European Journal of Biology and Medical Science Research
Vol.10, No.2, pp.45-56, 2022
Print ISSN: ISSN 2053-406X,
Online ISSN: ISSN 2053-4078

STUDY OF THE ASTRINGENT ACTIVITY OF THE AQUEOUS EXTRACT OF *TERMINALIA CATAPPA* (COMBRETACEAE) LEAVES IN MAMMALS

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Citation : OUATTARA-SORO Fatou Shcherazade, TEI Emmanuel Kouassi Éric, ABIZI Georges, KOUADIO Kouakou John, YAO Konan Bertin, THANON Mariam (2022) Study of the Astringent Activity of the Aqueous Extract of Terminalia Catappa (Combretaceae) Leaves in Mammals, European Journal of Biology and Medical Science Research, Vol.10, No.2, pp.45-57

ABSTRACT: *Terminalia catappa (Combretaceae) is a plant used in traditional medicine in the treatment of inflammatory, diabetic, bacterial pathologies as well as skin infections. This study aimed to evaluate the astringent activity of the aqueous extract of Terminalia catappa leaves in rats. The phytochemical study revealed flavonoids, polyphenols, saponosides, sterols and polyterpenes and the absence of tannins and quinone substances. The healing activity of this extract was evaluated after experimentally inducing wounds on rats. The healing effect of Terminalia catappa was compared to natural healing and artificial healing by the action of cicatryl, a pharmaceutical reference ointment.Thus, during this study, the rats treated with the different ointments of T. catappa at doses of 250; 500 and 1000 mg/kg of P.C, saw their wounds healed during eighteen days of treatment against 22 days of treatment for the control rats and 20 to 21 days of treatment for the rats treated with cicatryl, pharmaceutical ointment of reference. The results of this study show the impotence of the use of T. catappa leaves in the healing of skin wounds in traditional environment.*

KEY WORDS: *Terminalia catappa*, astringent activity, wound, healing

INTRODUCTION

The injury of a given organ, leaves a wound that usually leads to an alteration of its function and promotes the penetration of pathogens into the body, source of various diseases (Bacar., 2018). These wounds, are further classified according to the following parameters: size, location, tissue damage caused, as well as their level of contamination (Johnston., 1990). According to this classification, nearly 6 million people suffer from chronic wounds and about 12 million patients are affected by acute wounds worldwide (Perrier, 2012).

Depending on the severity of the wound, healing can be achieved through the mechanism of natural or artificial healing. Indeed, the natural biological process of healing occurs without external input of drugs through the process of tissue regeneration which is a mechanism unique to living organisms (Djoko *et al.*,2019).

Concerning artificial healing, traditional medicine, often underestimated by modern health care, is increasingly used by populations. This medicine exists in almost every country in the world and the demand for services in this area is increasing (WHO, 2013).

The populations have more recourse to medicinal plants because of the high cost of certain pharmaceutical products and also because of their socio-cultural habits (Duke *et al.*, 1993). Thus, medicinal plants are of great socio-economic importance in most African countries because they contain active components used in the treatment of various common diseases such as diabetes, hypertension, malaria, diarrhea etc. However, there are also reports in the literature on wound healing studies using medicinal plants (Logeeswari *et al.*, 2012). Thus, the wound healing properties of several medicinal plants have been highlighted among which we have *Terminalia catappa* (Combretaceae) known as badamier (Khan *et al.*, 2014).

Terminalia catappa (Combretaceae), belongs to the Combretaceae family and is widely used in traditional medicine. This plant has several therapeutic properties among which are cited anti-inflammatory, hepato-protective, antimicrobial and wound healing properties (Khan *et al.*, 2014).

The aim of this study was to evaluate the healing properties of the aqueous extract of *T. catappa* leaves of wounds caused from excision of the skin of Wistar strain rat in the dorsal region.

