



Research Article

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## Topical treatment with yellow-ipe extract (*Tabebuia aurea*) in wound healing by secondary intention in rats

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### ABSTRACT

The objective of the research was to study the healing in vivopotential, from the topical administration of the ethanol extract of the leaves of *Tabebuia aurea* (EETA). Twenty-seven *Rattus Norvegicus Albinus Wistar* were used in this study, divided into three groups with  $n = 9$ . The animals were followed for fourteen days, the post-production of the wounds. Group 1 was treated with 5% dexpanthenol, group 2 with based cream and group 3 was treated with 5% EETA. It was performed a macroscopic assessment of wounds, to monitor the size of the injury area, contraction percentage, histopathology analysis and biochemical evaluation. The results showed that the evolution of the group treated with EETA showed no scar development better than control groups, and presenting crust on the macroscopic assessment and the absence of totalre-epithelialization, at the end of fourteen days of treatment.

**Keywords:** Healing; *Tabebuia*; VegetalExtracts; Nursing.

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### INTRODUCTION

After the skin lesions occurs biochemical and physiological events for the restoration of injured tissue. Wound healing encompasses a cascade of linked events and which overlap, involving the inflammatory phase, proliferative and remodeling. Choosing the right substance to be dispensed on the wound is related to successful treatment [1].

The use of vegetal extracts for medicinal purposes is one of the oldest health care forms used by humanity [2] and some research were performed to evaluate the potential of vegetal extracts as healing [1,3].

The *Tabebuia aurea* is a tree native of Brazil, occurring in the Amazon biome, scrubland, and Atlantic Forest. Popularly known as yellow-ipe, *Tabebuia aurea*, “pau d’arco” and “paratudo” [4]. When used as a medicinal plant, the ethnobotanical research found that *Tabebuia* genre is mainly employed for the treatment of pain and inflammation, however, is also used for the treatment of cancer, malaria, tuberculosis, stomach problems, antimicrobial, antiseptic, wounds treatment and healing [5-9].

Basic experimental research showed biological activities for the genre, such as anti-inflammatory [10] antimicrobial [11] antioxidant [12] and wounds healing [13-14], however, there are few reports in the literature studying the biological activities of *T. aurea* species.

Aiming to expand the possibilities of therapeutic resources for the treatment of skin wounds, the study sought to evaluate the *in vivo* healing activity, from the topical administration of the ethanol extract of leaves of *Tabebuia aurea* (EETA).

## EXPERIMENTAL SECTION

### Collection and identification of vegetal sample

The vegetal sample of leaves *T. aurea* was collected in the city of Maceió-AL, forest zone region and vegetation of the Atlantic Forest, in January 2015, at the Federal University of Alagoas (Geographical coordinates: S9 ° 33' 23.6" W35° 46'39.6"). The sample was deposited in the Herbarium of the Environment Institute of Alagoas State, under MAC Registration<sup>o</sup>21433.

### Extract preparation

*T. aurea* leaves were dried at 38 °C and crushed. The leaves extracts were obtained by cold maceration method with ethyl alcohol of 97%, during nine days by exhaustive maceration and concentrated in a rotary evaporator at 45 °C [15].

### Experimental animals

Twenty seven *Rattus norvegicus albinos* were used, of Wistar lineage, females, with approximately four months old, weighing between 170-220 grams, provided by the Vivarium of the State University of Health Sciences (UNCISAL) and transferred to the Research and Wound Care Laboratory - LpTF of the Federal University of Alagoas, respecting the ethical principles in animal experimentation. This research was submitted to the Ethics Committee for Animal Use of the Federal University of Alagoas (CEUA/UFAL) and approved under process number 011/2014.

The rats were kept in plastic cages, covered with sterile wood shavings, minimal noise, a light-dark cycle of 12 hours, a temperature of 21 ± 2 °C, adequate ventilation, suitable food for the species (Nuvilab®) and free access to water. The rats were separated from their living group, one animal per cage and kept under observation for twenty one days before the bioassay. After this period, they were randomly divided into 3 groups of nine animals each, identified with the therapy: **group 1:** positive control (PC) - dexpanthenol 5%; **group 2:** negative control (NC) - based cream, nonionic; and **group 3:** treated with Ethanol Extract of *T. aurea* leaves (EETA) 5% incorporated into the cream base.

