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Full Length Research Paper

Hydroalcoholic extract of *Adansonia digitata's* trunk bark, in topical application, possess *in vivo* wound healing and *in vitro* anti-inflammatory and antioxidant activities

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Adansonia digitata **is a tree plant used in west African local pharmacopoeia. This work aimed to evaluate the wound healing properties of its trunk bark's hydroethanolic extract in wistar rats. The model of excision wound was used. Wistar rats were treated post-excision topically with neutral carbopol gel (Control), carbopol gels containing 2.5 and 5% of *A. digitata* hydroethanolic trunk bark extract (ADHE) and L-Mesitran. Pictures were taken post excision for analysis. Biopsies were realized for histological examination of the skin excised and for hydroxyproline determination. In vitro membrane stabilization anti-inflammatory test, DPPH and FRAP antioxidant tests, and phytochemical tests were performed. The results show a better rate of contraction in ADHE 2.5 and 5% animals (+56 ± 0.52% and +68 ± 0.24% respectively) as earlier as on day three post-excision. The hydroxyproline assay confirmed a better collagen production in ADHE 2.5% and 5% animals (+102.5 ± 2.9%, and +107 ± 3.0% respectively). Histological analysis showed a rapid re-epithelialization in ADHE animals. The anti-inflammatory test indicated an IC50 of 16.521 ± 0.525 µg/mL, very close to Aspirin (16.826 ± 0.341 µg/mL), showing a possible anti-inflammatory activity of ADHE. The extract reduced DPPH radical (IC50 of 344.985 ± 17.139 µg/mL) and ferric ions (EC50 = 178.689 ± 0.250 µg/mL). Phytochemical investigations revealed the presence of alkaloids, terpenoids, and polyphenolic compounds. Flavonoids in particular, with an amount of 51.694 ± 0.174 mgER/g, should explain the wound healing activity observed, making the hydroethanolic extract of *A. digitata* a potential remedy for excision wounds.**

Key words: *Adansonia digitata*, wound healing, Wistar rat, antioxidant, anti-inflammatory.

INTRODUCTION

Skin wounds greatly affect the global healthcare system, creating a significant burden on the economy and society (Raziyeva et al., 2021). Healthy skin serves as an effective barrier to protect internal organs from pathogen pathogen invasion, ultraviolet radiation and other exogenous factors (Chen et al., 2020). The loss of tissue

continuity of this organ, altering its function, constitutes the wound, which thereby becomes a point of entry for pathogens into the body. Fortunately, skin can spontaneously repair itself to restore its structural and functional integrity. But this process can be disturbed by several factors such as infections, inflammation and oedema, leading to complications. When the healing process does not progress in a timely and orderly manner, acute wounds can convert into more difficult manageable problem, chronic wounds (Velnar et al., 2009). The use of herbal systems, especially topical systems to accelerate the wound healing process, has gained importance in recent years with the search for bioactive substances that have an effective role in improving tissue repair (Rizzi et al., 2017). Traditional medical knowledge of medicinal plants and their use by indigenous healers is not only useful for the conservation of cultural traditions and community health care, but also contributes to the development of medicines in the future (Kantati et al., 2016). In Togo, a low-income country of West Africa, *Adansonia digitata* is one of the plants species commonly used in traditional pharmacopoeia. The different parts of the plant (leaves, bark, root, pulp, seeds) are used as a panacea (Kamatou et al., 2011). *A. digitata* has been subjected to various studies for its pharmacological activities such as antimicrobial (Yusha'u et al., 2010; Abiona et al., 2015), antidepressant (Shehu et al., 2018) of its bark, hepatoprotective (Al-Qarawi et al., 2003), antipyretic and analgesic (Ramadan et al., 1994), anti-inflammatory (Ayele et al., 2013), and antiviral (Hudson et al., 2000), antioxidant, and antidiabetic (Braca et al., 2018) properties of its pulp. Its trunk bark particularly has been cited in various ethnopharmacological surveys for its wound healing properties (Inngjerdingen et al., 2004; Kébenzikato et al., 2015). However, no pharmacological study has been conducted up to date to verify the wound healing properties of *A. digitata* trunk bark. The purpose of the present study is to evaluate the wound healing capacities of the hydroethanolic extract of *A. digitata*'s trunk bark (ADHE) in Wistar rats, and its *in vitro* antioxidant and anti-inflammatory properties.

MATERIALS AND METHODS

Plant

Preparation of A. digitata hydroethanolic trunk bark extract (ADHE)

A. digitata trunk bark was collected in district of Lomé (Togo) in December 2020. A plant specimen was identified with the help of botanists of Botany and Plant Ecology Laboratory, University of Lomé (Togo). *A. digitata* trunk barks were then cut into pieces, washed, dried under conditioning air, and reduced to powder before maceration. 400 g of *A. digitata* trunk bark powder was macerated in 4 L of a mixture of ethanol (95%) and water (50:50, v/v), at room temperature (25 ± 2°C), for 72 h. This mixture was stirred for 10 min, once a day. The obtained macerate was filtered on hydrophilic cotton, evaporated to dryness using a rotavapor (Buchi R- 210), and weighed to calculate the yield of the extraction according to the formula:

Yield (%) = (Mass of the final dry extract (g)×100) / Mass of the plant material (g)

ADHE gel preparation

ADHE gel was prepared using Carbopol 974P NF (Goodrich, USA). Carbopol (0.50 g) was dispersed in 49.5 mL of distilled water. The mixture was stirred continuously with a magnetic stirrer (IKA Magnetic stirrer IKA Combimag RCT) at 800 rpm for 1 h. The mixture, still stirred, was then neutralized by adding 1 mol/l NaOH solution, leading to a translucent gel as described by Darré et al. (2014). Two types of gels containing 2.5% (ADHE 2.5%) and 5% (ADHE 5%) of extract were prepared.