MATERIAL AND METHODS

Biological and technical material

Plant material

As plant material, leaves of *Terminalia catappa* (Combrétaceaes) were used. They were collected in Abidjan in September 2020, identified and authenticated at the Centre National Floristique (CNF) of the Université Felix Houphouët-Boigny of Cocody under the herbarium specimen No UCJ003136. These leaves were dried at room temperature for 3 weeks, then pulverized, and used for aqueous extraction.

Animal material

Male and female laboratory wistar rats of body weight between 137 and 250 g were used for the different tests. Six nulliparous, non-pregnant females were used for the acute toxicity evaluation and twenty for the astringent activity study in accordance with the Organisation for Economic Co-operation and Development guidelines 402 and 423. The animals were raised in the vivarium of the École Normale Supérieure (ENS). All animals were treated and handled according to the standards of the manuals on the care and use of experimental animals. Their diet consisted of pellets and dried bread. They had access to tap water contained in bottles. The rearing environment was characterized by a photoperiod and a natural temperature suitable for the animals' well-being. They were placed in polypropylene cages with a wire mesh roof and a bedding made of wood shavings on the bottom. These cages were regularly cleaned with a daily renewal of the litter.

Chemical material

As chemical material, total aqueous extract of *T. catappa* leaves was used for the experimental study, ethanol as disinfectant, Thiopental and Formol 10% for anesthesia. An antiseptic and the

pharmaceutical ointment Cicatryl 2g known as a drug that accelerates wound healing were also used. Distilled water was used as a solvent.

Methods

Extraction

The leaves of *T. catappa* were carefully cleaned and then dried in the shade at room temperature, i.e. out of the sun. The dried leaves were ground with an electric mill of the brand IKA A 10 Laboortechnik (Germany) in order to obtain a powder. Fifty grams (50g) of this powder was added to one liter (1L) of distilled water. The resulting mixture was blended with a blender and filtered twice on clean poplin cloth and five times on absorbent cotton. The filtrate obtained was placed in the Memmert (Germany) oven at 50°C for forty-eight hours (48h).

Phytochemical screening

A qualitative phytochemical screening was performed on the powder samples obtained, following extraction with an aqueous solvent. The analytical techniques described in the work of **Békro *et al* (2007)** were used to highlight the relatively abundant presence of chemical compounds such as flavonoids, polyphenols, tannins, alkaloids, sterols and polyterpene.

Formulation of the ointment

The ointment was formulated from the powder of *T. catappa*, obtained after evaporation of the aqueous extract in the oven. To the different quantities of powder namely 250; 500 and 1000 mg; 10 g of shea butter were added. The resulting mixture was homogenized with a pastula until a paste was obtained that was free of granules and easily applied to the wounds. Doses of 250, 500 and 1000 mg/kg were randomly selected to monitor the progress of wound healing.

Toxicological studies

Acute oral toxicity

The acute toxicity of the plant was carried out with the extract at the dose of 2000 mg/kg w.b. on rats. The rats were fasted overnight prior to the experiment. Food intake was suppressed and water intake was maintained for 3 to 4 hours. Before and after the fasting period, the animals were weighed, marked and divided into two batches of three (3) rats each. The substances distilled water or aqueous extract of *Terminalia catappa* was administered to them using a gavage cannula as follows:

- batch 1 control received 1 ml/kg bw of distilled water;

- treated batch 2 received the aqueous extract at 2000 mg/kg bw.

Behavioural observation was carried out regularly, 30 min, 4 h and every 24 h over a period of 14 days, after administration of distilled water and the aqueous extract of *Terminalia catappa* leaves. Thus, during the 14-day period, the animals were observed for signs of toxicity, including changes in coat, motility, tremors, body mass, grooming, respiration, stool appearance, and mobility (OECD 423, 2001).

Acute skin toxicity

Prior to the application of *Terminalia catappa* paste extract to the skin, the dorsal thoracic region of the animals was shaved under Thiopental anesthesia (OECD 402, 2017). The anesthetized animal, was placed prone on the table and held in this position with tape from the tips of the fore and hind limbs.