### Wound confection and treatment

Each animal underwent an examination of body weight for anesthesia calculation. The anesthesia was performed by intraperitoneal injection with 50 mg/kg of ketamine at 10% at 10 mg/kg of xylazine at 2%, administering 0.1 ml per 100 g of body weight of the animal.

All animals were weighed for the calculation of the anesthetic, which was performed intraperitoneally with 50 mg/kg of ketamine at 10% and 10 mg/kg of xylazine at 2%, administering 0.1 ml per 100 g of body weight of the animal [16].

When the necessary anesthetic depth was achieved, back epilation was held followed by skin antiseptics with chlorhexidine degreasing 2%. With a 12 mm metallic *punch* was made a single dorsal excisional injury to the level of aponeurotic tissue and then, the lesions were cleaned with 0.9% saline and covered with gauze and bandage, with aseptic technique and waiting 24 hours to start the therapy [1]. Between 1-14 postoperative days, followed the body temperature verification, the realization of dressings with a cover according to the division of the treatment groups.

During the treatment, 0.3 ml of cream was dispensed into each wound of all animals according to the therapy. On days 3, 7 and 14 occurred euthanasia of animals. At every euthanasia, three animals per group were sacrificed, to remove the wound and blood collection for biochemical evaluation of the following serum tests: aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine. The animals were euthanized at three times the dose used for the surgical procedure of wound confection. 2 to 5 ml of blood was removed by cardiac puncture and the wound was withdrawn (obtaining a fragment of the ellipsoid shape also covering the perilesional area).

### Macroscopic evaluation

On days 3, 7, 11 and 14 the findings of macroscopic wound evaluations were inserted into an instrument for clinical analysis of the lesions and this instrument assessed the following characteristics: size of the lesion, perilesional redness, inflammation, presence of granulation tissue, extension of the crust, exudate and fibrin [1].

### Wound area and percentage of wounds contraction

The wound size measurements were performed on days 0, 3, 7, 11 and 14. The measurement of the major and minor diameters of the wound was performed in triplicate, with a manual caliper, and the mean obtained. The results were calculated using the following mathematical equation:  $A = R \times r \times \pi$ , where A represents the wound area, R representing the largest radius, r representing the smallest radius, and  $\pi$  a constant equivalent to 3.14. The contraction percentage (%C) of the wounds was calculated using the formula:  $\%C = [(A_i - A_f) \div A_i] \times 100$ , where  $A_i$  is the initial area (immediate postoperative) and  $A_f$  is the final area in the corresponding postoperative day [17].

### Histological analysis

The wound was withdrawn covering the adjacent full skin and fixed in 10% formalin and submitted to paraffin, held 5  $\mu$ m thickness cuts and stained by hematoxylin-eosin method [1]. The identification of blades did not show the groups that belonged, were only numbered from 1 to 27. The blades reading was performed by pathologist Dr. Ricardo Luiz Simoes Houly. The histological analysis adopted the wound healing phases for the evaluation: inflammatory, proliferative and remodeling. For analysis of the results were elected scores (Table 1), in which the intensity of the variables, with a certain score from +1 to +5, was multiplied by factors - positive or negative - based on their importance to the healing [1.18]. Made the calculation, the product corresponded to the total score for each animal, which was subsequently added to the scores of other animals in the group and the average obtained

**Table 1- Scores used for histopathology evaluation**

Variables	Absent	Present	Discreet	Moderate	Intense	Factor
Crust/Necrosis	+1	+2	-	-	-	-1
Acuteinflammation	-	-	+3	+4	+5	-4
GranulationTissue	+1	+2	-	-	-	+10
Fibroblastproliferation	-	-	+3	+4	+5	+5
Neovascularization	-	-	+3	+4	+5	+5
Reepithelization	-	-	+3	+4	+5	+5
Collagenfibers	+1	+2	-	-	-	+10

Source: Adapted[1.18]

### Statistical analysis

Statistical results were obtained by *GraphPad Prism 5*® program and numeric variables evaluated by the test *One-way ANOVA* (variance analysis) with *Tukey* and *Dunnet* post-test. The values were considered significant when  $p < 0.05$  and results were expressed as mean  $\pm$  mean standard error (M  $\pm$  SE).

## RESULTS AND DISCUSSION

### Healing activity

**Group 1:** positive control (PC) - dexpanthenol 5%; **group 2:** negative control (NC) - based cream, nonionic; and **group 3:** treated with Ethanol Extract of *T. aurea* leaves (EETA) 5% incorporated into the cream base.