In vivo protocols

Animals

For this study, Wistar rats (200-280 g) were used. Animals were raised in the animal house of the Department of Animal Physiology of the University of Lomé, housed in standard polypropylene cages and maintained under standard laboratory conditions (temperature 24-25°C, relative humidity and a 12/12 h light-dark cycle). They had free access to food and water. Institutional guidelines and ethical principles of Physiology/Pharmacology laboratory (University of Lomé-Togo, ref: 001/2012/ CB-FDS-UL) were followed.

Excision wound induction

The excision wound model was used to evaluate the wound healing activity of ADHE. Animals were randomly divided into four groups of six rats each. Animals of the control group were treated by topical application of 1% carbopol gel, when second and third groups were treated, respectively with ADHE 2.5% and ADHE 5%. L-Mesitran (Honey, Lanolin, Sunflower Oil, Cod Liver Oil, Calendula officinalis, Aloe barbadensis, Vitamins C & E and Zinc Oxide) was used topically in the reference group animals. Rats were anesthetized by the open mask method with diethyl ether. The backs of the animals (on the right flank) were then shaved with an electric clipper and cleaned with 70% alcohol. Using a surgical chisel, the area outlined with a 1.5 cm diameter ink pad was excised to a circular surface (Gilles et al., 2011). The wounds were left in the open air and observed daily. Treatments were applied once daily from wound induction until 12 days post excision.

Measurement of wounds surfaces

Photographs of the wounds were taken using the same instrument, a Samsung brand phone (Samsung Galaxy A5), at a fixed distance

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of the camera from the wound. The pictures were then analyzed, and the wounds surfaces were evaluated on days 0, 3, 6, 9 and 12 post-excisions using the ImageJ 1.48v freeware [(National Institutes of Health, USA; https://imagej.nih.gov/ij), as previously described by Metowogo et al. (2020). Contraction rates (Tx) were calculated using the formula:

$Tx = ((Sj0 – Sjx) \pm 100) / Sj0$

where Sjx represent the wound area on day x (3, 6, 9 or 12), and Sj0 is the wound surface on the initial day.

Hydroxyproline content

Hydroxyproline, a protein marker produced as a result of collagen synthesis, was assayed using the colorimetric method of Darré et al. (2014). On day 12, the animals in each group were sacrificed by overdosing anesthesia using ether. The scar samples were collected for the determination of hydroxyproline content. Briefly, it consisted in a hot hydrolysis of dried wound biopsies (60°C in the oven for 12 h) in concentrated acid medium (6 N HCl) for 4 h at 130°C, with the aim of releasing the amino acids including hydroxyproline. Hydrolysates in sealed glass tubes were recovered with 10 ml of distilled water, homogenized; mixed successively with 50 µl of $CuSO_4$ (0.01 M), 50 µl of NaOH (2.5 N) and 50 µl of H_20_2 (6%); incubated at 80°C in the oven for 5 min. After cooling tubes at room temperature, 2 ml H_2SO_4 (3N) and 1 ml P-dimethylaminobenzaldehyde 5% were added before reading with a spectrophotometer at 540 nm. Standard solutions at concentrations of 1.0, 2.0, 4.0, and 8.0 mg/mL hydroxyproline were also prepared in triplicate to establish a calibration curve.

Histological study

On days 10 and 12, two rats from each group were anesthetized with an overdose of ether and sacrificed. The wounds were collected for histological analysis. After dehydration in different ethyl alcohol baths of increasing degree, the samples undergo impregnation and coating. Skin wound samples were fixed in 10% neutral buffered formalin, processed and included in paraffin. Five-micrometer skin sections were cut and stained with hematoxylin-eosin (H&E). The tissues were qualitatively assessed under light microscopy (Olympus BX 51) at 200x magnification. Parameters such as granulation, epithelialization, vascularization and inflammatory cells were highlighted.

In vitro tests

Anti-inflammatory activity

Hypotonicity induced hemolysis of red blood cells membrane was used to assess membrane stabilization ability of ADHE. The slightly modified protocol of Moualek et al. (2016) was used. Wistar rat blood sample was centrifuged at 3000 rpm for 10 min. The pellet obtained was washed three times with isotonic saline and a 10% v/v suspension was made with isosaline. Different concentrations of ADHE (100-500 µg/mL), reference drugs (Aspirin 500 mg and Diclofenac sodium 50 mg) samples and control were separately mixed with 1 mL of phosphate buffer, 2 mL of hyposaline (0.36% NaCl) and 0.5 mL of pellet suspension. All the assay mixtures were incubated at 37°C for 30 min and centrifuged at 3 000 rpm. Hemoglobin content was estimated by a spectrophotometer at 540 nm in the supernatant liquid decanted. The percentage inhibition of hemolysis was calculated using the formula:

(% of inhibition) = ((Abs control – Abs x) × 100) / Abs control

where Abs is the absorbance of the sample, and x corresponds to ADHE or reference drugs. The concentration of the extract corresponding to 50% inhibition of hemolysis (IC50) was determined by the dose-response curve.

