Full Length Research Paper

Topical wound healing activity of an ointment based hydroethanolic leaf extract of *Anogeissus leiocarpus*

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*Anogeissus leiocarpus* is a plant used in the local West African pharmacopeia. This study aimed to evaluate the healing properties of the hydro-ethanol extract of its leaves in Wistar rats. A 1.5 cm diameter excision wound model was used. Wistar rats were treated post-excision by the topical application of petroleum jelly alone, also known as ‘Vaseline’, petroleum jelly supplemented with 3% (ALHE-3%) and 10% (ALHE-10%) of *A. leiocarpus* leaves’ hydroethanolic extract, or sulfadiazine (reference drug), or no treatment (untreated or Control group). Photographs were taken after excision. Wound surfaces were assessed daily using ImageJ software. Biopsies were taken for histological examination of the excised skin and hydroxyproline determination. The results showed a significant reduction in wound surfaces of animals treated with ALHE-3% and ALHE-10% (33.10 ± 2.54 and 31.14 ± 1.71 mm², respectively) as early as the sixth day after excision. The dosage of hydroxyproline showed a significant difference in collagen production in animals treated with ALHE-3% and ALHE-10%, compared to the Control (P < 0.001) and Vaseline (P < 0.01) groups at day 14. Histological analysis showed rapid re-epithelialization in animals treated with the extracts. *A. leiocarpus* leaves’ hydroethanolic extract could be a potential natural remedy for treating wounds.

Key words: *Anogeissus leiocarpus*, excision wound, healing, Wistar rat, hydroxyproline.

INTRODUCTION

A wound is a disruption or break in the anatomical or cellular continuity of living tissue (Baidoo et al., 2021). The skin plays a protective role against external agents and ensures communication between the organism and its surrounding environment (Azame et al., 2020; Albahri et al., 2023). Altering its function may constitute a gateway for pathogens to enter the organism. To compensate for the loss of tissue, the body initiates a
healing process. This dynamic process unfolds through physiologically distinct stages of coagulation, inflammation, re-epithelialization, and tissue remodeling (Azame et al., 2020; El-Sherbeni and Negm, 2023).

Despite the existence of several wound-healing products, wounds often become chronic, which remains a public health problem to this day (Falanga et al., 2022). Scientists and clinicians are therefore actively researching alternative and more effective products to treat and manage chronic wounds. In developing countries, wounds have a considerable impact on the economy, physical and social health (Nussbaum et al., 2018) and constitute a global public health issue (Li et al., 2005).

The treatment of chronic wounds imposes an immense financial burden on the patient (Kayır et al., 2018) and may sometimes reduce the patient's quality of life (Järbrink et al., 2017).

A. leiocarpus is a medicinal plant that has been subjected to several scientific studies. Its stem barks have been used in the treatment of microbial diseases (Sanogo et al., 2016), diarrhea (Tagne et al., 2019), helminthiasis, trypanosomiasis and malaria (Okpekon et al., 2004) (Mukhtar et al., 2017), skin infections (Ganfon et al., 2019), hepatoprotective, antidiabetic and antioxidant activities (Motto et al., 2022). In the Togolese traditional medicine, it is used against fungal infections such as dermatitis and mycosis, and leaf decoction is also used against stomach infections (Batawila et al., 2005). However, to date, no pharmacological studies have been conducted to verify the wound healing properties of A. leiocarpus leaf extract. The present study aimed to evaluate the wound-healing properties of A. leiocarpus leaves hydroethanolic extract in Wistar rats.

**MATERIALS AND METHODS**

**Plant material**

The plant material used is A. leiocarpus leaves. The leaves were collected in January 2022 from the village of Kolo-kopé, district of Anié in the Plateau Region (Togo). The plant was identified with the help of botanists at the Laboratory of Botany and Plants Ecology (LBEV), Faculty of Science – the University of Lomé in Togo. A voucher specimen was deposited under the number TOGO 15483. The samples were dried in the dark for two weeks before being used for the extraction procedure.

**Extraction procedure**

One hundred grams of A. leiocarpus leaves powder were macerated in 1000 ml of an ethanol/water mixture (70/30) under continuous stirring for 48 h. The whole mixture was dried on Whatman No. 1 paper. The filtrate obtained was evaporated in a Heidolph Rotavapor type 94200 and freeze-dried. The dry extract was stored at 4°C until used to prepare ointments for pharmacological tests (Hoekou et al., 2017; Ganfon et al., 2019).