The animals did not show weight change, auto-aggression, and irritability during the procedure. The macroscopic evaluation of lesions allowed to follow the healing events in its stages, assessing the therapy progress used to treat them.

Group 1, positive control (PC) treated with dexpanthenol, presented moderate hyperemia of the wound edges, inflammation, exudate, and fibrin till the 7th day. The fibrin was abundant on the 3rd day but easily removed during treatment. There was granulation tissue formation after 3 days in some animals. The wounds were found with moist appearance, with the absence of crusting and progressive evolution of contraction (Figure 1A).

Group 2, negative control (NC) treated with base cream, nonionic presented in all animals, inflammatory characteristics, greater perilesional redness, and fibrin on the 3rd day of treatment and without the presence of exudate. The wounds possessed drying characteristics, remaining until the 4th day. The wound edges, until the 3rd day, showed greater area than the other groups. There was no presence of crust in any animal at any evaluation day, observing the granulation tissue from day 7 (Figure 1B).

Group 3, treated with ethanol extract of *T. aurea* leaves (EETA) 5% incorporated into the base cream was what showed less inflammatory process, hyperemia absence and exudate from the 7th day, when compared to control groups. The crust was partially present, in all animals of this group until day 3, progressing to full extension crust in all animals after 14 days of treatment. On the 11th day, some animals began to show part of the detachable crust on the wound edges and from this period, the contraction of the lesions was more significant (Figure 1C).

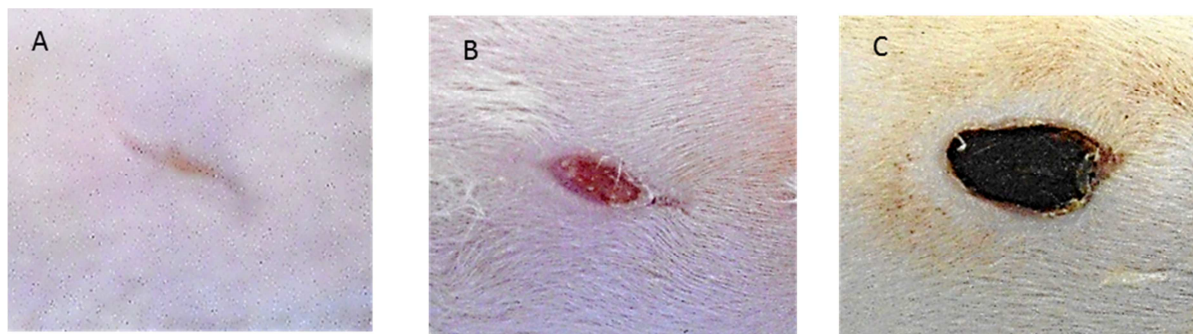


Figure 1 - Macroscopic aspects of wounds of rats after 14 days of treatment. A - Group 1; B - Group 2; C - Group 3

The crusts are commonly found in the proliferative phase [19]. It is a devitalized tissue caused by dryness and cellular dehydration. It can be serous, purulent or hematic, depending on the exudate presented on the surface of the lesion. Initially, this formation is important because it serves as a barrier against microorganisms.

Compared to experiments involving Bignoniaceae, the presence of crust was found in other studies. A study evaluated extract of leaves and roots of *Memora gnarled*, found crust in all groups from the 7th day, with a loss of crust on the 12th day [20]. A study using an ointment with 10% *T. Avellanae* extract, noted the presence of intense crust in 40% of animals on the 3rd day, 60% at 7 days, decreasing the intensity on the 10th day, being absent after 14 days of treatment. The re-epithelialization was complete at 14 days [21]. Another study evaluated the *T. avellanae* extract in the first three days of experimentation, found in the group treated with the plant, serous crust, soft and thin, with less swollen edges compared with the other groups [14].

The wounds contraction process also follows three stages, similar to wound healing [22] which are: 1) *Initial phase of centrifuge decrease*, with increase of the lesioned area due to loss of elastic tension of the adjacent skin, the formation of edema, the loss of adhesion to the deep fascia and a characteristic of the skin of the rat, that is its mobility [23-24]. 2) *Rapid contraction phase with centripetal force*, i.e. toward the center of the lesion [22]; and 3) *Slow contraction phase* when the wound is in the final stage of healing [22].