Antioxidant activities

DPPH reduction test: 2,2-diphenyl 1-picrylhydrazyl (DPPH) is a stable purple free radical in solution, which discolors to yellow when reduced to diphenyl picryl hydrazine by a compound possessing free radical scavenging properties. An equal volume of 100 µM DPPH in methanol was mixed with different concentrations of ADHE (5-200 µg/mL), or standards (Ascorbic acid and Quercetin). The mixture was incubated in the dark for 30 min. The absorbance was measured at 517 nm with a spectrophotometer. The percentage of DPPH trapping effect was calculated according to the formula of Kpemissi et al. (2019):

(% of inhibition DPPH) = ((Abs control – Abs x) × 100) / Abs control

where Abs is the absorbance of the sample, and x correspond to ADHE or standards. Quercetin and Ascorbic acid were used as reference antioxidants. The concentration of the extract corresponding to 50% inhibition rate (IC50) was determined using the dose-response curve.

Ferric ions reduction test (FRAP)

The ability to reduce ferric ions was measured using the method described by Nishaa et al. (2012) and slightly modified. The FRAP reagent was generated by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM TPTZ (Tripyridyl triazine) solution, and 20.0 mM $FeCl_3.6H_2O$ solution in a 10:1:1 volume ratio. ADHE at different concentrations (50, 100, 250, 500 and 1000 µg/ml) were then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37°C for 30 min. The increase in absorbance at 593 nm was measured. Fresh working solutions of $FeSO_4$ were used for calibration. Antioxidant capacity based on the ability to reduce ferric ions in the sample was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mM) per gram of sample.

Phytochemical investigations

Qualitative tests

The compound classes were characterized using appropriate techniques and specific reagents according to the respective methods described by Fankibe et al. (2020).

Research of tannins

ADHE (1 mL) in a test tube was treated with Molisch's reagent (0.5 mL) followed by 3 drops of ferric chloride, giving a green color in a condensed form, and indicating the presence of tannins.

Alkaloids

This research is based on the ability of alkaloids to precipitate by combining with metals and metalloids. 100 mg of ADHE were added to 5 ml of 10% sulfuric acid. The whole mixture was homogenized for 5 min and filtered. The mixture was treated with 0.5 ml of the reagents (Wagner, Hager, and Mayer) in different

tubes. The appearance of an orange precipitate, a yellowish precipitate, and a brown precipitate, respectively, indicated the presence of alkaloids.

Saponins

ADHE (4 mL) mixed with distilled water in a test tube was vigorously shaken for 2 min. A foam of about 5 cm formed and stable after about 10 min showed the presence of saponins.

Flavonoids

ADHE spiked with hydrochloric acid (HCl 2.5 mL) was mixed with 1.5 mL of sodium hydroxide (NaOH) in a test tube. The appearance of a yellow color indicated the presence of flavonoids.

Polyphenols

A few drops of 5% ferric chloride $FeCl_3$ were added to 5 mL of ADHE. The formation of an intense blackish coloration indicated the presence of polyphenols.

Sterols and triterpenes

One milliliter of acetic anhydride and 1 ml of chloroform were added to 100 mg of ADHE. After shaking, the resulting solution was separated into two test tubes. One part was used as a control. After addition of 1 ml of concentrated sulfuric acid, the formation of a brownish red or purple ring after a few minutes at the contact zone of the two phases indicates the presence of metabolites. The appearance of a gray or purple coloration indicated the presence of triterpenes, while a blue to gray-green coloration showed the presence of sterols.

Quantitative tests

Determination of total phenolic content

Total phenolic content was determined calorimetrically, basing on the reduction by total polyphenols of phosphotungstic acid and phosphomolybdic acid reagents (Folin reagent) (Kpemissi et al., 2019). The extract was prepared in a distilled water-methanol mixture (V: V). 400 µL of ADHE were mixed with 1 mL of diluted Folin-Ciocalteu reagent (1:10 in water). After 4 min, this mixture received 1.5 mL of saturated sodium carbonate solution (6%), followed by a 15-min incubation at room temperature (protected from light). The absorbance was measured at 765 nm. Gallic acid (0-100 µg/mL) was used as a standard. Results were expressed as mg gallic acid equivalents (mgGAE)/g dry weight of plant extract.

Determination of tannin content

Total tannin content was determined by the Folin-Ciocalteu method as described previously. Polyvinylpolypyrrolidone (PVPP) was weighed (6 mg) in a test tube and 400 µL of ADHE dissolved in distilled water (1 mg/mL) were added. After incubation for 15 min at 4°C, the tubes were vortexed and centrifuged at 3000 rpm for 10 min. Aliquots of supernatant (200 µL) were transferred to test tubes and unabsorbed phenols were determined as described earlier. The calculated values were subtracted from the total polyphenol contents, and the total tannin contents were expressed in mg gallic acid/100 g dry plant matter. All measurements were repeated 3 times.

