

In vivo Evaluation of the Hemostatic and Cicatrising Activities of a Gel Based on Vernonia Conferta Benth. On Post-extraction Wounds in Wistar Rats

Nokam Abena Marie Elvire^{1*}, Soppo Lobe Vanessa Charlotte², Boko Yolaine Fabienne, Mendimi Nkodo Joseph Marie³ and Nnanga Nga²

¹Department of Oral, Maxillofacial and Periodontal Surgery of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1, Cameroon.

²Department of Galenic Pharmacy and Pharmaceutical Legislation of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1, Cameroon.

³Department of Morphological Sciences Anatomy Pathology of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1, Cameroon.

***Correspondence:**

Nokam Taguemne Spouse ABENA Marie Elvire, Head of the Odontostomatology Department of the Central Hospital Yaounde, Cameroon, Tel: (237)699975202.

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ABSTRACT

Introduction: Despite the progress in oral surgical techniques, the occurrence of post operative haemorrhage can lead to delayed healing. It is with this in mind that we proposed to explore other therapeutic avenues to try to provide other solutions through traditional African medicine.

The Aim of the Study : To evaluate the haemostatic and healing activity of a Vernonia conferta-based gel on post-extractonal wounds induced in Wistar rats.

Methodology: An experimental study was conducted over a period of eight months during 2021 at the multidisciplinary laboratory of Galenic Pharmacy and Pharmaceutical Legislation of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1. A gel formulation test carried out in standard proportions for a 2% concentration such as the Elugel reference gel used as a positive control. To evaluate the haemostatic and healing activity, we made up three batches of five female wistar rats with a body mass of between 100 and 130 grams, acclimatised for three days before the experiment. This test consisted of performing an avulsion of the central incisor in the rat and then applying our active principle for 5 successive days on one of the batches and our reference gel on the other batch, while the last batch will be under observation receiving no treatment and considered as a negative control. The observation was carried out during 14 days while respecting the feeding conditions of the animals. The haemostatic activity was evaluated using a stopwatch or we took the bleeding time after tooth extraction in the constituted batches. The healing activity was evaluated by taking a tissue sample from the extraction site on days 3, 7 and 14 for histological analysis.

Results: The aqueous extract-based gel, made in standard proportions at a 2% concentration like the reference gel used as a positive control, stopped bleeding almost instantly, and clot formation occurred within 1-3 minutes after application. As for the healing activity, the batch of rats that received our test gel showed a rapid re-epithelialization observed on day 14^{eme}, testifying to the restitution of the tissue injured during avulsion.

Conclusion: The adhesion of Vernonia conferta aqueous leaf gel to the post-extraction alveolar mucosa in vivo in wistar rats has a favourable profile, as it allows faster haemostasis and accelerated healing.

Keywords

Vernonia conferta, Avulsion, Rats, Haemostatic, Healing.

Introduction

Haemorrhages can be of traumatic origin (caused by a blow, a shock, a fall, the removal of an organ; these are external haemorrhages) or of non-traumatic origin (cardiovascular, digestive, gynaecological and obstetrical causes, primary coagulation disorders, tumours, iatrogenic secondary coagulation disorders...these are internal haemorrhages) [1]. The occurrence of post-extraction haemorrhage in oral surgery leads to delayed healing, despite advances in surgical techniques [2].

After tooth extraction, a clot is formed, consisting mainly of platelets, a fibrin network, red and white blood cells and avascular cellular elements. The formation of this clot is the first stage of healing. The second stage begins around the third day after extraction and is marked initially by the appearance of a highly vascularised granulation tissue composed of endothelial cells and neo-vessels, then in a second stage by the lysis of the clot. The third stage corresponds to the progressive replacement of the granulation tissue by a scar tissue or provisional matrix visible after the 7th day. The number of residual desmodontal fibres appears to be significantly reduced and the fibres appear to be more elongated to fit into the scar tissue. The scar tissue contains neo-vessels, various types of leukocytes and collagen fibres, residual cells from the desmodont, clot, inflammatory infiltrate and marrow spaces, and mesenchymal stem cells from the neo-vascularisation, marrow spaces and the multi-volumetric potential space of the desmodont. This cell mass formed is called a scar cell infiltrate. Its maturation into a tissue with osteogenic competence is mediated by cytokines released by the extracellular matrix, endothelial cells, and the scar cell infiltrate's own cells. At the end of this phase, osteoclastic cells are recruited and lead to the resorption of the alveolar walls and any bone sequestration in the alveolus [3]. After 14 days of healing, the marginal portion of the alveolus is covered by connective tissue rich in inflammatory cells and vessels, which is partly bordered by epithelial cells. In the fourth stage, calcifications of osteoid tissue can be seen, which begin at the bottom and edges of the alveolus. However, osteoid tissue is already present between the 7th and 10th day after extraction. The mineralisation of the osteoid tissue therefore results in immature, non-functional bone tissue without trabecular architecture, which forms from the alveolar walls towards the centre of the alveolus. By day 14, the alveolus consists of provisional matrix and immature cancellous bone which corresponds to reticulated fibrous bone [3].

