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Research Article
Healing, Anti-inflammatory and Analgesic Activities of the Hydro-Methanolic Extract of *Acacia nilotica* Pods (*Mimosaceae*)

[1]Madieye Sene, [2]Ndiaga Diop, [1]Mamadou Oury Diallo, [3]Abdou Sarr, [1]Firmin Sylva Barboza, [1]Mamadou Ndiaye, [1]Awa Ndiaye-Sy and [1]Guata Yoro Sy

[1]Laboratory of Pharmacology and Pharmacodynamics, Faculty of Medicine, Pharmacy and Odonto-Stomatology, Universite Cheikh Anta Diop, BP 5005, Dakar-Fann, Senegal
[2]Laboratory of Histology-Embryology and Human Cytogenetics, Universite Cheikh Anta Diop, BP 5005, Dakar-Fann, Senegal
[3]Laboratory of Pharmacognosy and Botany, Faculty of Medicine, Pharmacy and Odonto-Stomatology, Universite Cheikh Anta Diop, BP 5005, Dakar-Fann, Senegal

Abstract

Background and Objective: The traditional African pharmacopoeia includes many plants used by the populations to combat inflammation and pain. Some of them, beyond inflammation and pain, is used in the treatment of wounds and burns. This is the case of *Acacia nilotica* var adstringens. The present research aimed to evaluate the healing, anti-inflammatory and analgesic activities of hydro-methanolic extract of *Acacia nilotica* pods (*Mimosaceae*). **Materials and Methods:** The pods were dried and then separated from their seeds and pulverized. Extraction of pods was carried out in methanol/water and the extract was characterized. The experiments were conducted in different models of experimental burn in rats, inflammation carrageenan-induced rat paw edema and pain by writhing test in mice. **Results:** Phytochemical study shows the presence of polyphenols, tannins, flavonoids and saponosides. Daily application of ointments promotes concentration-dependent healing. The cicatrization rate is higher with hydro-methanolic extract of *Acacia nilotica* pods 10% in petroleum jelly, which induces near complete tissue repair after 16 days of treatment. The ointment reduces inflammation and pain and increases angiogenesis, re-epithelialization and keratinocyte migration and promotes the synthesis of collagens. **Conclusion:** The results of the present study justify the traditional use of *Acacia nilotica* pods in the treatment of wounds and burns.

Key words: *Acacia nilotica*, pods, burns, cicatrization, inflammation and pain, epithelialization, keratinocyte

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Corresponding Author: Madieye Sene, Laboratory of Pharmacology and Pharmacodynamics, Faculty of Medicine, Pharmacy and Odonto-Stomatology, Universite Cheikh Anta Diop, BP 5005, Dakar-Fann, Senegal

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The healing process is classically described in three interconnected phases: Vascular and inflammatory, proliferative and remodelling. The vascular and inflammatory phase includes hemostasis, production of inflammatory factors, fibrin deposition and migration of monocytes, neutrophils and lymphocytes. A proliferative phase follows during which the formation of new blood vessels (angiogenesis), the precipitation of collagen, the creation of granulation tissue, epithelialization, contraction of the wound and finally remodelling occur[1]. Pain is thought to be an alarming sign of actual or apparent tissue damage[2]. Different pathways and molecules of inflammation are involved in nociception, defining so-called inflammatory pain[3].

The traditional African pharmacopoeia includes many plants used by the populations to combat inflammation and pain. Some of them, beyond inflammation and pain, is used in the treatment of wounds and burns[4]. This is the case of *Acacia nilotica* var adstringens.

Acacia nilotica is a very drought-resistant tree. It has a very wide range from coastal to sub-alpine regions and from high rainfall to arid areas, thus covering Africa, Australia, South America and other temperate regions in the world. In Africa, it is found from Senegal to Egypt and southwards from East Africa to Mozambique, South Africa (in Natal) and the Indian Ocean Islands. In Senegal *A. nilotica* forms stands in the river valley[5-8].

Various parts of this Acacia tree including the leaves, bark, seeds, roots, gum, flowers, fruits and young pods have anticancer, antimutagenic, antispasmodic, antipyretic, antidiabetic, antifungal, antiviral, antibacterial, antihypertensive, antioxidant, wound, anti-inflammatory and antinociceptive activities[9,10].

In the present research, the aim was to evaluate the healing, anti-inflammatory and analgesic activities of hydro-methanolic extract of *A. nilotica* pods (*Mimosaceae*).

MATERIALS AND METHODS

Study area: The study was carried out at Pharmacology Laboratory at the Faculty of Medicine and Pharmacy of Dakar University, Senegal from October, 2020 to July, 2022.

Drugs, chemicals and solvents: Petroleum jelly (Valdafrique Laboratory), Sulfadiazine (pharmacy), sodium benzoate, carrageenan, acetylsalicylic acid, acetic acid and extraction solvents were obtained from Sigma/BES (Dakar, Senegal).

Plant material: *Acacia nilotica* pods were collected from Fatick, in the centre region of Senegal. Botanical samples were identified at the Department of Botany and Pharmacognosy, Faculty of Medicine and Pharmacy, University of Dakar, where the voucher specimen (DPB/AN-10/02/21) was deposited. The pods were dried in the pharmacology laboratory at room temperature (25-30°C) for 4 weeks and then separated from their seeds and pulverized. A brown-coloured powder is obtained.

