groups in addition to blocking its excessive synthesis by slowing or delaying the regulated deposition of collagen to minimize further hypertrophic healing. Healing by secondary intention is characterized by an open wound with an excessive loss of tissue where the granulation reaction is necessary. These results are of great importance for the understanding of the biological activity of the HESE of *V. guianensis* in the process of repair.

The analysis of the results obtained in the present study suggested that the HESE of *V. guianensis* decreased the intensity of the inflammatory reaction on day 7 with a remarkable infiltration of lymphocytes in all groups. However, lymphocyte infiltration was more evident in the G1 and G3 groups, which improved the healing process. In addition, the HESE of *V. guianensis* modulated the velocity of collagen synthesis because parallel collagen fibers, which are indicative of type I collagen, were observed in the G2 and G5 groups, and HESE stimulated the synthesis of collagen type III in the G1, G3 and G4 groups. Therefore, it is preliminarily suggested that the studied extract may have clinical application, especially in the prevention of hypertrophic scarring by slow collagen stimulation and the blockade of the excessive synthesis of this protein. However, further studies should be performed to elucidate the pathophysiological mechanisms that are responsible for the possible bio-modulating activity of the HESE of *V. guianensis* during wound healing.

The results obtained in the evaluation of acute toxicity suggested that the extract has low oral toxicity in mice because no animals died and any noteworthy signs and symptoms of toxicity were recorded after the administration of doses of 2000 mg/kg and 5000 mg/kg.

CONCLUSION

The HESE obtained from the seeds of *Vatairea guianensis* interfered positively with the healing process by decreasing the intensity of the inflammatory reaction and stimulating the synthesis of collagen.



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