After application, the same observations made in the acute oral toxicity study were made.

Induced wounds and wound care

The animal's back was shaved and the skin disinfected with 70% ethyl alcohol. At the shaved area, a 2 cm diameter circle was made as well as skin excisions using a pair of scissors and forceps (Oryan *et al.*, 2010). The induced wounds were cleaned with a sterile compress soaked in 70% ethyl alcohol. During the experiment, the wounds were not protected with a dressing.

Every other day for eighteen days at the same time, a water-soaked compress was placed on the wound to soften the scab, then removed and the wound was dried with blotting paper. The three different concentrations of the paste extract namely 250mg/kg, 500 mg/kg and 1000 mg/kg were applied by gentle circular massage to the wounds of the rats divided into different batches. These applications were made every other day until the wounds were completely healed and the scores were evaluated every day for 24 days.

Histological studies

From the 16th day of experimentation, using forceps and scissors, a skin flap covering the entire wound surface was removed from each treated rat. The specimen was then immersed in 10% formalin until histological analysis. All jars containing the flaps were identified according to rat batch and type of treatment.

The histological study consisted in highlighting the different histological sections showing the evolution of the healing process in the control, *T. catappa* aqueous leaf extract and cicatryl treated batches.

Data analysis

The comparison of means between the weights of control and treated rats was performed by student's t-test. The means of the areas, the percentages of contractions as well as their standard deviations were calculated and analyzed using One way ANOVA in GraphPad prism 5.03 software.

Statistical significance was determined by analysis of variance (Anova). A value of $p < 0.05$ was considered the threshold for significance.

RESULTS AND DISCUSSION

Results

Phytochemical Screening

The results of the phytochemical analysis allowed to highlight the presence or absence of some groups of compounds of therapeutic interest. The presence of chemical compounds such as

sterols, polyphenols, flavonoids, alkaloids and saponosides was relatively abundant. A negligible amount of tannins and quinone substances were observed.

Acute oral and dermal toxicity

Oral administration and dermal application of 2000 mg/kg w.b. of T. catappa E.T.A. to animals did not result in pathological changes or death. During the 14 days of experimentation, the follow-up of the weight evolution showed the presence of a slight increase in weight in the animals treated with the dose 2000 mg/kg of w.b. of the aqueous extract of leaves of *T. catappa* thus, not significant ($p > 0,05$) compared to the control animals having received distilled water (**Azame et al., 2020**). Thus, at this same dose, no significant variations in food intake were observed.

Cicatrisation

Variation in wound surfaces

In general, a reduction in wound area was observed. These areas all decreased over time for both the control rats and the rats treated with the different *T. catappa* ointments at the doses of 250, 500 and 1000 mg/kg wb. The wound area for rats treated with cicatryl also decreased (Figure 1).

Variation of the percentage of contraction

Figure 2 shows a gradual increase in the percentage of contraction until total wound healing for both control rats and rats treated with the different *T. catappa* ointments at doses of 250; 500 and 1000 mg/kg w.b. The percentage of wound contraction for rats treated with cicatryl also increased gradually. The macroscopic evolution of wound healing was also observed (Figure 3).

Re-epithelialization of the skin

The process of re-epithelialization of the skin during the healing mechanism was observed from different histological sections (figures 4 and 5). During these histological observations, it was noted an absence of tissue necrosis in all the rats treated with the different ointments of *T. catappa* at the doses of 250; 500 and 1000 mg/kg wb.

Concerning the control rats, from the 10th to the 18th day of the healing process, it was observed an absence of epithelialization and thus an incomplete healing (Figure 4). The same result was observed in rats treated with Cicatryl, the reference pharmaceutical ointment.