In table 2 and figure 2, this phases of contraction can be evidenced by starting with an increase in the 3rd day, rapid contraction phase on day 7 (mainly group 1) and then, slow contraction of the edge in the final stage.

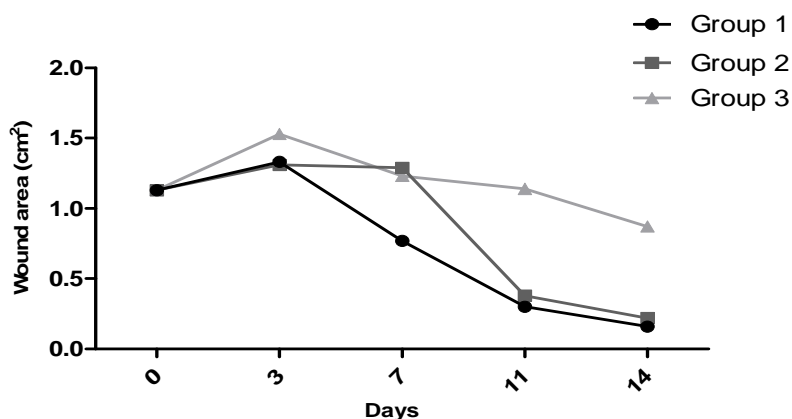


Figure 2–The average variation of the wounds area (cm<sup>2</sup>) according to the treatment group and evaluation day

Figure 2 shows a noticeable increase in the wound area in the period from D0 to D3 and the difference of the wound contraction evolution of the group 3 compared to other groups from D7, however, only in D11 and D14 is demonstrated statistical significance, with values of  $p = 0.0026$  and  $p = 0.0040$ , respectively.

**Table 2 -Wound contraction percentage (%) compared to the original size, shown by groups, and days of macroscopic evaluation**

Groups	Contractionpercentage (%)		
	D3	D7	D14
1	-18.19	31.86	85.54
2	-16.12	-14.16	80.23
3	-35.40	-8.85	22.71
p-value	p= 0.5174	p=0.8117	p= 0.0040*

ANOVA, Tukey test ( $p < 0.05$ ) Group 3 \*  $p < 0.001$ , compared to groups 1 and 2; group 1 = positive (PC) and negative control (NC), group 3 = EETA, control.

There was not, until the 14th day, the total detachment of the crust in any animal of EETA group and it was only possible to observe some positive percentage of wound contraction at the end of the experiment (Table 2) as a consequence of the crust part detachment from the edges of the lesion, which occurred on the 7th day of evaluation. The group treated with dexpanthenol obtained positive contraction percentage, from the 7th day and the group 2 from the 11th day.

### Histological analysis

The histopathological analysis confirms the macroscopic result, showing significance only in the remodeling phase (Table 3). The data shown in Table 3 suggest that the crust formed in the group using the extract, interfered in the total re-epithelialization of this group in the 14-day period, and the other groups achieved full re-epithelialization. However, when performing the overall average of healing by a group, there was no statistical significance between them.

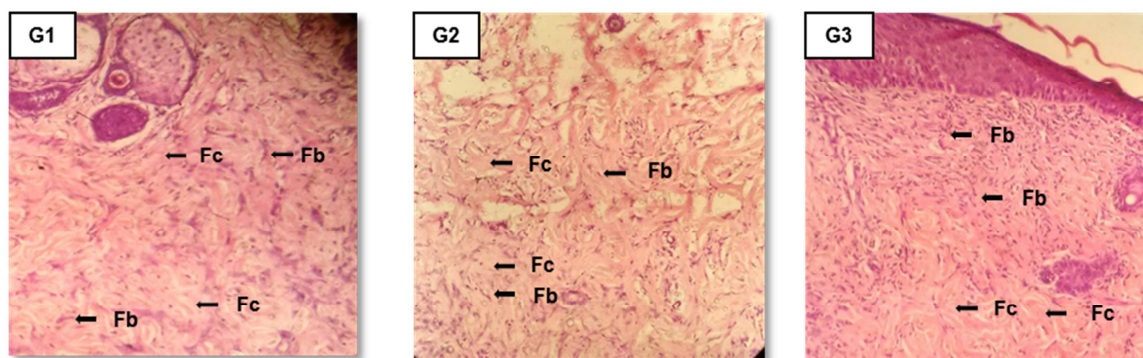
**Table 3 - Scores of histopathological evaluation of the wounds of groups treated with 5%*T. aurea* extract, positive control and negative control**

Euthanasia days	Scores			p values
	Group 1	Group 2	Group 3	
3°	41.00	44.67	46.00	0.809
7°	91.33	90.00	76.33	0.681
14°	115.7	119.0	106.3	0.039*
<b>Average</b>	82.68	84.56	76.21	0.955

ANOVA, Tukey test ( $p < 0.05$ ) Group 3 \*  $p < 0.05$ , compared to groups 1 and 2; group 1 = positive (PC) and negative control (NC), group 3 = EETA, control.