Determination of flavonoid content

This assay is based on the property of flavonoids to form aluminium chelates with aluminium chloride. 2 mL of crystalline aluminium chloride ($AlCl_3$, 20 mg/mL) and 6 mL of crystalline sodium acetate (40 mg/mL) were dissolved in 10 mL of extraction solvent, and the mixture was added to 200 µL of ADHE dissolved in distilled water (1 mg/mL). The absorbance was recorded at 430 nm against a blank (2 mL of solution analyzed plus 5 mL of water). Amount of flavonoids was calculated as rutin equivalent from the calibration curve of rutin standard solutions and expressed as mg rutin/g plant material.

Statistical analysis

Results are expressed as mean M ± SEM, for all tests performed. The data were statistically analyzed by the software GraphPad Prism version 8.4.2. Analysis of variance was performed using the ANOVA test. Differences were considered significant at $P < 0.05$.

RESULTS

Extraction efficiency

Dry extract (16.78 g) were obtained, corresponding to a yield of 4.20%.

Evolution of the surface area and contraction rates of wounds

Figure 1 shows the appearance of the wounds just after excision at D0, whereas Figure 2 shows the evolution of these wounds in Wistar rats at D0, 3, 6, 9, and D12 in all groups. Macroscopic observation of the wounds and analysis of their surfaces obtained using "Image J" software (Figure 3A) showed a decrease in wounds areas from an average of 3.36 ± 0.11 cm^2 at D0 in almost all animals to 0.347 ± 0.081 cm^2 at D12 in control animals, 0.094 ± 0.042 and 0.041 ± 0.018 cm^2 at D12 in animals treated with ADHE 2.5 and 5%, respectively, and 0.090 ± 0.036 cm^2 at D12 in those treated with L-Mesitran. These observations are confirmed by calculation of the corresponding contraction rates. The best contraction rates were obtained between D3 and D6 (Figure 3B) in animals treated with ADHE 5% (+68 ± 0.24 at D3, $P < 0.001$; and +88 ± 0.11% at D6, $P < 0.001$ compared with control animals).

Hydroxyproline content

The measurement of hydroxyproline is generally used as an indicator to determine the collagen level. In this study, the amount of hydroxyproline in scar fragments in rats

Figure 1. Photograph showing the appearance at D0 of the excisional wounds.
Source: Authors

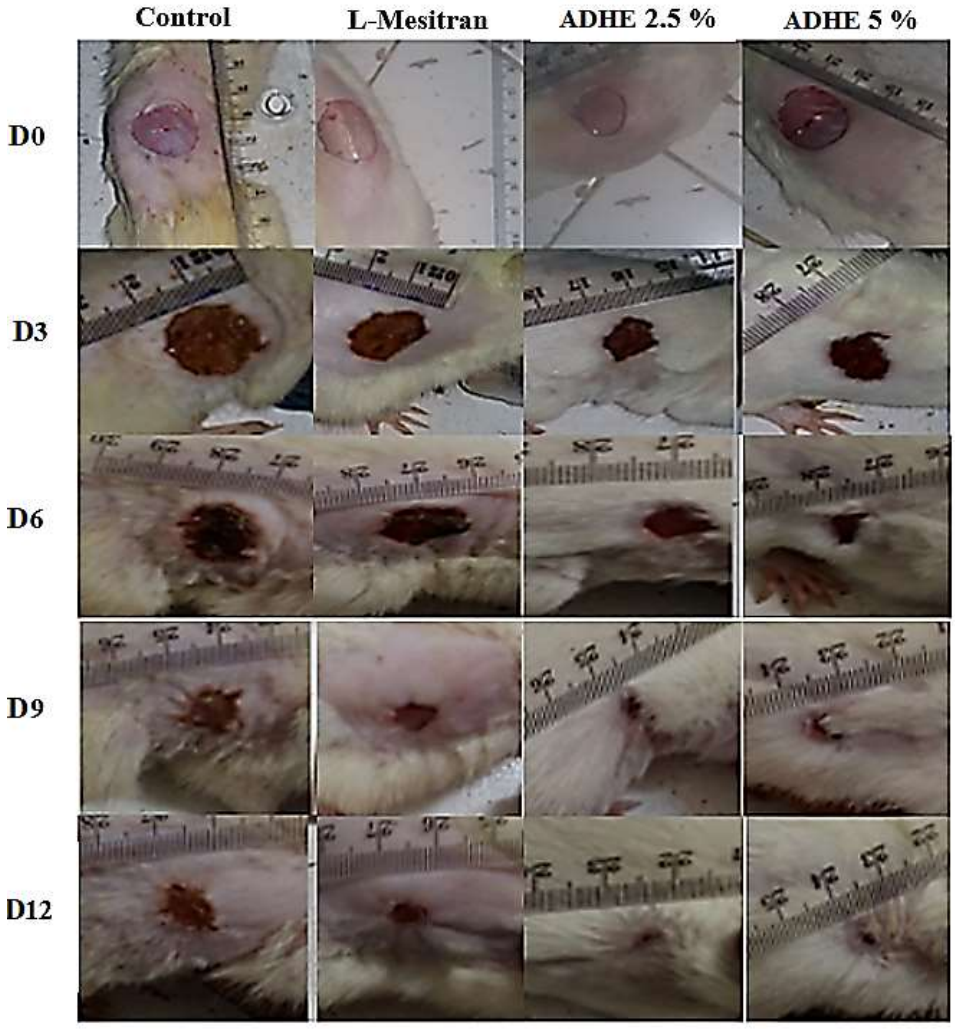

Figure 2. Photographs showing the evolution of the excisional wounds. From top to bottom: all groups at D0, D3, D6, D9 and D12. From left to right: different groups (Control; L-Mesitran; ADHE 2.5% gel, and ADHE 5% gel). Scale: 1 cm (graduated ruler).
Source: Authors