All the rats treated with the different *T. catappa* ointments at doses of 250, 500 and 1000 mg/kg wb. showed a rapid evolution of the healing process. Histological sections taken on the 10th day in the rats treated with the 250 mg/kg *T. catappa* ointment revealed the presence of epidermal cells and the beginning of epithelialization. On the 18th day, a very advanced epithelialization with the formation of hair follicles as well as a migration of fibroblasts were observed (Figure 5). Concerning the rats treated with the 500 mg/kg *T. catappa* ointment, the sections showed epidermal cells and very advanced epithelialization at day 10. Strong epithelialization with the presence of more fibroblasts and hair follicles were observed on day 18 (Figure 5). For rats treated with 1000 mg/kg *T. catappa* ointment, histological sections taken on the 10th day of treatment also revealed the presence of epidermal cells with very advanced

epithelialization. At the 18th day, epithelialization was highly elaborated with a large number of fibroblasts and hair follicles (Figure 5).

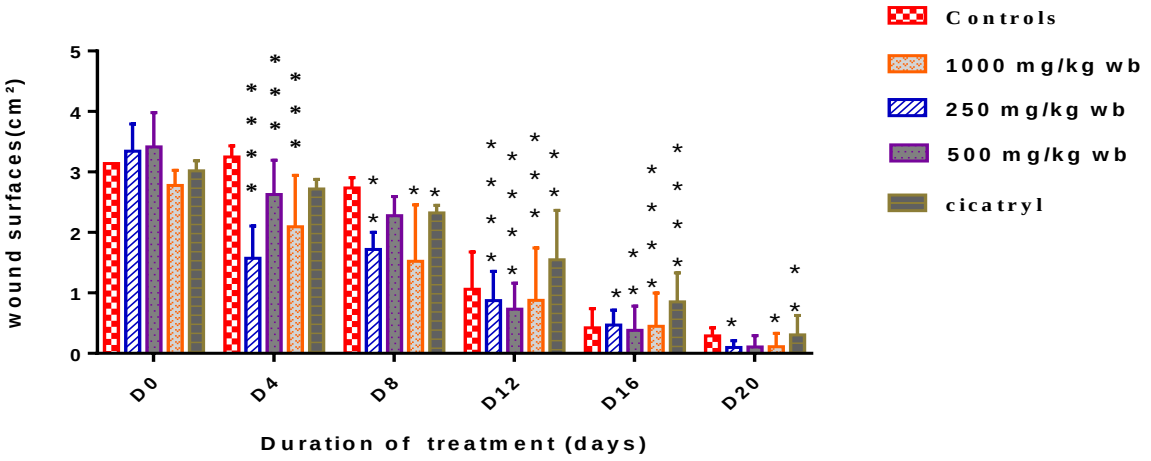

Figure 1: Retraction of skin wound surfaces as a function of time.