Animals: A total of 85 rats and 9 mice from the Pharmacology Laboratory, Faculty of Medicine and Pharmacy were used. The weights of the rats varied between 125 and 283 g and those of the mice between 15 and 25 g. The animals were housed in a cage under conditions of 25±2°C temperature, 12 hrs light cycle and provided with food and water *ad libitum*. For the rats, 60 were used for the study of the healing activity (of which 35 are sacrificed for the needs of the histological tests) and 25 for the study of the anti-inflammatory activity.

The experimental protocols were conducted following the guidelines of the Institutional Ethics Committee (Research Ethics Committee of Cheikh Anta DIOP University).

Experimental procedures

Extractions: A total of 50 g of *A. nilotica* pods powder were subjected to a decoction in 400 mL of methanol and 100 mL of boiling water for 5 min. After cooling, the decoction was filtered (filtrate 1) and the residue was exhausted twice with the same volumes of methanol and water on each pass (filtrates 2 and 3).

The combined filtrates were then concentrated in a rotavapor and then dried in an oven at a temperature of 40°C. The hydro-methanolic extract thus obtained was then ground to give a homogeneous dry powder, used for the preparation of the ointments necessary for the pharmacological tests.

Phytochemical characterizations: The purpose of the phytochemical study was to research the chemical groups present in the pod powder of *A. nilotica* to have an idea of the chemical nature of the active ingredients. This research was carried out, using the classic methods of characterization of the major chemical families[11]. The main chemical groups that were searched were:

- Flavonoids (Shibata reaction)
- Tannins (Stiasny reaction)
- Alkaloids (Bouchardat, Dragendorff, Valser-Mayer Reactions)
- Sterols and triterpenes (Liebermann Reaction)

Table 1: Composition of different ointments

Composition (g)	Ointment (3%)	Ointment (10%)	Ointment (30%)
HMEANP	1.5	5	15
Sodium benzoate	0.075	0.075	0.075
Petroleum jelly	48.425	44.925	34.925
Total (g)	50	50	50

Hydro-methanolic extract of *A. nilotica* pods (HMEANP)

Table 2: Scores of the evolution of experimental burns

Score	Evaluation of the healing process
0	Healing is complete and tissue repair is complete
1	Tissue healing is almost complete
2	Remnants of the crust remain the size of the lesion decreases (skin reconstruction)
3	All dead tissues (scabs) are removed, wounds and oozing
4	Necrotic skin is partially removed, ulcerated and oozing
5	Necrotic skin completely covers the burned area

Table 3: Different products tested according to the doses used

Groups	Products	Acronyms	Doses
1	Normal saline	Control	10 mL kg^{-1}
2	Acetyl salicylic acid	ASA	30 mg kg^{-1}
3	Hydro-methanolic extract of *A. nilotica* pods	HMEANP	10 mg kg^{-1}
			30 mg kg^{-1}
			100 mg kg^{-1}

Ointment formulation: From the powder of Hydro-methanolic extract of *A. nilotica* pods (HMEANP) 03 ointments at 3, 10 and 30% in petroleum jelly were prepared at 50 g. Sodium benzoate was used as a reference preservative at a rate of 1.5 g/1000 g of ointment. Sodium benzoate and extract powder were crushed in a mortar. Petroleum jelly was added gradually by gently crushing until the mixture was homogenized. An ointment at 3, 10 or 30% were obtained depending on the proportions (Table 1). The ointments were packaged in hermetically sealed jars and then stored at room temperature away from light.

Burn induction: Healing activity was evaluated in the experimental burn model[12]. Sixty rats were divided into 5 groups of 12 as follows:

- **Group 1:** Untreated rats
- **Group 2:** Sulfadiazine
- **Group 3:** Rats treated with an ointment of 3% hydro-methanolic extract of *A. nilotica* pods in petroleum jelly (HMEANP-3%)
- **Group 4:** Rats treated with an ointment of 10% hydro-methanolic extract of *A. nilotica* pods in petroleum jelly (HMEANP-10%)
- **Group 5:** Rats treated with an ointment of 30% hydro-methanolic extract of *A. nilotica* pods in petroleum jelly (HMEANP-30%)

Sulfadiazine is an antibacterial sulfonamide, used topically in preclinical and clinical studies to demonstrate the healing activity of a new product[13,14].

The rats were then anaesthetized with a 3% chloral solution by intra-peritoneal injection (1 mL/100 g). The dorsal flanks of the rats were shaved and cleaned. Experimental burns have been induced using a 3 cm diameter metal cylinder and heated for 5 min. The cylinder was applied for 20 sec by slight pressing on the surface of the shaved skin of the rats[15].

Evaluation of the healing activity of the HMEANP: Healing activity was evaluated according to Kamoshida's Method, which assigns scores ranging from 1-5 depending on the importance of the burn (Table 2)[16]. Evaluation of the scores has been done daily for 28 days.

