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Evaluation of cytotoxicity and wound healing activity of *Avicennia schaueriana* in cream

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Abstract: *Avicennia schaueriana* is found in Brazilian mangrove coast. The cytotoxicity in vitro of the Aqueous Extract of Leaves of *Avicennia schaueriana* (AELAs) and the healing activity of the plant in cream on mice skin wounds were evaluated in this study. The cytotoxic evaluation was performed on Vero cells. The healing activity was evaluated on mice treated during 5, 10 and 15 days with cream at 5%, solution of sodium chloride at 0.9% and dexpanthenol in cream at 5%. The extract did not show cytotoxicity, but showed mitogenic activity (100μg/ml). In morphometric analysis, the percentage of wound contraction after 10 days was higher in dexpanthenol group (93.41%). In 15 days, the lowest percentage of contraction was observed in the dexpanthenol group (94.41%) and the highest in the AELAs cream group (98.50%). In histomorphometry the dexpanthenol showed the lowest length of re-epithelialization in 10 days. In 15 days, the AELAs cream group showed 100% of re-epithelialization. The number of fibroblasts found in AELAs cream group was higher than the saline solution in 10 days. In 15 days, AELAs cream group maintained a higher amount of fibroblasts when compared to the others. *A. schaueriana* did not show cytotoxicity. Furthermore, topical application of AELAs cream decreased the wound area, stimulated the re-epithelialization and increased the number of fibroblasts. The species *A. schaueriana* could become a topical treatment in tissue repair process.

Key words: *Avicennia schaueriana*, *in vitro* techniques, re-epithelialization, Vero cells, wound healing.

INTRODUCTION

Recently, the study of wound healing has become an important topic, as many people suffer from surgical or traumatic wounds every year, with the imminent need of treatment (Li et al. 2015). The loss of epithelium and the exposure of the connective tissue that characterize the ulcers cause pain and discomfort, affecting the quality of life of the patients (Duarte et al. 2011).

The healing of skin wounds is an intrinsic process of cellular structure reconstructions and layers of lost tissues (Li et al. 2015) providing

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a perfectly coordinated cascade of cellular and molecular events that interact to promote tissue repair (Duarte et al. 2011). This process is systemic and dynamic and it is directly related to the general conditions of the organism (Broughton et al. 2006).

The predominant cell population for proper wound healing are keratinocytes, fibroblasts and endothelial cells (Barrientos et al. 2008). Fibroblasts are connective tissue cells responsible for collagen deposition that is required to repair tissue damage (Ross 1969). If there is no repair modifying factor in the wounds, the healing process takes place in an orderly and efficient sequence of events (Campos et al. 2007, Diegelmann and Evans 2004). The wound healing is usually divided into three main phases: inflammatory phase, proliferation or granulation phase and remodeling or maturation phase (Clark 2005, Gurtner et al. 2008). However, some authors, such as Diegelmann and Evans (2004) claim that the healing process is characterized by four distinct and overlapping phases: hemostasis, inflammation, proliferation, and remodeling.

Researches in new medicines that can accelerate the healing process of wounds reduce the painful symptoms and show a great cost-benefit relation have been investigated (Duarte et al. 2011). Studies have suggested that antioxidants may play an important role in the healing process of injuries (Kim et al. 2008).

It is important to highlight that, from the clinical point of view the topical application of medication throughout the entire thickness of the injury is interesting due to the reduction of adverse effects on other organs (Li et al. 2015). Topical treatment of ulcers consists of restoring the physiological environment of the wound, to maintain proper humidity, temperature, pH, control of bacterial load, non-viable tissue removal (debridement), odor control, and minimization of the pain and protection of the skin in the affected area. Those conditions, once adjusted, will contribute to the repair and restoration of tissue function (Rolstad et al. 2012).

Plants have been a source of inspiration for new pharmaceutical compounds, which have great contributions to human health because of their therapeutic values (Panda et al. 2009, Vadlapudi 2012). According to the World Health Organization, plants are a source of compounds, which have the ability to combat diseases, with antimicrobial, antiviral and antifungal activities (Gazim et al. 2008, Nascimento et al. 2000). However, herbal products can only be introduced in the society if the laboratory and specific clinical studies prove their efficacy and safety (Agra et al. 2007). Therefore, the proper use of medicinal plants represents an important step and a medication option to be dedicated to the people with the intent to improve their health and quality of life (Silva et al. 2006).