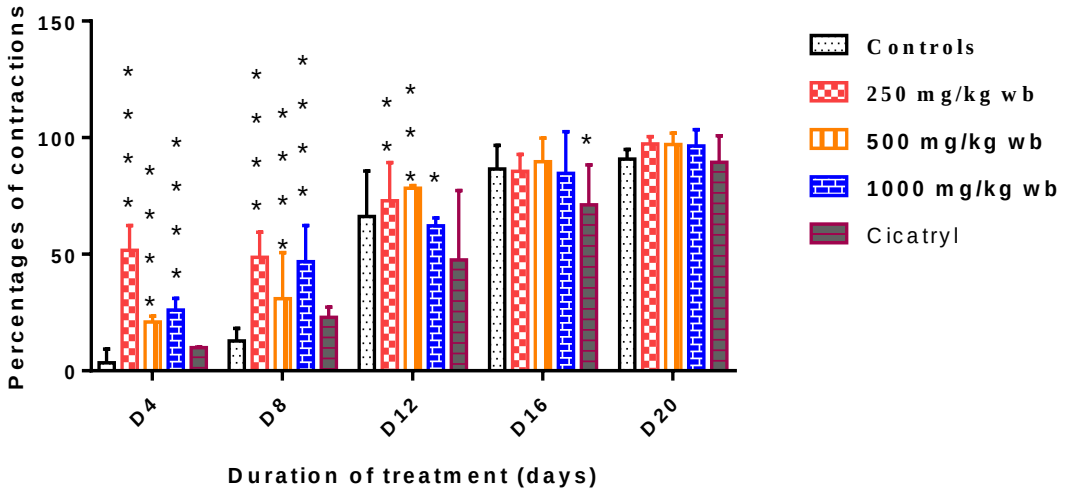

Figure 2: Percentage of skin wound contraction as a function of time

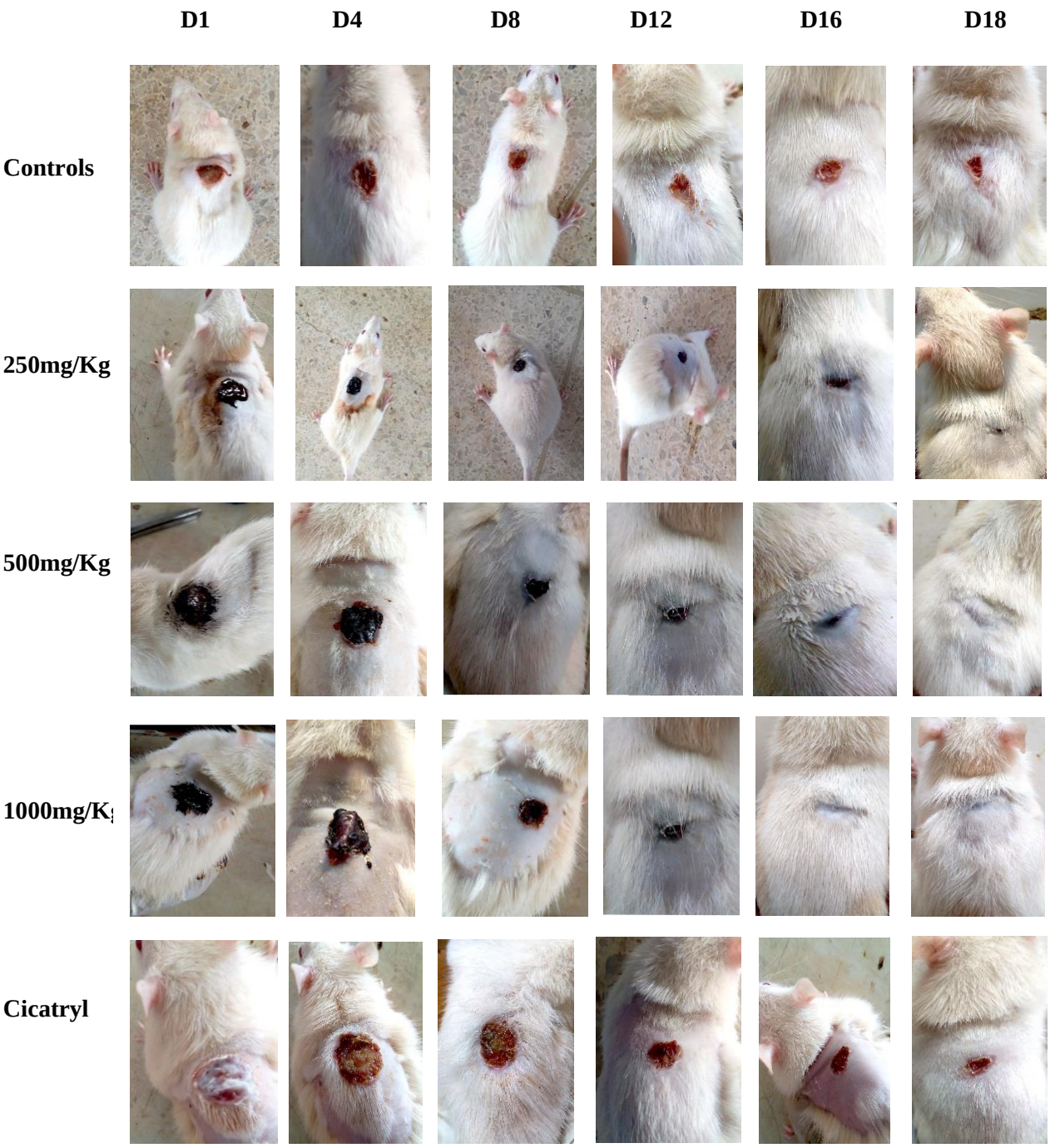

Figure 3: Macroscopic observation of the healing as a function of the dose of *T. catappa* applied to the wounds induced