Also, the epithelialization time was identified. The latter was defined as the number of days required for the remnants of dead tissue to shed without any residual raw wounds[17].

Histological study of the wound: For each group of rats, we performed after a macroscopic study a periodic incisional biopsy to remove a part of the lesion. The fragment was then fixed by dipping it in the tube containing 10% formalin for 48 hrs. Histological sections were obtained after the following steps:

- The dehydration of the samples by successive baths of alcohol of increasing degrees
- The inclusion of fragments by melted kerosene at 58°C
- Embedding: The sample is cast in a kerosene block
- The making of thin cuts of 3 microns thickness thanks to the microtome
- Finally, the sections were stained using Hematoxylin Eosin and Masson's Trichrome stain

Anti-inflammatory activity: The anti-inflammatory activity study was carried out following the carrageenan-induced rat paw edema method, described by Winter *et al.*[18]. The rats were divided into 5 groups 5 (Table 3). Then, they had fasted for 12 hrs before the tests. For each rat, the initial diameter (D0) of the left hind paw was measured using a digital calliper.

The rat paw edema was induced by injection of carrageenan solution 1% (100 µL) underneath the planter region of the left hind paw of the rats 1 hr after oral administration with the different solutions. The increased edema was measured using a digital calliper at 60, 180 and 300 min 1, 3 and 5 hrs after carrageenan injection.

The importance of oedema was assessed by determining the mean percentage increase (% INC) of the diameter of the rat paw according to formula[19]:

$$INC\ (\%) = \frac{Dt - D0}{D0} \times 100$$

Where:

Dt = Paw diameter at t time
D0 = Initial paw diameter

Analgesic activity: The writhing test in mice was used[20]. Contortions were induced by intraperitoneal injection of 3% acetic acid. Animals were divided into 3 groups of 3 mice each. They then fasted 12 hrs before tests.

Mice were treated with the following solutions:

- Group 1 (control): Normal saline (10 mL kg^{-1}, per os)
- Group 2 (reference): Acetyl salicylic acid (ASA) (30 mg kg^{-1}, per os)
- Group 3 (treated): HMEANP (30 mg kg^{-1}, per os)

Intraperitoneal injection of 3% acetic acid solution was performed 1 hr after gavage. The pain sensitivity was evaluated by the contortions number counted 30 min after latency time.

Statistical analysis: All data were expressed as Mean±Standard Error of the Mean (SEM) and analyzed by GraphPad 6.0 software. The significance was evaluated using a One-way Analysis of Variance (ANOVA) followed by Dunnett's *post hoc* Test compared with the control group. Values of $p<0.05$ were considered significantly different. The n is number of animals in each group.

RESULTS

Phytochemical characterizations: The phytochemical study showed the presence of polyphenols, tannins, flavonoids, saponosides, alkaloids, sterols and triterpenes were not present (Table 4).

Healing activity: The induction of the experimental burn according to the method described above causes a deep second-degree burn. The compared histological sections of normal skin (control) and after induction of the burn were shown in Fig. 1a-b. The normal skin of the control group consists of a thin epidermis, formed by 3-4 layers of cells surmounted by keratin blades. The dermis is composed of

Table 4: Phytochemical constituents in different HMEANP

Groups	HMEANP
Polyphenols	+
Condensed tannins	+
Hydrolyzable tannins	+
Flavonoids	+
Saponosides	+ (Im = 100)
Alkaloids	-
Sterols and triterpenes	-

+: Presence and -: Absence

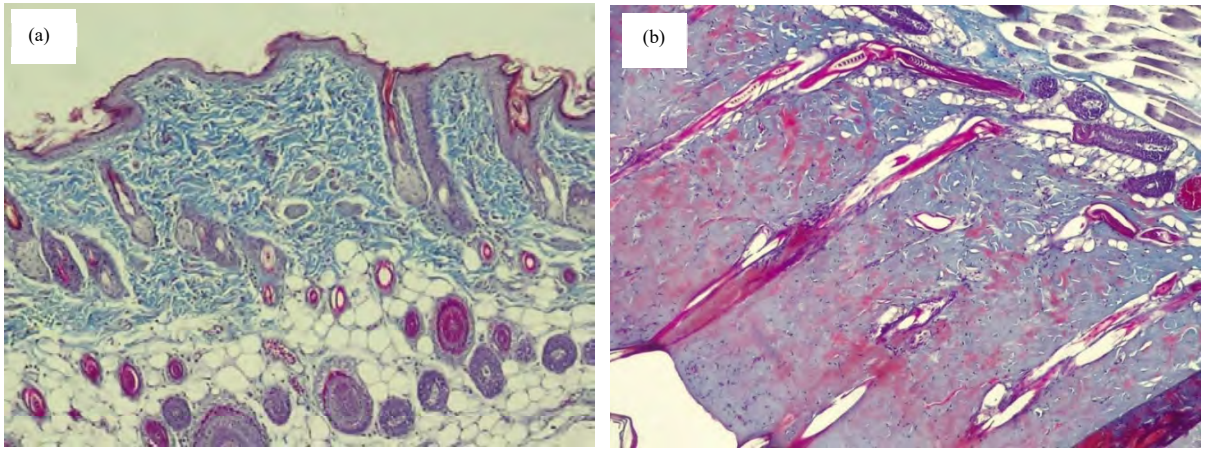

Fig. 1(a-b): Histological section of normal and burned skin at Day 0, (a) Normal skin of the control group, the dermis is composed of loose connective tissue with numerous sebaceous glands and hair follicles and (b) Histological examination shows the destruction of the epidermal basal layer with damage to the deep dermis of the burn site: 2e deep degree
Masson's Trichrome Staining and GX100