The extracts of different mangrove plants are reported to have several medicinal properties (Agoramoorthy et al. 2007, Bandaranayake 1998). In the pharmacological properties of the Verbenaceae family, according to Bandaranayake (1998), the mangrove plants *A. alba, A. African, A. germinans* and *A. marina* show therapeutic compounds that can be used for the treatment of various diseases, including ulcers. Sumithra et al. (2011) showed anti-inflammatory activity of methanolic extract from leaves of *A. officinalis* and it was primarily used for the treatment of rheumatism, paralysis, asthma, skin diseases and ulcers (Kathiresan and Ramanathan 1997, Ramanathan 2000).

The *Avicennia* gender has two species in Brazil, *Avicennia schaueriana* and *Avicennia germinans* (Profice et al. 2010). The *Avicennia schaueriana*, popularly known as black-mangrove or siriúba, is an endemic species from mangrove belonging to the family Verbenaceae (Schaeffer-Novelli 1995). The extract of those plant showed antibacterial activity against *Staphylococcus aureus* (ATTC 6835), *Micrococcus luteus* (ATCC 9341) and *Klebsiella pneumoniae* (ATCC 700603)

(Santos et al. 2010). Furthermore, *A. schaueriana* proved to be promising for isolation of substances with antifungal potential (Fardin and Young 2015).

The species of the *Avicennia* gender are widely used by traditional communities for several diseases (Santos et al. 2010); however, there are still no scientific reports on the wound healing potential of the plant. The aim of this study was to evaluate the cytotoxicity *in vitro* and the wound healing activity of Aqueous Extract of Leaves of *Avicennia schaueriana* (AELAs) in cream on skin lesions in mice.

MATERIALS AND METHODS

BIOLOGICAL MATERIAL

Plant

The leaves of *Avicennia schaueriana* species were collected in October 2013, in Itamaracá mangrove, located in Vila Velha, Northern coast of the State of Pernambuco, Brazil, LAT 07°48.716' S and LONG 34°51.347' W. A voucher specimen was deposited at Geraldo Mariz Herbarium in the Universidade Federal de Pernambuco (UFPE) under the registration number UFP 75.458.

Cell culture

Vero cells (fibroblasts) from kidney of African green monkey or old world monkey (*Cercopithecus aethiops*) were used. The cell line (CCL-81, Rio de Janeiro, Brazil) was obtained from the Department of Histology and Embryology (UFPE). Vero cells were grown in Eagle culture medium Modified by Dulbeco (DMEM - Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% of Fetal Bovine Serum and 1% of antibiotic-antimycotic solution (10,000 units of penicillin, 10 mg of streptomycin in 0.9% of sodium chloride; Sigma). They were maintained at 37 °C in humidified atmosphere with 5% of CO_2.

Experimental animals

Forty five *Wistar* female mice aged 8 and 12 weeks, weighing 230 ± 20 g were used. They were obtained from the Department of Antibiotics of UFPE. Experiments with animals were performed with the approval of the Ethics Committee for Animal Experimentation of the Universidade Federal de Pernambuco (UFPE) under number 23076025194/2012-10.

AQUEOUS EXTRACT OF LEAVES OF *A. schaueriana* (AELAs)

The aqueous extract was prepared by infusion from 500g of fresh leaves of *Avicennia schaueriana*. The material was weighed, grounded and extracted with water at 40 °C for 20 minutes. The solid residue was removed by filtration and the water by lyophilization. The dried material was stored at -20 °C. The yield of the aqueous extract was 4% and it was used for healing and cytotoxic activity (Nascimento et al. 2016, adapted).

CREAM OF AQUEOUS EXTRACT OF LEAVES OF *A. schaueriana* (AELAs CREAM)

The AELAs was weighted on digital analytical scale (Shimadzu ATY 224) with the use of waxed paper until achieving 3g and poured into porcelain mortar. It was solubilized with distilled water and homogenized. In a watch glass, the anionic emulsion was weighed until achieving 60g and it was poured into the mortar containing the extract of *A. schaueriana* until solubilization. The pH was measured and maintained between 5.5 and 6.5. It was packed in a plastic jar containing the extract in cream at 5%.