**Preparation of A. leiocarpus hydroethanolic leaf extracts (ALHE)**

From the crude extract of A. leiocarpus leaves, two ointments at 3% and 10% in pure petroleum jelly Vaseline were prepared (Qsp 50 g). Sodium benzoate was used as a reference preservative at a rate of 0.075 g per 50 g of ointment. Sodium benzoate and crude extract powder were triturated in a mortar using a pestle. Vaseline was added gradually, with gentle stirring, until the mixture was homogenized. A 3 or 10% ointment was obtained, depending on the proportions (Table 1). Ointments were packaged in hermetically sealed jars and stored at room temperature out of direct sunlight (Sene et al., 2020).

**In vivo protocols**

**Animals**

Wistar rats aged 8-10 weeks with mean weights ranging from 155 to 181 g were used for this study. The animals were bred in the animal house of the Faculty of Sciences, University of Lomé (Togo). The rats were acclimatized to laboratory conditions for two weeks before starting the test. They were housed in polypropylene cages and maintained under standard laboratory conditions (temperature 25-30°C, relative humidity and a 12h/12h light-dark cycle). The animals had free access to food and water. The principles of laboratory’s animals care as described in the institutional guidelines and ethics of the Physiology and Pharmacology laboratory of the University of Lomé-Togo (ref: 001/2012/CA-FDS-UL) were followed.

**Chemicals and reagents**

Pure petroleum jelly (Vaseline) and Sodium benzoate came from Sigma (Merck KGaA, Darmstadt, Germany) when Sulfadiazine was provided by Thermo Fisher Scientific (Waltham, USA).

**Excision wound induction**

Healing activity was assessed in a slightly modified model of
experimental excisional wounding in Wistar rats (Shafei et al., 2020). Twenty-five rats were divided into 5 groups of 5 rats each as follows: Untreated rat group; the control group (pure Vaseline, used as excipient); 3% extract ointment (ALHE-3%) group; 10% extract ointment (ALHE-10%) group; and Reference drug sulfadiazine group. The rats were then anesthetized by exposure to diethyl ether, and their dorsal skin was shaved before surfaces of 1.5 cm diameter were excised using scissors.

Wound treatment

Treatments were administrated daily for 15 days by topical application. Wound photographs were taken on the first day (D0) immediately after induction, and then every 48 h for 15 days, using a high-resolution Android phone (Google Pixel 4XL; 12.2 Megapixels) at the same fixed distance from the wound camera. Wound diameters were taken with a graduated ruler every day before treatment application. Wound areas were calculated using Image J 1.48v freeware (National Institutes of Health, USA). The following formula was used to determine the contraction rate of each wound (Azame et al., 2020).

\[
\text{Percentage of the contraction} = \left( \frac{\text{Surface J}_0 - \text{Surface J}_n}{\text{Surface J}_0} \right) \times 100
\]

where J0 is the original wound area on the first day, and Jn is the wound surface area on days x after wound induction.

Determination of hydroxyproline

Hydroxyproline is an amino acid index of collagen synthesis, was measured on day 15 using methods previously described by Darré et al. (2014) and repeated by Metowogo et al. (2020). Briefly, fragments were dried in an incubator at 60°C for 12 h. Samples were then digested in 6 N HCl for 4 h at 130°C in hermetically sealed glass tubes. The hydrolysates were topped up with 10 ml distilled water, and to this volume was successively added 0.5 mL CuSO\(_4\) (0.01 M), 0.5 ml NaOH (2.5 N) and 0.5 ml H\(_2\)O\(_2\) (6%). The resulting solutions were vortexed and incubated in an oven at 80°C for sixteen min. After cooling in the temperature room, 2 ml H\(_2\)SO\(_4\) (3 N) was added, and the solutions were vortexed. Following this, 1 ml of a 5% P-dimethyl-amino-benzaldehyde solution was added, and absorbance was read at 540 nm using a spectrophotometer. Standard solutions of 1.0, 2.0, 4.0, 8.0 and 16.0 mg/ml hydroxyproline were also prepared in triplicate to make a calibration curve.

Histological studies

On day 6, two rats from each group were anesthetized with diethyl ether and sacrificed. Wounds were collected for histological examination and hydroxyproline analysis. On day 15, the remaining rats in all groups were sacrificed by dislocation for histological examination and hydroxyproline assay. Biopsies intended for histological testing were stored in 10% formalin. Skin wound samples were fixed in 10% neutral buffered formalin, processed and embedded in kerosene. Five-micrometer skin sections were cut and stained with hematoxylin-eosin (H&E). Tissues were qualitatively assessed under a light microscope (Olympus BX 51) at 200x magnification. Parameters such as granulation, epithelialization, vascularization and inflammatory cells were highlighted.