The traditional pharmacopoeia is rich in herbal precepts and remains the primary therapeutic option in our African context [4]. After centuries of consuming plant extracts, modern pharmacology is increasingly turning to synthetic molecules based on medicinal plants, which seem to have a better efficacy with fewer side effects, than chemical molecules synthesised in the laboratory [5].

Among the plants with great therapeutic potential we have *Vernonia conferta* Benth. is a shrub of the *Asteraceae* family, which grows in tropical forests, swampy areas, savannahs and plains [5]. This plant has antimicrobial, anti lithiasis and wound healing properties [6] and healing properties for incurable wounds [7].

In Cameroon, the *Vernonia conferta* plant is widely used by people living along the Nyong River to effectively treat Buruli ulcer. With this in mind, we attempted to explore its haemostatic and healing properties on post-extraction dental wounds. The aim of this work was first to formulate a gel with good adhesion to the oral mucosa and then to test this gel on albino wistar rats in which dental extraction was performed.

Methodology

This was an experimental study conducted from November 2020 to June 2021, at the multidisciplinary laboratory of Galenic Pharmacy and Pharmaceutical Legislation of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé1. The study population was fifteen female wistar rats, aged 40 to 60 days with 100g-150g of weight, divided into three batches. Their diets consisted of maize; soya; fish; wheat bran; palm kernel; bone; premix.

□ Method for Obtaining the Aqueous Extract

The leaves of *Vernonia conferta* were collected with a botanist in Nkolafamba in the central region, Mefou-et-Afamba department (Figure 1A). The leaves were dried (Figure 1B) in a dark and airy place for 21 days, then pulverised, before being used for the preparation of the aqueous extract. The decoction was made by mixing 500g of powder in 5L of distilled water, boiled for 15 minutes, then allowed to cool to room temperature before filtering. The procedure was repeated three times. The filtrate was placed in an oven to dry for 10 days.

□ Phytochemical Screening

The plant extract was obtained by decoction. The identification of secondary metabolites was carried out according to the method of Harbone (1998) [8].

□ Formulation of the Oral Gel

For the formulation of a 2% gel, 2g of dried extract was mixed with 100ml of distilled water.

□ Dental Extraction

The animals were anaesthetised intraperitoneally with 75mg/kg per body weight of ketamine added to 5mg/kg of Diazepam. Once asleep, dental extraction of the rat's lower incisor was carried out in the conventional manner, using instruments of convenience [9].

Assessment of Haemostatic and Healing Activity [9]

The evaluation of this activity was carried out in the multidisciplinary laboratory of the Department of Galenic Pharmacy and Pharmaceutical Legislation, on 15 female rats of wistar strain, weighing between 100 mg and 150 mg, in which the extraction of lower incisors was performed, divided into three batches of five.



Figure 1: A) *Vernonia conferta* plant growing in the wild in Nkolafamba. B) The dried leaves of *Vernonia conferta*.

A test batch of 5 rats on which the gel formulated with leaf extracts from our plant was applied for 5 days. A positive control lot consisting of 5 rats in which the Elugel reference gel was applied. A negative control lot consisting of 5 rats in which no active ingredient was applied. Macroscopic and microscopic observation and analysis by means of histological examination made it possible to evaluate the haemostatic and healing activities.

Results

At the end of our work, the expected results were:

- Identification of secondary metabolites
- Formulation of the oral gel
- Improved scar and haemostatic profiles after tooth extraction.

□ Performance

The filtrate obtained from 1.5 L after decoction was oven dried at 45° for 7 days. The yield obtained was 15.3g of dried *Vernonia conferta* extract (Figure 2A).