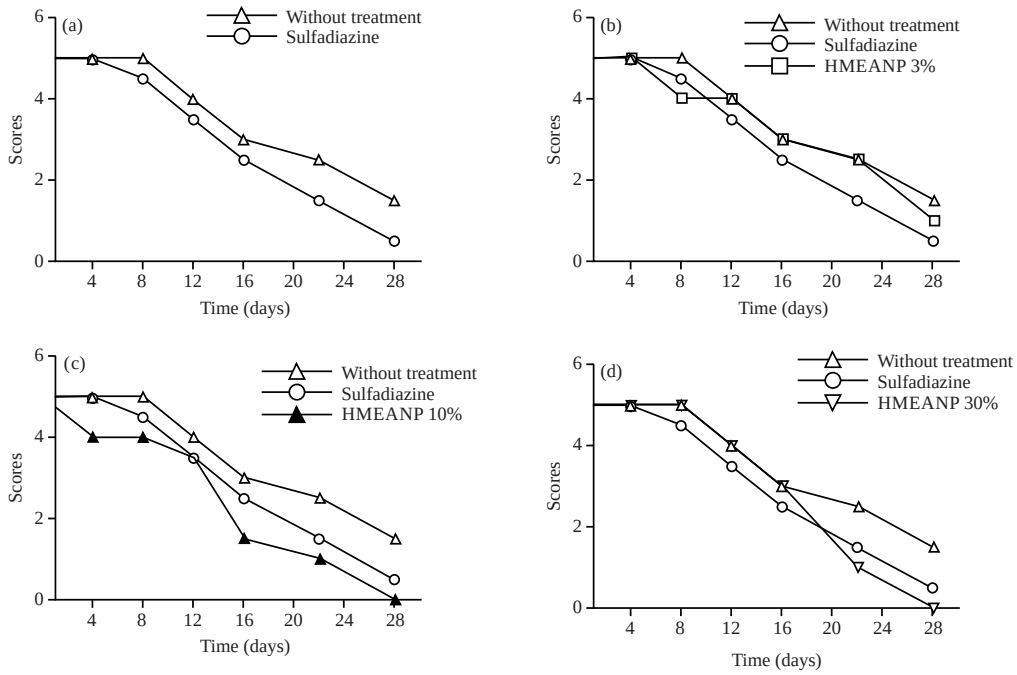

Fig. 2(a-d): Healing effect of HMEANP experimental deep second-degree burns, (a) Without treatment and treated with reference ointment (sulfadiazine), (b) Treated with HMEANP 3%, (c) Treated with HMEANP 10% and (d) Treated with HMEANP 30%

loose connective tissue with numerous sebaceous glands and hair follicles. Histological section of the burn site: 2e deep degree. One hour after the induction of the burn, the macroscopic examination of the burn shows a rounded lesion of about 3 cm in size, the background is yellowish-white. The phanera are not adherent. There is no dermal bleeding on the wound. Histological examination shows the destruction of the epidermal basal layer with damage to the deep dermis, indicating a deep second-degree burn. Vascular congestion was also noted.

Evolution of experimental deep second-degree burn scores in rats without treatment: Up to eight days after induction of the experimental burn, the burnt part is still covered with necrotic skin, corresponding to a score equal to 5. Ulceration and oozing are observed from the 12th-22nd day after induction. The scores are 4 and 3. The skin begins to reconstitute on the 26th day with a reduction of the lesion (score 2). Healing is not complete after 28 days of treatment (Fig. 2a).

Evolution of experimental deep second-degree burn scores in rats after daily application of sulfadiazine: The burn of rats treated with sulfadiazine develops substantially identically to that of untreated rats except that here healing is almost complete after 22 days of treatment (Fig. 2a).

Scores evolution of experimental deep second-degree burns after treatment with HMEANP-based ointment: Daily application of HMEANP 3% ointment in petroleum jelly was not associated with rapid healing. The evolution of the scores was similar to that of the untreated control group. Indeed, after 22 days of application, the wound was still oozing with a score of 3. Healing was not complete until after 28 days of treatment, with a score of 1, which was higher than that obtained with the reference lot that received the sulfadiazine (Fig. 2b).

The evolution of the scores in the rats treated with the 10% ointment is better. Daily application of 10% HMEANP resulted in almost complete healing after 16 days of treatment. The average score was 1.5 (Fig. 2c).

Application of HMEANP 30% ointment is associated with complete healing after 22 days. The average score was 1 (Fig. 2d).