CYTOTOXICITY TEST

The evaluation of the cytotoxic activity was performed using the bromide colorimetric method (3-[4.5-dimethylthiazol-2-il]-2.5-tetrazolium diphenyl) (MTT) (Geran et al. 1972, Mosmann

1983). The methodology used to conduct this test followed the rules of the International Standard Organization (ISO 10993-5 2009).

The cells at the concentration of $2x10^5$/mL of DMEM per well were distributed into 96-well plates (TPP, Darmstadt, Germany) and incubated for 24 hours at 37 °C with atmosphere enriched with 5% of CO_2 and 95% of air for stabilization. After this period, the AELAs, previously dissolved in phosphate-buffered saline (PBS) and filtered (0.22µm syringe filter - TPP, Darmstadt, Germany) in different concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL, was added to the wells with Vero cells. PBS and DMEM culture medium were used as control. After incubation for 24 hours of contact of the cells with the extract, 25µl (5mg/mL) of MTT solution was added to each well and the plate was incubated for 3 hours. The MTT and culture medium were removed and 25µl of dimethylsulfoxide (DMSO) was added to each well to dissolve the formazan crystals. Subsequently, the spectrophotometer reading was performed (Bio-Rad, São Paulo, Brazil) with a wavelength of 570nm. The test was performed in duplicate.

WOUND HEALING ACTIVITY

Division of groups

To evaluate the wound healing activity, the mice were randomly divided into 3 groups according to the treatment proposed for the ulcers induced in each animal. The control group (15 animals) received saline solution at 0.9%. The standard group (15 animals) was treated with 5% dexpanthenol in cream and the AELAs cream group (15 animals) was treated with AELAs cream at 5%. Each group was divided into 3 subgroups of 5 animals supervised during 5, 10 and 15 days after the induction of dorsal ulcer.

Surgical procedures in vivo

The animals were previously weighed and anesthetized with ketamine hydrochloride (10 mg/kg Ketamin®), xylazine hydrochloride (0.5 mg/kg, Anasedan®) and 0.9% saline solution, associated in the same syringe and administered intramuscularly. The animals were subjected to the demarcation of the area to wound induction with subsequent trichotomy in the dorsal region and positioned on the operating table in prone position. After asepsis of the dorsal region with alcohol 70%, the induction of a rectangular wound with 2.3/2.0 cm was conducted on dorsal region. A skin fragment was removed from the center of the shaved area to display the dorsal muscular fascia using a #15 scalpel (Silva et. al 2016).

Post-operative

After surgery, the mice were subjected to the corresponding treatment and they were kept in cages. The ulcers did not receive occlusive dressings. The application of the medication relating to each group was performed daily, once a day, around 11:00 a.m. until the end of the experiment. The aspect of the wound was described during the research in different groups.

On the 5th day, the measurement was performed on the wound area (length and width) using a caliper, but without removing the skin fragment. After 10 and 15 days, measurement of the lesion area and withdrawing of the skin fragment containing the wound were conducted. The animals were anesthetized and a scalpel #15 was used to remove surgical pieces formed by scar or skin lesion with a margin of 1 cm of skin around the lesion and to the dorsal musculature of the animal. The removed tissue was placed in formalin at 10% during 24 hours at room temperature and processed for light microscopy. After the fragment collection procedure, the animals were euthanized by cervical dislocation (Silva et al. 2016).

Morphometric analysis of the wound

For the morphometric analysis, initial and final measurements for each wound in different groups and periods were performed using a caliper to calculate the rate of healing of ulcers. It was used a degree of contraction expressed in percentage by the equation proposed by Ramsey et al. (1995), where Wo is the initial area and Wi is the final area:

$$\% \text{ of contraction} = (Wo - Wi)/Wo \times 100$$

Histomorphometric study

After fixing the removed specimens, samples were sent for processing in conventional standard of histological technique for light microscopy and embedded in paraffin. Serial sections of 5 μm were stained with Hematoxylin and Eosin (HE), fitted with "entellan" and observed under an optical microscope.

Selected histological sections were viewed in a slides scanner (3DHISTECH) for capturing images. For the evaluation of re-epithelialization, the distances non epithelialized of the wounds were measured with the Pannoramic Viewer program, with an increase of 2x, in the samples collected from each animal with 10 days and 15 days of treatment. Moreover, fibroblast count was performed. Five fields were acquired by preparation under 40 times magnification and quantified using ImageJ 1.48 software (Zur and Klement 2015).