Legend: D1- D18 = Day 1 of treatment to Day 18 of treatment

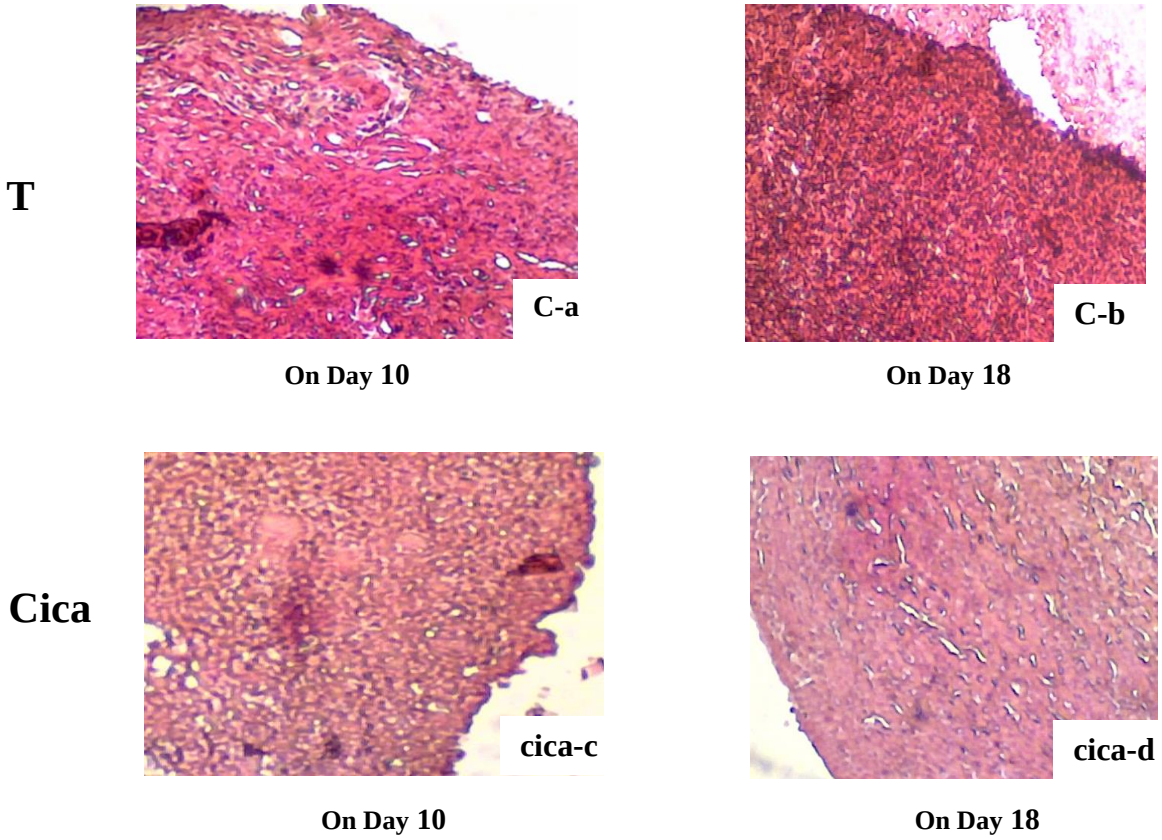

Figure 4: Histological sections showing the evolution of the healing process (control **(T)** and treated with cicatryl **(Cica),** pharmaceutical reference ointment)

Legend: C-a = absence of epithelialization; **C-b** = absence of epithelialization; **cica-c** = absence of epithelialization; **cica-d** = absence of epithelialization

Magnification x 100.