Epithelialization time of experimental deep second-degree burn wounds in rats: Wound epithelialization time is better in the group treated with HMEANP 10% ointment. Indeed, it is

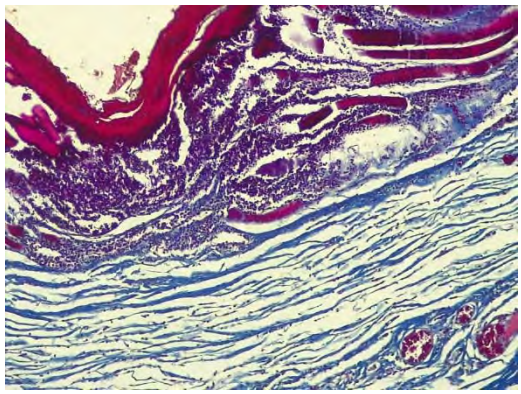

Fig. 3: Histological appearance of the burn site in the group of rats treated with HMEANP 10% ointment on day 4
Masson's Trichrome Staining and GX100

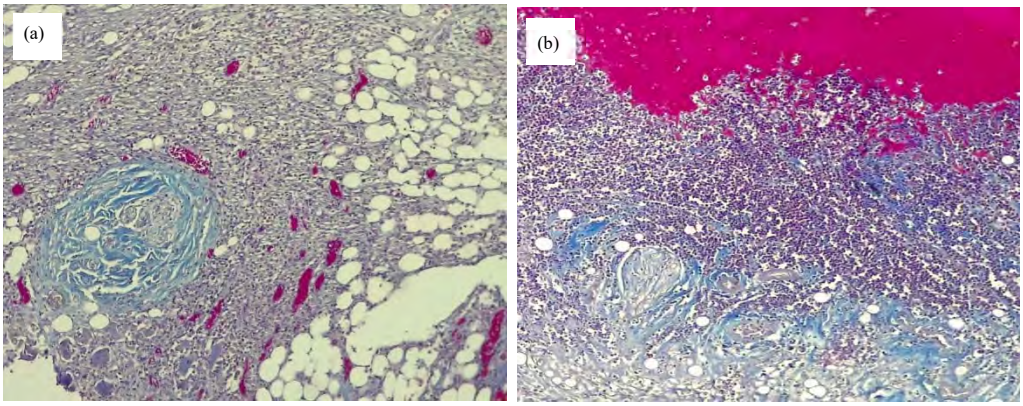

Fig. 4(a-b): Histological section of the burn site in the rat group treated on day 12, (a) HMEANP 10% ointment and (b) HMEANP 3% ointment
Masson's Trichrome Staining and GX100

16 days against 22 days in the batches treated with 30% ointment and sulfadiazine. On the other hand, it is only observed at 28 days in the untreated batches having received the 3% ointment.

Histological evolution of experimental deep second-degree burns after treatment with ointments: On day 4 after induction histological examination shows an edematous congestive dermis and presents a diffuse lymphoplasmacytic and polynuclear infiltrate extending to the panniculus carnosus. This infiltrate was intense in the untreated control subjects, the sulfadiazine-treated references and in the group treated with HMEANP 3 and 10% ointment, whereas it is moderate in the group treated with HMEANP 30% ointment. The histological appearance of the burn site in the group of rats treated with HMEANP 10% ointment was shown in Fig. 3.

On day 12, we observed the constitution of a fleshy bud in the group treated with HMEANP 10% ointment and the references treated with sulfadiazine. The fleshy bud comprises a loose extracellular matrix in which granulation tissue leukocytes, fibroblasts, myofibroblasts and blood neo-vessels are observed. The fleshy bud of the group treated with HMEANP 10% ointment was richer in collagenous fibres (Fig. 4a). Inflammation was persistent in the other rat groups. Fleshy bud formation was delayed (Fig. 4b).

On day 21, the healing was almost complete in the HMEANP 10% group. The beginning of epithelial regeneration was observed, the dermis is dense in collagen and the pilosebaceous and sweat annexes are absent (Fig. 5a, b).

The healing progress of the burns in rats treated with HMEANP-based ointment was resumed in Fig. 6a1-e7. Re-epithelialization is complete on day 16 in the group treated with HMEANP 10% (Part D5: Fig. 6), unlike the other groups where the wounds are still oozing.