STATISTICAL ANALYSIS

The *in vitro* study data were expressed through statistical measurements: average, standard deviation, coefficient of variation, median, minimum and maximum value. The data were evaluated according to F (ANOVA) test with multiple comparisons of Tukey. To verify the hypothesis of equality of variances, the F Levene test was performed.

The data from the morphometric and histomorphometric analysis and from the counting of fibroblasts were expressed as average ± SE (standard error of the average) and median. In the morphometric study, we used the F (ANOVA) test on the contrast between the groups (saline solution, dexpanthenol and AELAs cream), with multiple Tukey comparisons to evaluate the contraction percentage with 5, 10 and 15 days and the t-Student test paired in the comparison between the initial and final measurements. In the histomorphometric study and in the fibroblasts counting, the data was evaluated by the Kruskal-Wallis statistical tests in the comparison between groups and Mann-Whitney for the comparison between the evaluation times (10 and 15 days). Statistical calculations were performed using the program SPSS, version 21.0, and the margin of error used in the decisions was 5.0%.

RESULTS

CYTOTOXICITY

The AELAs (25μg/mL) and the PBS control showed an average number of Vero cells of 0.437 and 0.438 respectively with statistical difference when compared to the AELAs concentrations of 100 μg/mL (0.686) and 6.25 μg/mL (0.710) ($p < 0.001$). The AELAs (50 μg/mL and 12.5 μg/mL) and DMEM control did not show statistical difference when compared to the other groups with average number of Vero cells of 0.567, 0.626 and 0.533 respectively.

MACROSCOPIC DESCRIPTION OF WOUNDS

During treatments in 5, 10 and 15 days it was evaluated the macroscopic aspect of the wounds (general aspect of wounds, granulation tissue and scab and scar formation). Figure 1 shows the healing process of wounds induced in mice. Digital images showed the evolution of the wound areas in different experimental times, according to the treatment.