Staining: hematoxylin-eosin

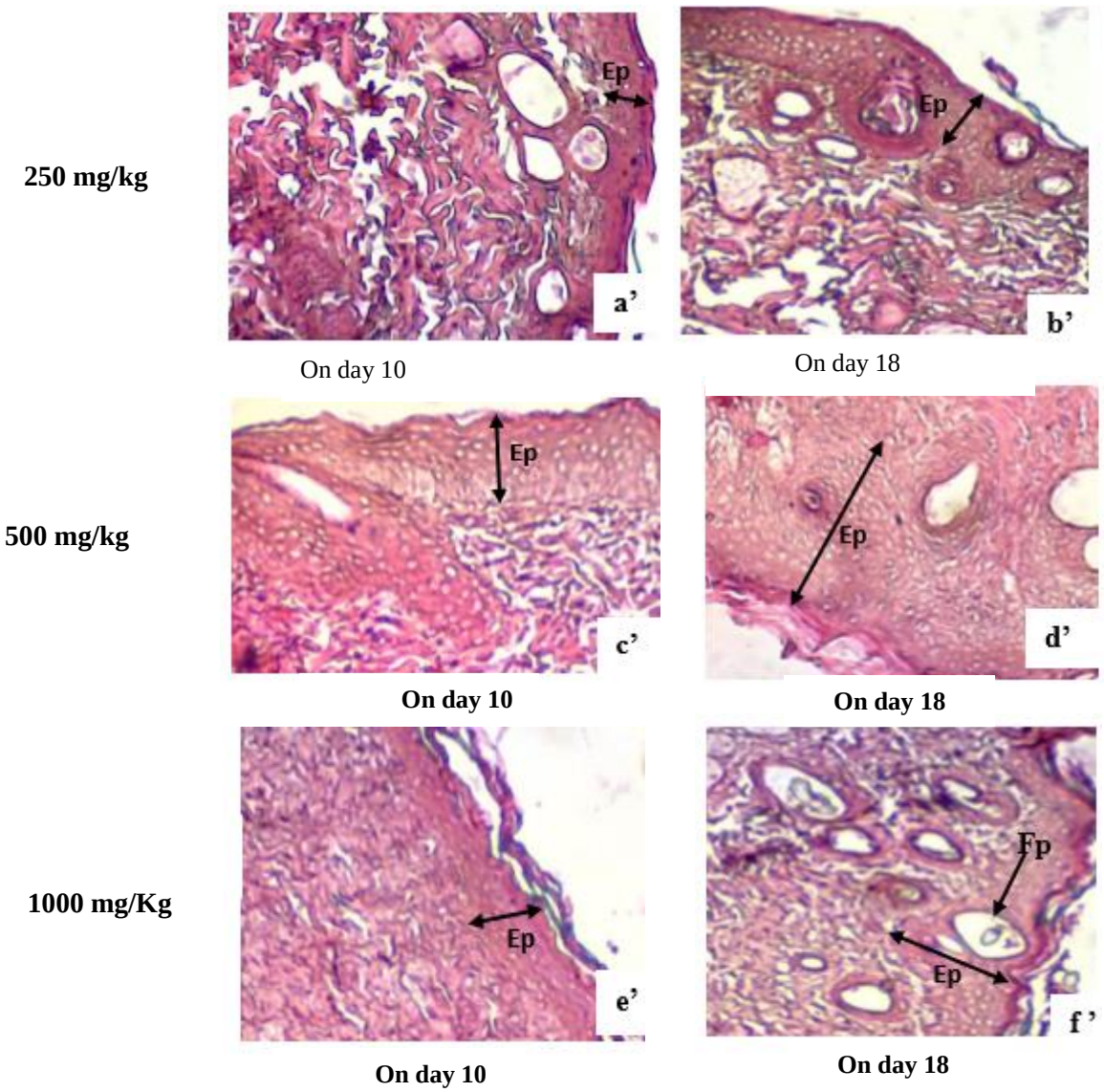

Figure 5: Histological sections showing the evolution of the wound healing process in rats treated with the total aqueous extract of *T. catappa.*