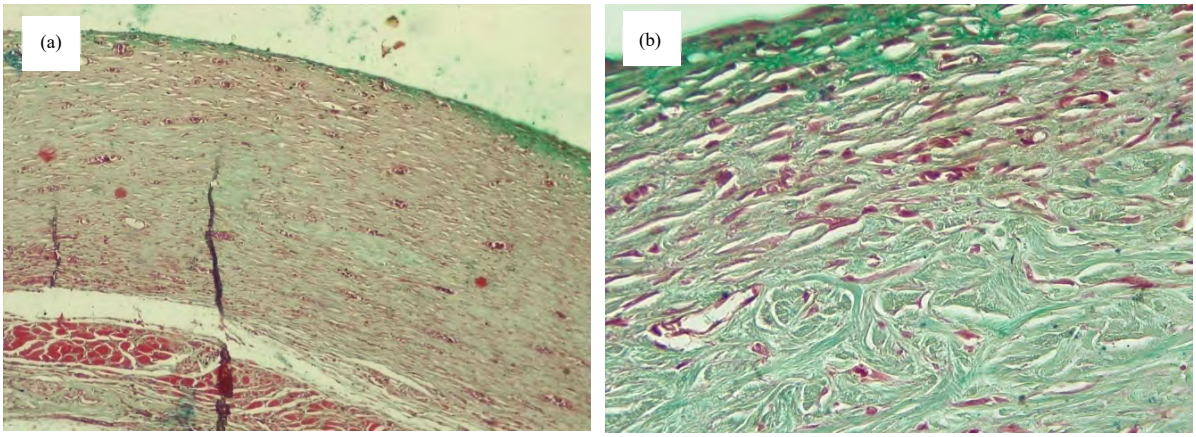

Fig. 5(a-b): Histological section of the burn site in the rat group treated on day 21, (a) HMEANP 3% ointment and (b) HMEANP 10% ointment

Masson's Trichrome Staining and GX400

Table 5: Percentage increase of edema in the different batches on carrageenan-induced paw model in rats

		Increased rat paw edema		
Treated groups	Dose	1 hr	3 hrs	5 hrs
Control	10 mL kg^{-1}	41.16±3.8	62.39±8.52	84.65±8.83
ASA	30 mg kg^{-1}	14.70±3.08**	36.13±3.52	33.88±5.81**
HMEANP	10 mg kg^{-1}	32.02±6.78	74.34±19.99	66.51±15.50
	30 mg kg^{-1}	14.81±3.33**	18.73±5.17*	22.25±7.98***
	100 mg kg^{-1}	33.09±4.69	29.48±6.12	33.15±7.38**

*p<0.05, **p<0.01, ***p<0.001 vs. control group, ASA: Acetylsalicylic acid, HMEANP: Hydro-methanolic extract of *A. nilotica* pods

Anti-inflammatory activity

Induction of rat paw inflammatory edema in the control group: Carrageenan 1% in rat paw after pre-treatment with normal saline-induced edema. The significant increase of rat paws was 41.16±3.8, 62.39±8.52 and 84.65±8.83%, respectively at 1, 3 and 5 hrs after carrageenan administration. The percentage increase in edema of the different batches was summarized in Table 5 (p<0.05 vs. baseline, n = 5).

Effect of HMEANP: Oral administration of HMEANP at doses of 10, 30 and 100 mg kg^{-1} significantly prevents inflammatory edema. Prevention of edema is better at a dose of 30 mg kg^{-1}. Indeed, the percentages of increase in edema are 14.81±3.33, 18.73±5.17 and 22.25±7.98, respectively at 1, 3 and 5 hrs after carrageenan administration (p<0.05 vs. baseline, n = 5). ASA is used as a reference molecule (Fig. 7).

Anti-inflammatory activity was evaluated by the carrageenan-induced rat paw edema method. The increase in edema was assessed at doses of 30 and 100 mg kg^{-1}, compared to the control group. ASA was used as a reference molecule. The *p<0.05, **p<0.01, ***p<0.001 vs. control group, n = 5.

Analgesic activity of acetylsalicylic (ASA), HMEANP on contortions induced with acetic acid 1% in mice: The administration of ASA (30 mg kg^{-1}, per os) significantly prevented the occurrence of contortions in mice. The number of contortions is 30.50±4.06. The HMEANP significantly prevented contortions induced by intraperitoneal administration of 3 % acetic acid in mice. The analgesic effect of HMEANP (30 mg kg^{-1}, per os) was similar to that observed with ASA, there was no significant difference between the 2 groups. The number of contortions after HMEANP administration was 40±8.39 (Fig. 8).

DISCUSSION

Acacia nilotica is a plant widely known to traditional medicine practitioners around the world, who use all of its parts as a remedy for the management of many diseases[21,22].

The present study aims to evaluate the healing, anti-inflammatory and analgesic activities of the hydro-methanolic extract of the pods of this plant, in experimental models of deep second-degree burns and inflammation in rats as well as pain in mice.