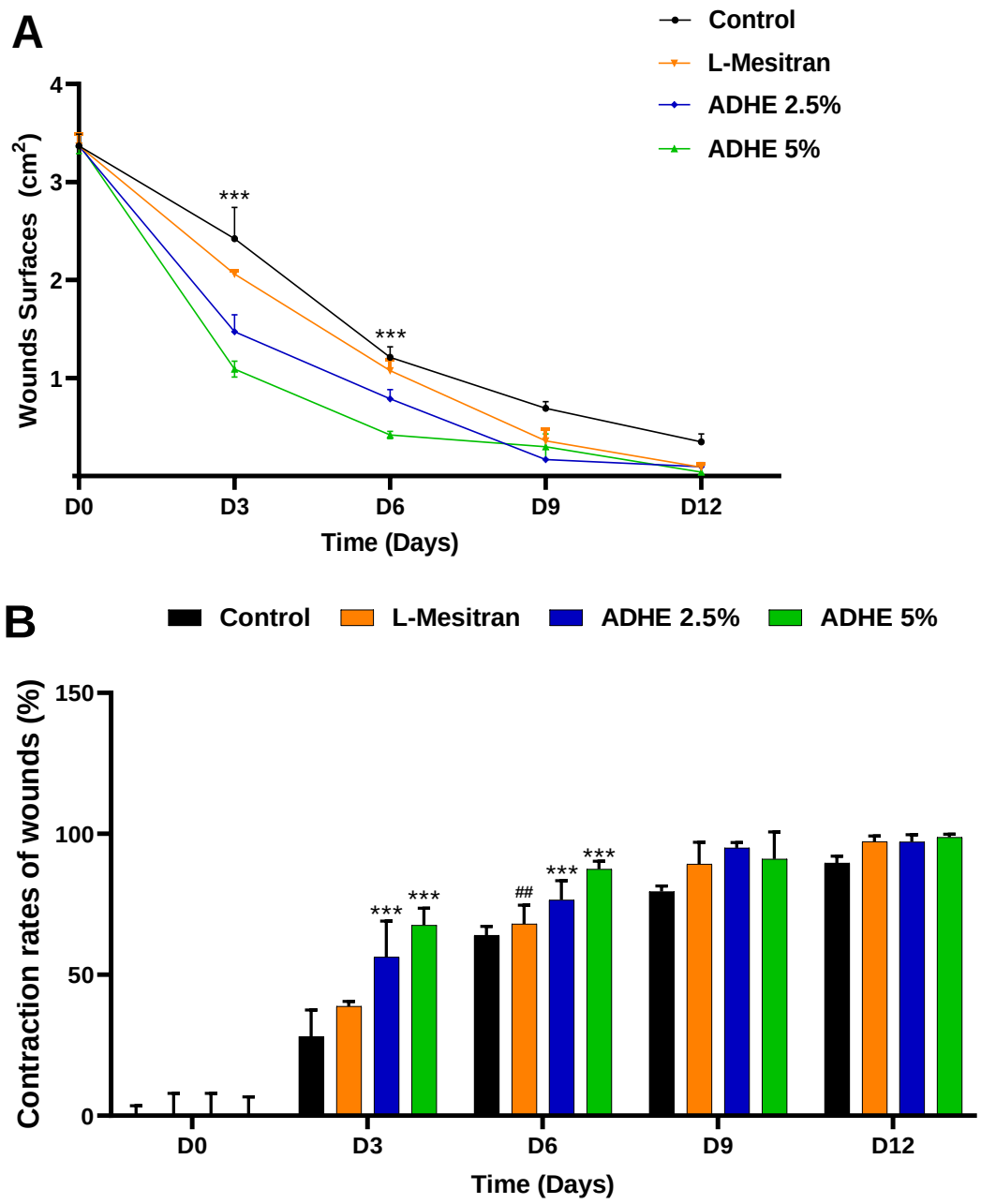

Figure 3. Evolution of wound area (**A**) and corresponding contraction rates (**B**). Values are expressed as Means ± SEM, n = 6. Units: Cm2. ***p< 0.001: ADHE 2.5 and 5% vs Control at D3 and D6. ##p< 0.01: L- Mesitran vs Control at D6.
Source: Authors

treated with ADHE was significantly increased (P < 0.05 at 2.5% dose, and P < 0.01 at 5% dose), compared with the control group (Figure 4).