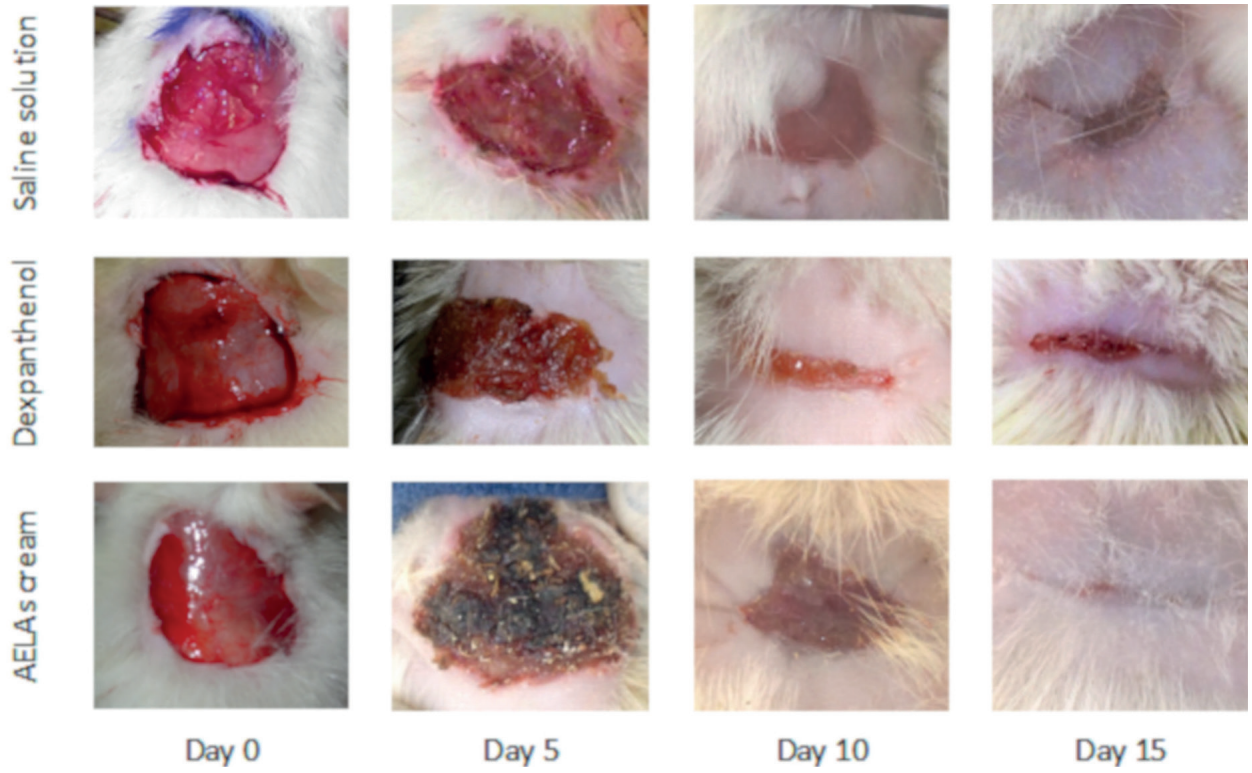

Figure 1 - Wound healing process in different days of treatments.

The treatment with saline solution showed mice with moist infected bleeding wounds, granulation tissue from the 10th day and scab formation in 3 mice in 15 days. The dexpanthenol group showed two mice with infected wounds in 5 days of treatment, granulation tissue from the 5th day and scab formation in 10 days with presence of tissue granulation. The AELAs cream group in 5 days of treatment showed the presence of tissue granulation and it did not show infected wounds. In 10 and 15 days, there was an evolution to scab and scar formation in all mice.

MORPHOMETRIC ANALYSIS OF THE WOUNDS

Table I shows the average of the decreasing wound areas and an increase of wound contraction in each group. In the evaluation after 10 days, the group treated with dexpanthenol showed the lowest area (30.30 mm^2) with the average percentage of contraction of 93.41% ($p \leq 0.05$). However, in the analysis after 15 days, the average of the area was 25.70 mm^2 with the lowest average percentage of contraction (94.41%). The treatment with AELAs cream in 15 days showed the lowest area (6.90 mm^2) with the highest average percentage of contraction (98.50%) with statistical significant difference between those two groups ($p \leq 0.05$).

HISTOMORPHOMETRIC ANALYSIS AND FIBROBLAST COUNTING

Table II highlights that in 10 days, the average distance between the epithelia of the surgical wound was higher in the saline solution group (3498.66 µm) with $p \leq 0.05$. The lowest average was observed in the dexpanthenol group (706.28 µm) with no statistical difference when compared with AELAs cream group (833.04 µm). After 15 days, in all studied groups, it was verified that the average of distances between the epithelia

TABLE I
Measurements of wound areas (mm^2) and percentage of wound contraction according to the groups and time of evaluation after *in vivo* surgical procedure.

Evaluation time after wound induction	AELAs cream (n=15) Average + EPM (Median)	Saline Solution (n=15) Average + EPM (Median)	Dexpanthenol (n=15) Average + EPM (Median)	p value
Initial	460.00 ± 0.00 (460.00)	460.00 ± 0.00 (460.00)	460.00 ± 0.00 (460.00)	
5 days	262.30 ± 22.98 (256.50)	227.60 ± 22.55 (230.00)	238.04 ± 39.05 (260.00)	$p^{(1)} = 0.698$
10 days	93.31 ± 10.41 $^{(A)}$ (97.50)	93.60 ± 17.22 $^{(A)}$ (88.00)	30.30 ± 4.29 $^{(B)}$ (34.00)	$p^{(1)} = 0.003$*
15 days	6.90 ± 2.98 $^{(A)}$ (8.00)	17.71 ± 3.81 $^{(AB)}$ (16.00)	25.70 ± 3.47 $^{(B)}$ (24.00)	$p^{(1)} = 0.008$*
p value	$p^{(2)} = 0.001$* $p^{(3)} < 0.001$* $p^{(4)} < 0.001$*	$p^{(2)} = 0.001$* $p^{(3)} < 0.001$* $p^{(4)} < 0.001$*	$p^{(2)} = 0.005$* $p^{(3)} < 0.001$* $p^{(4)} < 0.001$*	
Contraction % with 5 days	42.98 ± 4.99 (44.24)	50.52 ± 4.90 (50.00)	48.25 ± 8.49 (43.48)	$p^{(1)} = 0.698$
Contraction % with 10 days	79.72 ± 2.26 $^{(A)}$ (78.80)	79.65 ± 4.74 $^{(A)}$ (80.87)	93.41 ± 0.93 $^{(B)}$ (92.60)	$p^{(1)} = 0.003$*
Contraction % with 15 days	98.50 ± 0.65 $^{(A)}$ (98.26)	96.15 ± 0.83 $^{(AB)}$ (96.52)	94.41 ± 0.75 $^{(B)}$ (94.78)	$p^{(1)} = 0.008$*