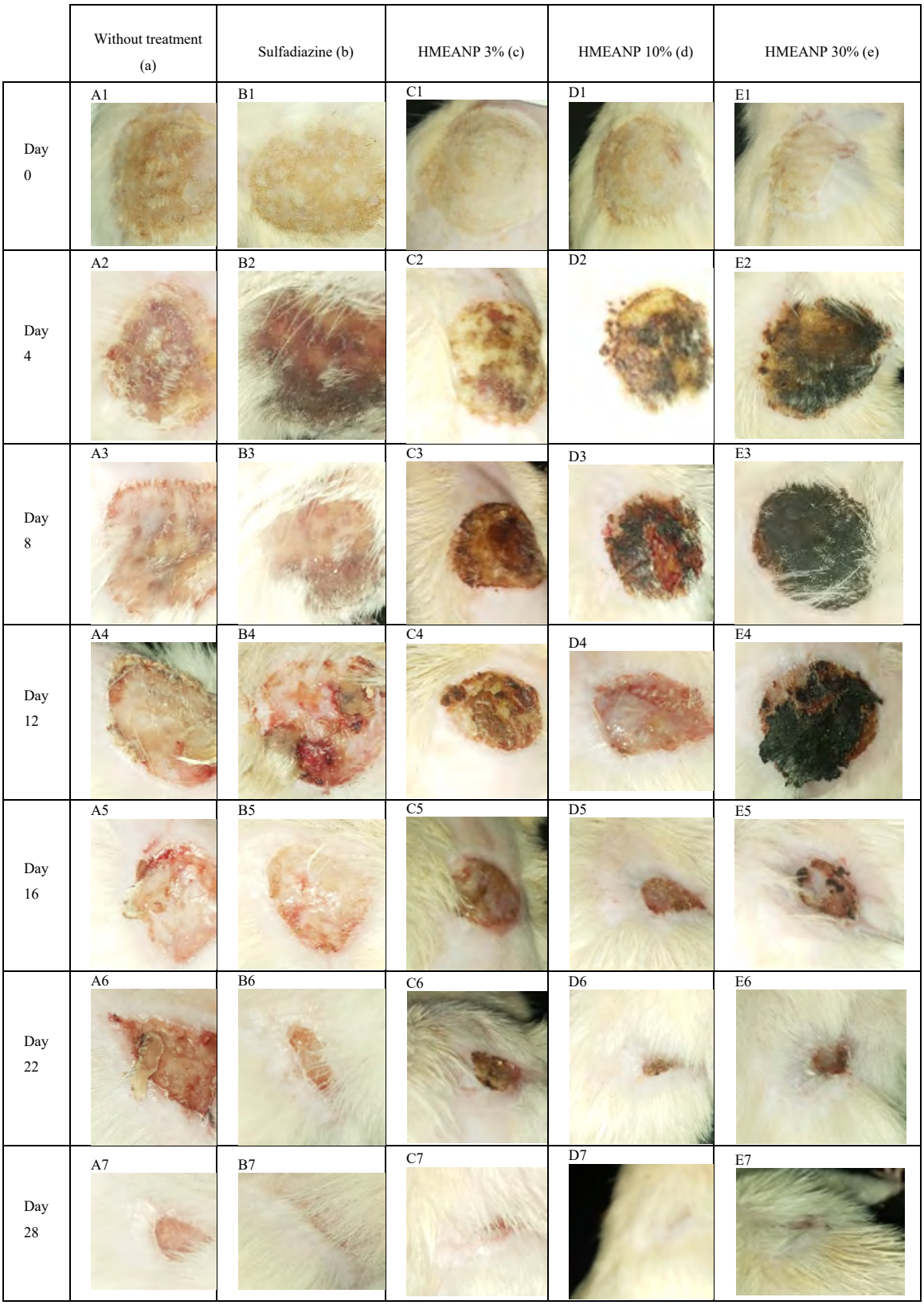

Fig. 6(a1-e7): Healing activity of HMEANP, images show concentration-dependent healing between 3 and 10% with complete healing after 16 days of treatment

An oozing wound is observed in the untreated control group (day 16)