Histological studies

Histological studies revealed an improved repair and remodeling of the wound area with a complete skin thickness and a healing close to normal skin (hyperplastic squamous epithelium) in animals treated with ADHE. In fact, as shown in Table 1 and Figure 5, on the tenth post-treatment day, the microscopic histological examination of the wounds showed neoformed vessels, fibroblasts, epithelialization, hair follicles in ADHE 2.5 and 5% rats compared to control animals which still showed

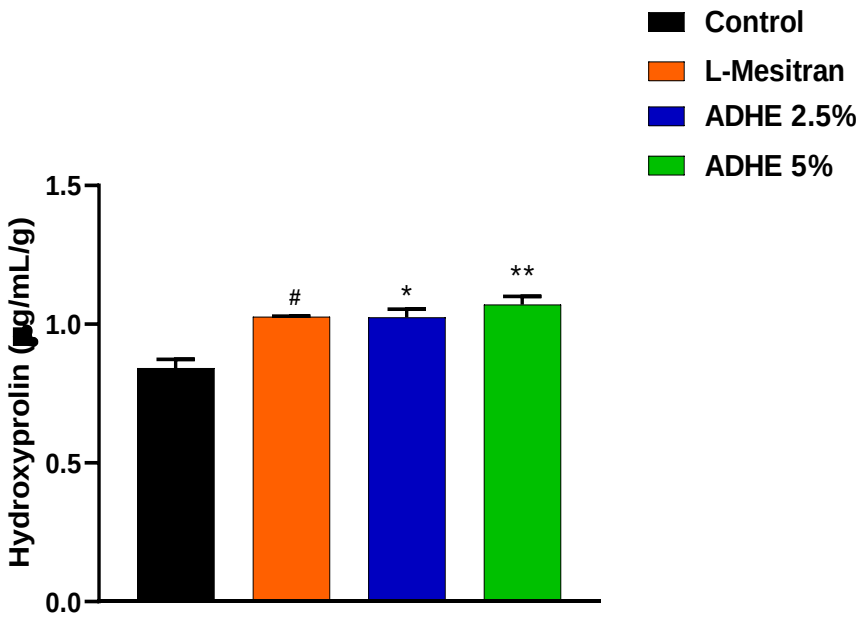

Figure 4. Hydroxyproline content of scar fragments from different groups at day 12. Values are expressed as Means ± SEM, n = 8. Units: µg/mg dry tissue. *p< 0.05: ADHE 2.5% vs. Control. **p< 0.01: ADHE 5% vs Control. #p< 0.05: L- Mesitran vs Control.
Source: Authors

Table 1. Histological evaluation at D10 after application of ADHE gels.

Treatment	Ed	Inf	Nv	Hf	Fb	U	Ep
Control	--	--	--	--	++	+++	++
L-Mesitran	--	--	+	+	+++	--	++
ADHE 2.5%	--	--	++	+	+++	--	++
ADHE 5%	--	--	++	++	+++	--	+++

(+++) = High; (++) = Normal; (--) = Absence; Ed = Edema; Inf = Inflammatory infiltrate; Nv = Neovascularization; Hf = Hair follicle; Fb= Fibrosis; U= Ulceration; Ep = Squamous epithelium.
Source: Authors

ulcerations. Already on the twelfth day, in ADHE 2.5 and 5% rats as well as L-Mesitran treated animals, epithelialization was noted with the presence of much more elaborate fibroblasts (Table 2 and Figure 6). At the same time, control animals revealed a weak proliferation of fibroblasts.

Anti-inflammatory activity of ADHE

Table 3 shows that the extract stabilized the red blood cell membrane against hemolysis induced by the hypotonic 0.36% NaCl solution. The IC50 found for ADHE (16.565 ± 0.223 µg/mL) is very close to that of Aspirin (16.826 ± 0.341 µg/mL), and lower than that of Diclofenac (19.935 ± 2.640 µg/mL).

Antioxidant activities of ADHE

The amount of ADHE that inhibited 50% of DPPH free radicals (IC50) was 344.985 ± 17.139 µg/mL as presented in Table 4. ADHE also clearly reduced ferric ions (Fe^{3+}) with an EC50 of 178.689 ± 0.250 µg/mL, although lower than that of the reference molecules (Ascorbic acid and Quercetin).

Phytochemical studies

Qualitative composition

Phytochemical screening of ADHE revealed the presence of tannins alkaloids triterpenes flavonoids and phenolic