(*): Significant difference ≤ 5%. (1): Through the F (ANOVA) test for comparison between the groups in each evaluation and for the concentration percentage with 5 days, 10 days and 15 days with Tukey comparisons. (2): Through t-Student test paired for comparisons between the evaluation of initial time and 5 days in each group. (3): Through t-Student test paired for comparisons between the evaluation of initial time and 10 days in each group. (4): Through t-Student test paired for comparisons between the evaluation of initial time and 15 days in each group. Note: The different letters in brackets indicate significant differences between the corresponding groups.

TABLE II
Distance between the epithelia of the surgical wound of the groups in relation to the evaluation time after *in vivo* surgical procedure.

Evaluation time after wound induction	AELAs cream (n=10) Average + EPM (Median)	Saline solution (n=10) Average + EPM (Median)	Dexpanthenol (n=10) Average + EPM (Median)	p value
10 days	833.04 ± 515.65 [(A)] (0.00)	3498.66 ± 232.37 [(B)] (3572.98)	706.28 ± 233.73 [(A)] (704.84)	p [(1)] = 0.002*
15 days	0.00 ± 0.00 (0.00)	968.89 ± 303.49 (905.04)	582.21 ± 370.34 (0.00)	p [(1)] = 0.078
p value	p [(2)] = 0.444	p [(2)] = 0.008*	p [(2)] = 0.683	

(*): Significant difference ≤ 5%. (1): Through the Kruskal-Wallis test for comparisons between the groups in each evaluation time with comparisons of the mentioned test. (2): Through Mann-Whitney test for comparisons between evaluation time in each group. Note: The different letters in brackets indicate significant differences between the corresponding groups.

were correspondingly smaller than after 10 days, indicating an increase in re-epithelialization. The saline solution group showed the highest distance. In 15 days, the average of distance between the epithelia of the surgical wound in AELAs cream group was null, indicating complete re-epithelialization of the wounds in all mice (Figure 2).

In Table III it is verified that within 10 days, the average of fibroblasts were higher in AELAs group (579.20) than the saline solution group (295.20) ($p \leq 0.05$). In the evaluation performed after 15 days of the wound induction, the average number of fibroblasts in AELAs cream group remained higher (568.40) compared to the others ($p \leq 0.05$).

DISCUSSION

Wound healing is a dynamic and interactive process initiated in response to an injury (Guo and Dipietro 2010), whose purpose is to restore the anatomical and functional continuity of the tissue. The process is essential for maintaining the body integrity (Barbul 1990, Broughton et al. 2006, Thornton et al. 1997). A large number of investigations and clinical trials have been conducted with the aim of improving the healing process of wounds (Das 2013, Duarte et al. 2011, Gál et al. 2009, Li et al. 2015, Ulger et al. 2016) and consequently quality of life.

This is the first study that addresses the therapeutic potential in relation to the healing of the *Avicennia schaueriana* species. It is important to note that the *Avicennia* gender has chemical constituents that may have different pharmacological properties, such as alkaloids, tannins, flavonoids, saponins and triterpenes (Abeysinghe 2010, Ghani 1998, Vadlapudi 2012), which contribute to the medicinal activity of the plant.