Statistical analysis

Data were entered and processed using Image J 1.48v freeware (National Institutes of Health, USA). Graph Pad Prism 6 software (Boston, USA) was used for one-way analysis of variance (ANOVA) followed by Bonferoni’s test at the significant threshold of P < 0.05. Results were presented as percentages and means with standard error on the mean (M ± SEM).

RESULTS

Assessment of wound surface

Macroscopic observations (Figure 1), followed by wound surface measures were performed. On the first day, Figure 2 show that all the different groups share similar wound surfaces (104.01 ± 0.15 mm\(^2\)) after wound induction. A reduction of wound surfaces was observed in all five groups after treatments, with high rates for groups treated with sulfadiazine, ALHE-10% and ALHE-3%. In fact, from days 2 to day 6 the wound surfaces were significantly reduced, with a maximum significant reduction (P < 0.05) observed on the sixth day in the groups treated with ALHE-3% (33.10 ± 2.54 mm\(^2\)), ALHE-10% (31.14 ± 1.71 mm\(^2\)), and Sulfadiazine (28.02 ± 2.81 mm\(^2\)).

The calculation of contraction rates shown in Figure 3 corroborates these observations and reveals after day 8 that there was no significant difference in the wound contraction process between groups. At this date, the best contraction rates were observed in ALHE-3% (81.04 ± 4.35%), ALHE-10% (81.32 ± 6.78%), and Sulfadiazine (82.62 ± 4.66%) groups (P < 0.05, P < 0.01 respectively when compared to Control and Vaseline groups). Differences were also found to be significant at day 8 in contraction rates of ALHE-3%, ALHE-10% and Sulfadiazine groups when compared to the vehicle Vaseline group.

Hydroxyproline content

The measurement of hydroxyproline is generally used as an indicator to determine collagen levels. According to Figure 4, A. leiocarpus leaves hydroethanolic extract in petroleum jelly (Vaseline) promoted collagen production, as shown by the hydroxyproline content. A significant difference was observed at day 14 between the hydroxyproline content of the control group and the groups treated with ALHE-3%, ALHE-10% and Sulfadiazine (P<0.001). Significant differences were also observed in collagen production of groups treated with ALHE-3%, ALHE-10% and Sulfadiazine when compared with the vehicle Vaseline group (P<0.01).

Histological studies

Histological sections taken on day 6 show the presence of hair follicles and squamous epithelium in rats treated...
Figure 1. Macroscopic observation of wound healing progress in the different groups. ALHE-3% = Vaseline supplemented with 3% A. leiocarpus leaves hydroethanolic extract; ALHE-10% = Vaseline supplemented with 10% A. leiocarpus leaves hydroethanolic extract.

Figure 2. Evolution of wounds area. Values are expressed as Means ± SEM, n = 5 Units: mm². D= Day; ALHE-3% = Vaseline supplemented with 3% A. leiocarpus leaves hydroethanolic extract; ALHE-10% = Vaseline supplemented with 10% A. leiocarpus leaves hydroethanolic extract. *P<0.05, ** P<0.01, *** P<0.001: Compared to the Control group on days 2, 4 and 6.
with ALHE-3%, ALHE-10% and reference cream Sulfadiazine, while an important influx of inflammatory cells is still observed in control rats at this date (Figure 5 and Table 2). On day 14, neovascularization and fibroblastic cells were observed in sections from rats treated with ALHE-3%, ALHE-10% and Sulfadiazine. In rats treated with Vaseline and control, there was only minor neovascularization (Figure 6 and Table 3). In addition, re-epithelialization was realized as early as day 6 in skin samples of ALHE-3%, ALHE-10% and Sulfadiazine treated rats. Conversely, in Control and vehicle Vaseline groups, the re-epithelialization was not markedly observable before the 14th day.