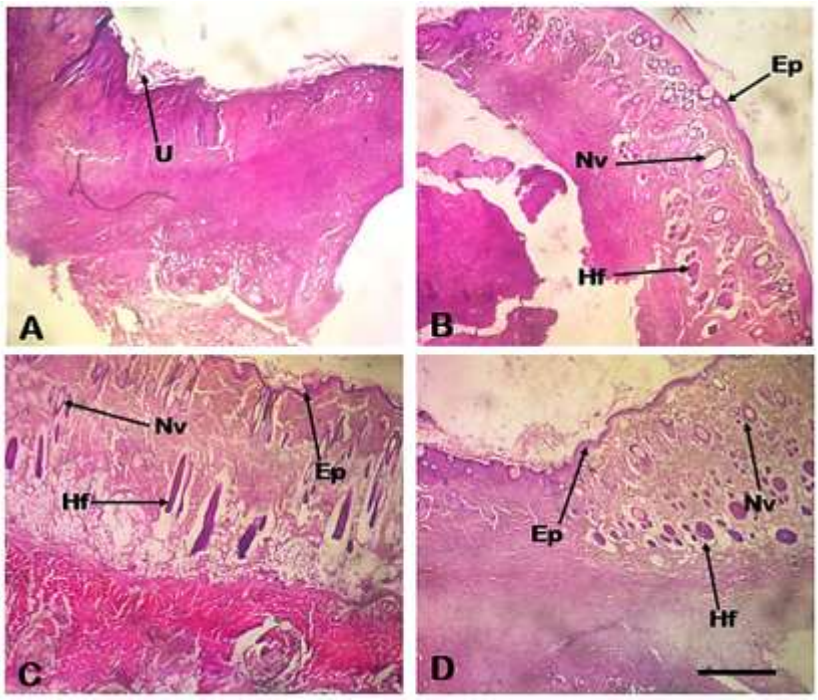

Figure 5. Histological observation of wounds at day 10. A = Control; B= L-Mesitran; C= ADHE 2.5%; D= ADHE 5%. Nv = Neovascularization; Hf = Hair follicle; Fb= Fibrosis; U= Ulceration; Ep = Squamous epithelium. Hematoxylin-eosin (H&E) stain. Olympus BX 51 Light microscope. Scale bar is 50 µm.
Source: Authors

Table 2. Histological evaluation at D12 after application of ADHE gels.

Treatment	Ed	Inf	Nv	Hf	Fb	U	Ep
Control	--	--	+	--	+++	++	+++
L-Mesitran	--	--	++	++	+++	--	+++
ADHE 2.5%	--	--	++	++	+++	--	+++
ADHE 5%	--	--	++	+++	+++	--	+++

(+++) = High; (++) = Normal; (--) = Absence; Ed = Edema; Inf = Inflammatory infiltrate; Nv = Neovascularization; Hf = Hair follicle; Fb= Fibrosis; U= Ulceration; Ep = Squamous epithelium.
Source: Authors

compounds (Table 5).

Phenolic compounds quantitative composition

The results of the quantitative determination of total phenolics, tannins, and flavonoids from rutin and gallic acid standards are shown in Table 6.

DISCUSSION

The skin provides protection and acts as an external barrier to the body's tissues and organs against assaults from the external environment. Any damage to the skin barrier should be quickly and effectively repaired through the healing process. Considering that wounds generally result from traumatic processes, the model of excisional wounds, which mimics very well skin barrier accidental rupture, has gained importance in studies of wound healing management (Chhabra et al., 2020; Kour et al., 2021). It is then on a suitable model of the wound healing process that ADHE properties have been tested in this study. Evaluation of the wounds surfaces and the rate of contraction of these wounds, a parameter that characterizes the closure of the wound (Darré et al., 2014), indicated that ADHE incorporated in Carbopol gel and administrated topically accelerated the wound

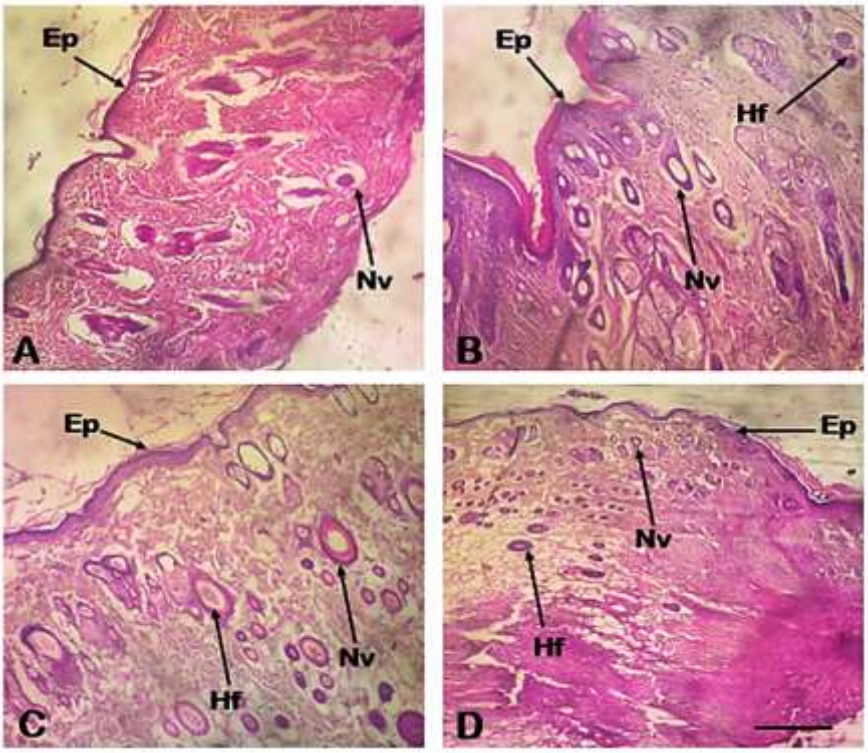

Figure 6. Histological observation of wounds at day 12. A = Control; B= L-Mesitran; C= ADHE 2.5%; D= ADHE 5%. Nv = Neovascularization; Hf = Hair follicle; Fb= Fibrosis; U= Ulceration; Ep = Squamous epithelium. Hematoxylin-eosin (H&E) stain. Olympus BX 51 Light microscope. Scale bar is 50 µm.
Source: Authors

Table 3. Hemolysis inhibiting capacity of ADHE and the standard molecules.