According to ISO 10993-1 (2018), the *in vitro* cytotoxicity test is one of the tests that serves to evaluate the biocompatibility of a particular material or extract, showing an important step for animal testing and clinical trials. The results of this research show that the Aqueous extract of leaves of *Avicennia schaueriana* (AELAs) did not show cytotoxic activity, since in all tested concentrations there was Vero cells proliferation. Studies with

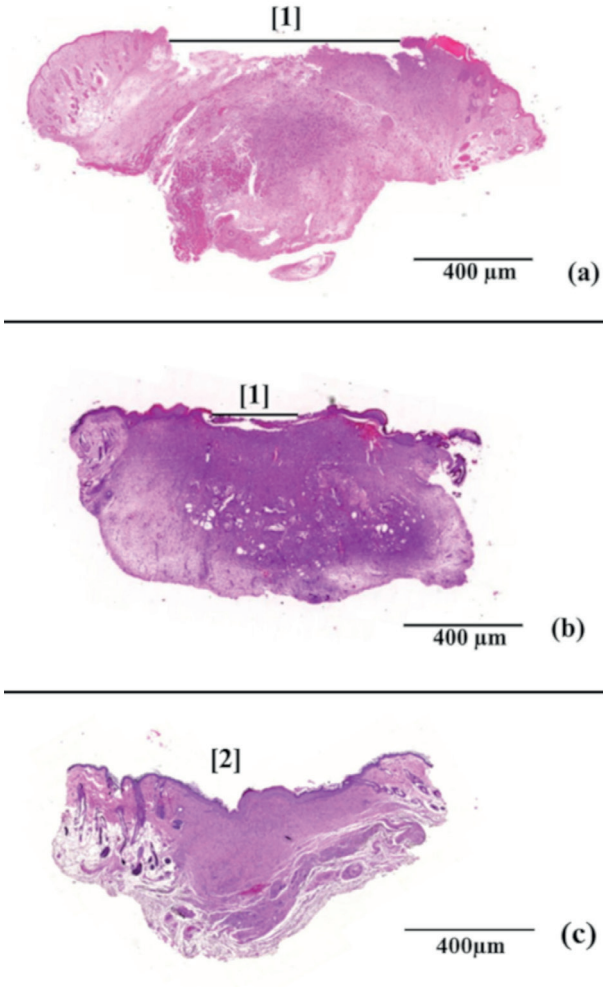

Figure 2 - Histological Sections of dorsal skin of mice showing the saline solution group (**a**), dexpanthenol group (**b**) and AELAs cream group (**c**). All images show the extent of re-epithelialization after 15 days of treatment: [1] distance between the epithelium and [2] total re-epithelialization. HE staining. Magnification 20x.

some species of the Verbenaceae family showed similar results. Akter et al. (2014), using a methanol extract of the leaves of *A. alba*, showed that this plant has an antitumor potential by cytotoxicity against cancer cells, however, it did not show cytotoxic activity on Vero cells. Behbahani et al. (2013) concluded that the methanolic extract of *A. marina* did not affect the viability of Vero cells, demonstrating no cytotoxic effect at concentrations equal to or less than 32 µg/mL. According to Bueno et al. (2014), tannins influence on the physiology of skin cells through their pharmacological properties, increasing cell proliferation. The result of this study suggests a mitogenic activity of AELAs possibly because of tannins presence.

In inspection of the surgical wounds, it was observed that the groups of saline solution and dexpanthenol showed scabs and granulation tissue with infectious processes of the wounds during the study period. However, the group of AELAs cream showed scabs and scars without infection in surgical wound. The results can be explained by different mechanisms of action of secondary compounds present in the plant, such as tannins and flavonoids, which are known to have antimicrobial and antioxidant properties (Ofori-Kwakye et al. 2011). Therefore, Santos et al. (2010) showed that the hydro-alcoholic extracts of the bark, leaves and roots of *A. schaueriana* showed antibacterial activity *in vitro*, which can contribute to the healing activity of this plant, reducing the risk of infection of the injury, which is the most likely cause of the delay in wound healing (Leaper et al. 2015).

The morphometric analysis revealed that the average of the wound areas decreased with the progression of evaluation time in all groups. It was observed that the dexpanthenol cream had an excellent performance in the healing of skin injuries of mice in the first 10 days compared to the other groups. Dexpanthenol cream is widely used because it indicates improvement in wound healing (Heise et al. 2012, Oguz et al. 2015, Ulger et al. 2016), since it easily penetrates the skin in high local concentrations. The most significant effects of formulations containing dexpanthenol include stimulation of epithelialization, granulation and itching relief (Ebner et al. 2002). However, after 15 days of treatment the AELAs cream group showed the highest percentage of wound healing. The studied plant significantly stimulated contraction of the wound, accelerating the healing process. This healing property is probably due to the high content

TABLE III
Fibroblast counting after *in vivo* surgical procedure in 10 and 15 days.