**DISCUSSION**

The healing activity of *A. leiocarpus* leaves...
hydroethanolic extract was evaluated using a model of excision wounds in Wistar rats. Treatment of excision wounds with the different formulations gave indications of the healing speed of the plant compared with the reference drug and the untreated batch. The increase in contraction rates after fourteen days of treatment in *A. leiocarpus* treated rats indicates that this plant accelerates skin restoration. Topical application of these ointments at 3 and 10% inhibited erythema, exudate and unpleasant odors fairly rapidly. Taken together, these phenomena explain the increase in healing speed it provokes. The reduction in erythema and exudate confirms that this plant could act on the inflammatory phase, as demonstrated by Dimo et al. (2006).

There was a significant difference in wound closure time between the group treated with 10% ointment (ALHE-10%) and the group treated with 3% ointment (ALHE-3%) at the same time. This shows that the extract induces dose-dependent wound healing in an excision wound model. The results of the present study concur with those of studies by Metowogo et al. (2020) who showed that another Togolese medicinal plant *Cochlospermum planchonii* could accelerate the burn wound healing process. In their study 5% Carbopol gel supplemented with *Cochlospermum planchonii* extract induced early burn wound closure in mice compared with
**Figure 6.** Histological observation of wounds on day 14. Light microscope structure of wounds. Hematoxylin-eosin (H&E) staining. x 200 growth, the scale bar is 50 μm. Abbreviations: A= control; B= Vaseline; C= ALHE-3%; D = ALHE-10%; E = Sulfadiazine; Fp = Hair follicles; Fb = Fibrosis; Ep = Re-epithelialization.

**Table 3.** Histological sections analysis at day 14.

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<th>Treatment</th>
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<th>NV</th>
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<td>Control</td>
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(--) = Absence; (+) = minimal presence; (++) = Normal; (+++) = high; (++++) = Very high; Fp= Hair follicles; Ed= Oedema; Inf= Inflammatory infiltrate; NV= Neovascularization; Fb= Fibrosis; U= Ulceration; Ep= Squamous epithelium

the control group after twelve days (P < 0.01). *Vitex pinnata* extract 50% (w/w), in another study, decreased wound surface with a topical ointment prepared in Vaseline by 98.7 ± 0.6% (Shafie et al., 2020). *Amaranthus spinosus* whole plant extract (65% in ethanol) was also shown to be active both *in vitro* on HaCaT and MEF cells and *in vivo* on a model of incision wound in Wistar rats (Paswan et al., 2023). Furthermore, from day 10 to 14, there was no significant difference between the different groups. This could be explained by the fact that wound healing is a natural phenomenon (Amegbor et al., 2012; Saleem et al., 2022), and any appropriate medication used to heal wounds should contribute to accelerating the healing speed to avoid possible infection. The observed improvement in the healing speed of *A. leiocarpus* leaves could be attributed to the presence of phytocomponents. These phytocomponents have antioxidant, antimicrobial and anti-inflammatory properties.

Phenolic compounds, alkaloids, and flavonoids (Kantati et al., 2016; Sanogo et al., 2016; Bamba et al., 2020), are known for their healing properties linked to their astringent effect. Tannins act as free radical scavengers (Barku et al., 2013). According to Azame et al. (2020), antibacterial properties are responsible for the rapid attenuation and
even disappearance of signs of inflammation, namely redness, heat, edema, exudation and pain. These observations were corroborated by hydroxyproline measurement and histopathological analysis. The measurement of hydroxyproline is frequently used as a reliable index to quantify collagen in tissues (Nagappan et al., 2012). Collagen is the main structural protein component of tissues and is well known to effectively enhance the healing process by promoting greater fibroblast proliferation (Süntar et al., 2011). In the present study, the hydroxyproline content of groups treated with ALHE-3%, ALHE-10% and sulfadiazine increased significantly compared to control batches, indicating that the extract may have provoked collagen synthesis by stimulating the proliferation of the fibroblasts responsible for collagen production. This observation was confirmed by wound histology, which revealed increased re-epithelialization, neovascularization and recurrence of hair follicles in the groups treated with ALHE-3%, ALHE-10% and sulfadiazine, while a delayed healing process was still observed in control animals after 14 days.

Conclusion

The present study aimed to investigate the healing effect of the hydroethanolic extract of *A. leiocarpus* leaves using a model of excision wounds in Wistar rats. The rate of contraction, the hydroxyproline levels and the nature of the histological tissue obtained after fourteen days of application of the hydroethanolic extracts of *A. leiocarpus*, allowed us to conclude that the hydroethanolic extract of *A. leiocarpus* leaves could be a potential natural remedy for treating wounds. Further studies will be carried out to elucidate factors such as toxicity, anti-inflammatory activity and effective dose for human use.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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