Treatment	IC50 (µg/mL)
Aspirin	16.826 ± 0.341
Diclofenac	19.935 ± 2.640
ADHE	16.565 ± 0.223

Values are expressed as Means ± SEM, n = 3. Units: µg/mL.
Source: Authors

Table 4. Antioxidant activities of ADHE.

Treatment	IC50 (µg/mL)	EC50 (µg/mL)
Ascorbic acid	45.129 ± 1.130	23.057 ± 0.210
Quercetin	21.899 ± 1.730	48.521 ± 2.026
ADHE	344.985 ± 17.139	178.689 ± 0.250

Values are expressed as Means ± SEM, n = 3. Units: µg/mL.
Source: Authors

healing process, compared to control animals, thus justifying its use in traditional medicine in Togo. The results in ADHE 5% animals in particular showed a better fasted healing process than in L-Mesitran (a

Table 5. The different families of compounds present in the extract.

Compounds	ADHE
Alkaloids	+
Flavonoids	+
Phenolic compounds	+
Sterols	-
Tannins	+
Triterpenes	+

(+) Presence; (-) Absence.
Source: Authors

Table 6. Total phenols, flavonoids and tannin contents of ADHE.

Compounds	ADHE	Reference compound
Flavonoids	51.694 ± 0.174 mgER/g	Rutin
Tannins	68.086 ± 2.44 mgEAG/g	Gallic acid
Total phenols	99.188 ± 2.129 mgEAG/g	Gallic acid

Values are expressed as Means ± SEM, n = 3. Units: total phenols and tannins are expressed as mg gallic acid equivalent/g of ADHE (mgEAG/g), while flavonoids are expressed as mg rutin equivalent/g ADHE (mgER/g).
Source: Authors

commercialized natural herbal remedy) treated animals. Considering that the cicatrization of an injured skin is a complex process involving multiple mechanisms such as collagen production, inflammatory cells signaling and free radicals management (Herman and Herman, 2020), we investigated *in vivo* the hydroxyproline production and *in vitro* the anti-inflammatory and antioxidant activities of ADHE. The measurement of hydroxyproline is frequently used as a reliable index to quantify collagen in tissues. Collagen is the main structural protein component of tissue (Nagappan et al., 2012), and is well known to effectively increase the healing process by promoting more orderly proliferation of fibroblasts (Süntar et al., 2011). In the present study, the hydroxyproline content of ADHE 2.5 and 5% groups significantly increased, indicating that the extract could have promoted collagen synthesis by stimulating the proliferation of fibroblasts which are responsible for collagen production. This observation was confirmed by wounds histology which revealed increased re-epithelialization, neovascularization and hair follicles recurrence in ADHE treated groups while a delayed healing process was still observed in control animals after 12 days.

Otherwise, in the sequence of events leading to wound reparation, inflammation occurs in the earlier steps. The present findings, with significant differences between ADHE groups and control animals already on the third day of treatment, suggest a precocious activity, then a possible effect on inflammation process. The inhibition of hypotonia-induced erythrocyte lysis was used in our study to examine the anti-inflammatory activity of ADHE. The IC50 of the extract, close to that found for Aspirin, indicates a possible anti-inflammatory activity which would also explain the observed early healing activity. This membrane stabilization property may be related to ADHE flavonoids, which have been described to interact with the polar head of phospholipids at the interface between water and lipids of the cell membrane, thus improving the mechanical stability of lipid bilayers (Huh et al., 1996; Phan et al., 2014). In addition, these polyphenolic compounds often possess antimicrobial activity, providing better and faster healing by forming a barrier against contamination (Süntar et al., 2011). Previous antimicrobial and antibacterial studies of *A. digitata* bark conducted elsewhere have proven its effectiveness (Yusha'u et al., 2010; Abiona et al., 2015), and provide an additional possible explanation for the healing effect of the extract by preventing wound infection. More interestingly, phenolic compounds present in ADHE, revealed by phytochemical studies, may ultimately act by antioxidants mechanisms. According to the results, ADHE is a good free radicals' scavenger, with an IC50 of 344.985 ± 17.139 µg/mL. The FRAP assay confirmed this antioxidant capacity of ADHE.

The improved wound healing, especially in the ADHE 5% groups, can then be attributed to satisfactory profile of the aforementioned studied parameters in the treated groups, supported by the phytochemical analysis of

ADHE revealing the presence of antioxidant and anti-inflammatory compounds flavonoids and tannins as the major active components.

Conclusion

The objective of the present study was to evaluate the wound healing property of the hydroethanolic extract of the trunk bark of *Adansonia digitata* in Wistar rats. The results obtained show a better profile of cicatrization in ADHE treated animals than control animals. ADHE may fast wound healing process through probable anti-inflammatory and antioxidant activities, correlated to the observed phytochemical content. All of these results allow us to confirm the healing potential of the hydroethanolic extract of the trunk bark of *A. digitata*. In perspective, further dermal toxicity and exploration of other possible mechanisms are needed before the presentation of this very interesting extract to local populations or to the pharmaceutical and/or cosmetic industry.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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