Evaluation time after wound induction	AELAs cream (n=10)	Saline solution (n=10)	Dexpanthenol (n=10)	p value
	Average + EPM (Median)	Average + EPM (Median)	Average + EPM (Median)	
10 days	579.20 ± 60.67 [(A)] (527.00)	295.20 ± 26.47 [(B)] (276.00)	508.60 ± 46.83 [(A)] (537.00)	p [(1)] = 0.004*
15 days	568.40 ± 53.60 [(A)] (543.00)	331.00 ± 35.57 [(B)] (338.00)	397.00 ± 45.40 [(B)] (421.00)	p [(1)] = 0.011*
p value	p [(2)] = 1.000	p [(2)] = 0.421	p [(2)] = 0.222	

(*): Significant difference ≤ 5%. (1): Through Kruskal-Wallis test for comparisons between the groups in each evaluation time with comparisons of the mentioned test. (2): Through Mann-Whitney test for comparisons between evaluation time in each group. Note: The different letters in brackets indicate significant differences between the corresponding groups.

of flavonoids (Vinothapooshan and Sundhar 2010), saponins (Jiang et al. 1991) and tannins present in the plant, because these secondary compounds have astringent and antimicrobial characteristics, which appear to be responsible for wound contraction and epithelialization rate increase (Deshmukh et al. 2009).

In the histomorphometric analysis, the average distance between the epithelia of the surgical wound was significantly higher in the group of saline solution after 10 days, and similar between the groups treated with dexpanthenol and AELAs cream. In the evaluation after 15 days of treatment, all animals treated with AELAs cream showed complete re-epithelialization of wounds. This can be attributed to the presence of tannins in this plant, because they contribute to the formation of a protective layer on the skin and mucous membranes, acting in inflammatory processes, causing the epithelium restructuring and neovascularization (Simões et al. 2010). This layer can exert a protective action isolating the wound from the environment, accelerating, significantly, the tissue repair in the group treated with the AELAs cream.

Additionally, in this study, there was a significant increase in the amount of fibroblasts in AELAs cream group compared to the other groups in 15 days. Ulger et al. (2016) believe that the improvement in wound healing rate is due to the increased proliferation of fibroblasts, as well as a rapid epithelization. In addition, other studies show the importance of fibroblasts in the healing process (Das 2013, Diegelmann and Evans 2004, Ebner et al. 2002, Khoshneviszadeh et al. 2014, Sonmez et al. 2015). It is suggested that this stimulatory activity of dermal fibroblasts in the group of the studied plant is due to the presence of hydrolyzable tannins (Bueno et al. 2014).

Therefore, we emphasize the importance of histomorphometry in the studies, since only from the microscopic analysis was possible to evaluate the degree of re-epithelialization of the wound, showing that the groups treated with dexpanthenol and AELAs cream were superior to the saline solution because they obtained a faster and more efficient healing process. In all analyzed groups, the

one that obtained the best results of healing was the AELAs cream group. However, several studies are still necessary to evaluate the medical potential of this plant.

CONCLUSIONS

This study showed that the *A. schaueriana* species did not show cytotoxic activity. In addition, topical application of the AELAs cream decreases the area of the wound, stimulates re-epithelialization and increases the number of fibroblasts, exhibiting a healing activity on skin injuries in mice more efficient than dexpanthenol cream. Therefore, new researches on this plant could contribute to a topical treatment in tissue repair process with benefits to the population.

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AUTHOR CONTRIBUTIONS

All authors made substantive intellectual contributions to the manuscript. Briefly, Caroline Maria Igrejas Lopes, Ivone Antônia de Souza, Erwelly Barros de Oliveira, Jéssica Guido de Araújo Sá, Marllon Alex Nascimento Santana and Pedro Paulo Marcelino Neto participated in the execution of the research and in the planning, analysis and preparation of the manuscript; Liriane Baratella-Evêncio supervised the project and participated in the planning, analysis and preparation of the manuscript; Eduarda Santos de Santana and Luzia Abílio da Silva contributed to the preparation of the manuscript; finally, Jeymesson Raphael Cardoso Vieira conceived the original idea, contributed to the methodological design and participated in the analysis of the results and critical review of the manuscript.

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