On the 3rd day, groups 1 and 2 had higher scores for fibroblast proliferation and the group 3 largest scores for granulation tissue. Group 2 was the only one with collagen fibers on the 3rd day of treatment. On the 7th day, the findings between groups were similar and in 14 days, the main difference was the total re-epithelialization of the groups 1 and 2 and partial reepithelialization of group 3.

The crust was found in all groups up to the 7th day of treatment, remaining in group 3 until the 14th day and absence in the control groups in this period.



**Figure 3 – Histopathological appearance of wounds at 14 days of treatment showing the presence of fibroblasts (Fb) and collagen fibers (Fc). G1 - Group 1; G2 - group 2; G3 - Group 3. HE with an increase of 10X**

The research showed in its histopathological results, in healing study in skin wounds in rats with 10% *T. avellanedae* extract, less inflammatory infiltration and presence of moderate crust when compared to control at the 3rd day of evaluation; on the 7th day showed collagen fibers more abundant than control and the 14th day total epithelialization of wounds. [21]

In another study that evaluated the ointment derived from the aqueous extract of *T. avellana* stem bark at 10%, the result of the histological investigation showed a greater presence of fibroblasts and newly formed vessels on the 7th day when compared to the control and on the 14th day complete epithelialization [10]. Literature data corroborate those of this research as regards the presence of crust in the groups treated with *T. avellana*. Histopathological evolution of skin wounds treated with EETA 5% possessed similar improvement compared to the controls until the 7th day, and the formation of the crust and partial epithelialization of this group were the differences compared to controls.

### Biochemical evaluation

The serum analysis of AST and ALT aminotransferase allows inferred hepatocellular acute lesion if there is an increase of these liver transaminases. To analyze urea and creatinine allows inferred renal lesion since they are metabolites usually eliminated in the urine after filtration by the kidneys and increased values of these metabolites suggests some renal damage. Biochemical values of animals used in this experiment are shown in Table 4 and were not statistically significant when compared with the untreated group (group 2).

**Table 4 - Results of biochemical values of treated groups with *T. aurea* extract 5% and controls expressed as mean (M) and mean standard error (SE)**

Biochemicals results				
Biochemical	Group 1 M ± SE	Group 2 M ± SE	Group 3 M ± SE	p value
AST (U/L)	98.50 ± 8.56	102.0 ± 11.11	82.88 ± 6.76	p= 0.2981
ALT (U/L)	53.50 ± 5.39	49.00 ± 3.61	42.75 ± 4.53	p= 0.2696
Urea (mg/dL)	40.53 ± 3.28	43.10 ± 3.25	46.29 ± 3.12	p= 0.4604
Creatinine (mg/dL)	0.24 ± 0.01	0.25 ± 0.02	0.23 ± 0.03	p= 0.8264

U/L= unity per liter; mg/dL= milligrames per deciliter.

Thus, compared to the untreated group, the biochemical values were not statistically significant and could be suggested that there are the following probabilities according to the obtained results: or treating topically is not absorbed systemically, or if absorbed, is not capable of causing, during the metabolism and excretion process, a liver and renal damage.

The study sought to determine the range of reference values for the hematological and biochemical parameters of untreated animals from the Vivarium of the Tiradentes University, Sergipe, Brazil, found results that are similar to this research [25].

### CONCLUSION

The macroscopic analysis of the healing of the group treated with 5% EETA showed less inflammation process in the initial healing related to the control groups and showed total extension crust at the end of 14 days in all animals. The histological evaluation was similar between the groups up to the 7th day, however, on the 14th day, the group treated with the extract had no full re-epithelialization, differently the control group who were all re-epithelialization. The biochemical evaluation showed normal parameters, suggesting not to influence the topical treatment with EETA to 5% of liver and renal tissues. The result of the healing of wounds treated with the extract was not superior to the controls.

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