Legend: 250 mg/kg-a': early epithelialization; **250 mg/kg-b':** slightly advanced epithelialization; **500 mg/kg-c'**: advanced epithelialization; **500 mg/kg- d'**: very advanced epithelialization beginning of hair follicle formation (Ep); **1000 mg/kg-e'**: advanced epithelialization; **1000 mg/kg-f'**: very advanced epithelialization with hair follicle formation (Fp)

Magnification x 100.

Staining: hematoxylin-eosin

DISCUSSION

The qualitative phytochemical study of the total aqueous extract of *Terminalia catappa* leaves showed that the leaves of this plant contain sterols, polyphenols, flavonoids, alkaloids and saponosides, similar result to **Ackah *et al*., (2016).**

Acute oral and dermal toxicity results in rats, showed that at the dose of 2000 mg/kg wb, *Terminalia catappa* administered orally and dermally, resulted in no mortality. No clinical signs in rats were observed in this study. This result, according to the OECD 423 guideline, would indicate that the LD50 of *Terminalia catappa* would be between 2000 and 5000 mg/kg or greater than 5000 kg/mg CP. (OECD 423, 2001). Thus, this result is in agreement with the work of **Lemba (2011)** whose study of the toxicity of the aqueous extract of Terminalia superba administered orally at the dose 2000 mg/kg bw did not result in death or behavioral changes during 14 days of experimentation. On the other hand, studies conducted by **Adeneye and Agbaje (2007),** on Cymbopogon citratus (Poaceae), revealed that the LD50 of the aqueous extract of this plant is greater than 5000 mg/kg body weight. The work of **Koné *et al*., (2009**), also showed that the LD50 of the total aqueous extract of Sacoglottis gabonensis is higher than 5000 mg/kg. The morphological parameters observed were normal for all animals.

Moreover, our results showed that the leaves of *Terminalia catappa* contain polyphenols. These polyphenols, having the property of activating several components of the immune system including complement, macrophages, B and T lymphocytes. responsible for the healing of external wounds, which gives *Terminalia catappa* a healing effect observed during this study **(Tsuchiya et al., 1996; Abadjian, 2004; Caesar, 2007; alahtavakoli *et al*., 2010)**

During this study, it is noteworthy that, the wounds of the rats treated with distilled water had a slight foul odor, due to the bacteria present in the necrotic tissues. The rats treated with the different ointments of *T. catappa* at the doses of 250; 500 and 1000 mg/kg of wb. as well as those treated with cicatryl did not present any nauseating odor. Thus, this result presents the antibacterial property of *T. catappa* according to the studies of **Khan *et al*., 2014.**

Furthermore, the observation of histological sections showed more elaborate epithelialization in *Terminalia catappa* treated rats. The amount of fibroblasts within the wounds of *Terminalia catappa*-treated rats was also more abundant, a result in agreement with that of **Tchin et al., (2014)** who showed that *T. catappa* promotes the fibroblastie, (**Tchin et al., 2014**). Thus, the increase in collagen-producing fibroblasts obsequious in this study, would promote a good quality scar.

CONCLUSIONS

The phytochemical study of the aqueous extract of *Terminalia catappa* leaves revealed the presence of polyphenols, flavonoids, saponins, sterols and polyterpenes and an absence of tannins as well as quinone substances. The study of the acute oral and cutaneous toxicity of the aqueous extract of the leaves of *Terminalia catappa* showed that this extract, administered orally and cutaneously, was not toxic with an estimated LD50 beyond 5000 mg/kg wb.

Our results showed that the aqueous extract of *Terminalia catappa* leaves has antimicrobial, anti-inflammatory and healing properties, as shown by many authors.

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