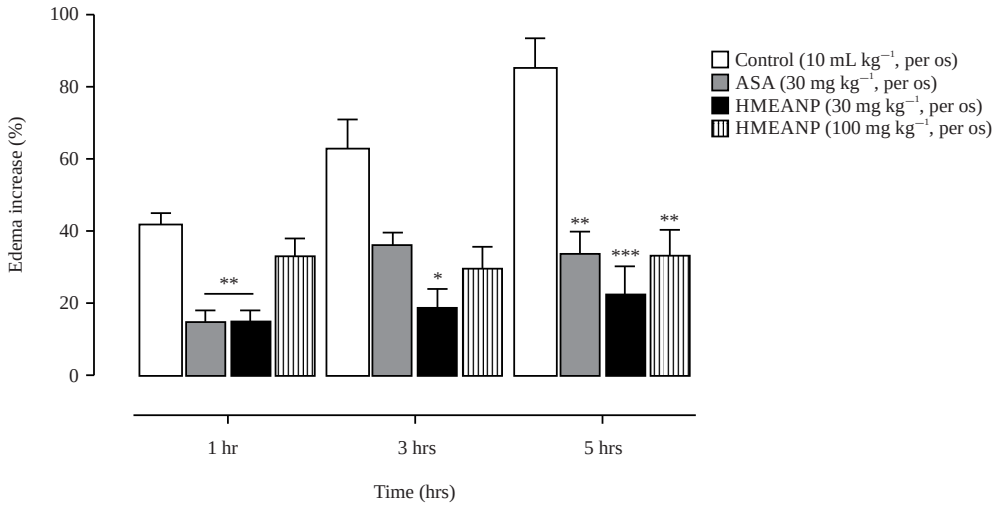

Fig. 7: Effect of HMEANP on carrageenan-induced inflammatory edema in rats

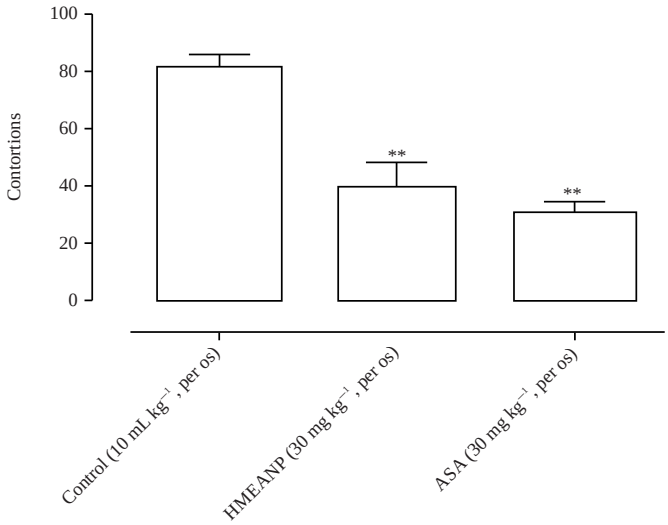

Fig. 8: Effect of HMEANP on contortions acetic acid-induced with acetic acid 3% in mice

The results showed that the absence of treatment is not associated with complete healing 28 days after burn induction. Also, HMEANP has a concentration-dependent healing activity between 3 and 10%, in the experimental deep second-degree burn model in the Wistar rat.

Ethnobotanical data have attributed healing effects to plants of the *Mimosaceae* family. A decoction of the root or bark of *Entada africana* is used by healers in Mali to wash wounds and as a healer[23]. The Malayali tribes of India use the stem and bark of *Albizzia lebbeck* (Linn.) Willd., the whole plant of *Mimosa pudica* (Linn.) to heal wounds[24].

Ethnopharmacological surveys carried out among the diola people in the south of Senegal have reported the use of plants of the *Mimosaceae* family in the treatment of wounds and burns. Indeed, the ashes of the new leaves and the powder of the dried peduncle of *Parkia biglobosa* are used for the treatment of burns[25]. The study by Diatta *et al.*[26] in the department of Tivaouane (East of Senegal) revealed that the plant most used for wound healing is *Acacia nilotica*.

Previous work has demonstrated the healing activity of species of the genus *Acacia*. Indeed, the ethanolic extract of *Acacia caesia* bark has healing activity on excision and incision wound models[27]. In the same models, ethanolic and aqueous extracts of *Acacia suma* Roxb leaves and *Acacia auriculiformis* A. Cunn stem bark is also healing[28,29].

The methanolic extracts of the leaves and aqueous extracts of the pods of *A. nilotica* showed healing activity in

the rat excision wound model. On the burn wound model, the hydro-ethanolic extract of the pods is healing[30,31].

Healing process includes three phases: Inflammatory, proliferative and remodelling. The inflammatory phase is associated with hemostasis, production of inflammatory factors, fibrin deposition and migration of monocytes, neutrophils and lymphocytes. A proliferative phase follows during which the formation of new blood vessels (angiogenesis), the precipitation of collagen, the creation of granulation tissue, epithelialization, contraction of the wound and finally remodelling occur[1].

The results of the present study in the deep second-degree burn wound model showed that HMEANP promotes complete healing (score 1.5) with re-epithelialization after 16 days of treatment with the 10% ointment. In the animals treated with sulfadiazine and the untreated control batch, the same score is obtained at 22 and 28 days, respectively as well as re-epithelialization.

The early stage of inflammation is an important phase in the wound healing process. It is indeed essential to fight against the surrounding bacteria and to create an environment conducive to healing[32]. However, when it is maintained, especially in chronic wounds, such as diabetic wounds, it becomes harmful. The introduction of anti-inflammatory agents or healing products with anti-inflammatory activity in wound management could be a beneficial strategy to improve wound healing[32,33].

Several works have shown the action of the phytochemical components highlighted in this present study in the healing mechanisms of wounds and burns in different animal models. Thus, flavonoids reduce inflammation and increase angiogenesis, re-epithelialization and keratinocyte migration[34]. Polyphenolic compounds like tannins are astringent and promote wound healing by chelating free radicals, contracting damaged tissues and increasing the formation of capillaries and fibroblasts[35,36]. Saponosides can enhance the synthesis of pro-collagens[37].

The main limitation of this study could be the absence of tests on a possible anti-infectious activity of the extract. Indeed, it could accelerate or facilitate the healing process.

CONCLUSION

The results of the present study justify the traditional use of *Acacia nilotica* pods in the treatment of wounds and burns. The continuation of this study should be directed towards a finer characterization of the healing properties of the phytochemicals present in the hydro-methanolic extract. Thus, a confirmation of the healing activity would open perspectives toward the evaluation of the interest of these extracts in the healing of gastroduodenal ulcerations and diabetic foot wounds.

SIGNIFICANCE STATEMENT

The present research aimed to evaluate the healing, anti-inflammatory and analgesic activities of hydro-methanolic extract of *Acacia nilotica* pods (*Mimosaceae*). Hydro-methanolic extract of *Acacia nilotica* pods has a concentration-dependent healing activity between 3 and 10%, in the experimental deep second-degree burn model in the Wistar rat. Hydro-methanolic extract of *Acacia nilotica* pods promotes complete healing in the deep second-degree burn wound model with re-epithelialization, angiogenesis and collagen production after 16 days of treatment with the 10% ointment. Healing products with anti-inflammatory activity in wound management could be a beneficial strategy to improve wound healing in the burn wound model.

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