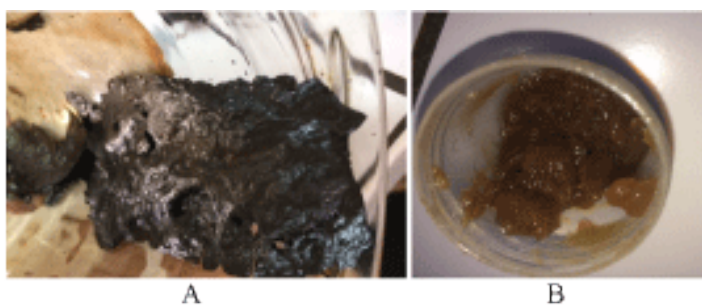


Figure 2: A) Dried extract of *V. conferta* leaves. B) Gel based on *Vernonia conferta* extract

□ Phytochemical Screening

The results of the phytochemical screening revealed the presence of some secondary metabolites in the aqueous extract of *Vernonia conferta* (Table 1). The extract of *Vernonia conferta* contains a predominance of phenols, polyphenols, flavonoids and tannins testifying to the analgesic, haemostatic, antimicrobial, astringent and healing activity of the leaves.

□ Gel formulation

To formulate the gel, a portion of 0.2g of extract diluted in 10ml of distilled water to a quantity of 1g of Carbopol diluted in 10ml

of distilled water were mixed to obtain the gel based on *Vernonia conferta* extract (Figure 2B). The gel obtained had good adhesion, dark brown colouration, slightly pungent taste, pH=7.8 (Table 2).

Table 1: Phytochemical composition of the aqueous extract of *Vernonia conferta* leaves.

Families of compounds	Aqueous extract of <i>Vernonia conferta</i> leaves
Alkaloids	+
Phenols	+++
Polyphenols	++
Tannins	++
Saponins	+
Flavonoids	++
Tri Terpenes and Steroids	+
Anthocyanins	-
Anthraquinones	-

Table 2: Characteristics of the *V.conferta*-based gel.

Features	Results
Colour	Dark brown
pH	7,8
Consistency	Soft
Smell	Slightly spicy
Accession	Good

□ Haemostatic and Healing Activities

The haemostatic process was observed in rats subjected to our test gel, Elugel and the negative test batch. The bleeding time was recorded between 2 and 5 minutes after tooth extraction (Figure 3), and photographs were taken.

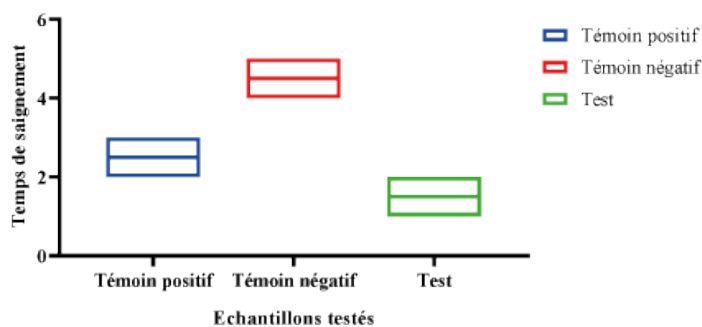


Figure 3: Bleeding time evaluation chart for the tested samples

The Macroscopic Analysis was carried out with the help of intra-oral photographs taken on days D1, D3, D7 and D14 after the surgical procedure showing the state of the mandible. On day D1 of the surgical procedure we observed the first reaction, the time taken to stop the bleeding (Figures 4A, 4B, 4C) and the second step which is coagulation.

The Microscopic Analysis was performed through tissue samples from the tooth extraction area placed in 10% formalin solution on days D3, D7, and D14 respectively after extraction. Histological examination of the socket revealed :

- **On day 3^e** : highly vascularised granulation tissue composed of endothelial cells and neo-vessels, following lysis of the blood clot.

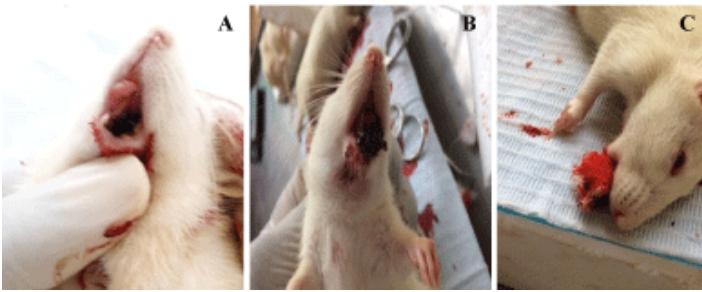


Figure 4A: Rat number 1 of the test batch in which after avulsion haemostasis was after 68 seconds

Figure 4B: Positive control rat number 1 in which after dental avulsion haemostasis was after 130 seconds

Figure 4C: Negative control rat number 1 in which after dental avulsion haemostasis was after 300 seconds

Test batch

The test batch of rats that received our extract-based gel showed a fleshy bud consisting of young connective tissue secreted by numerous fibroblasts, dissociated by neo-capillaries. The whole is infiltrated by inflammatory cells with numerous polynuclear cells. Endothelial cells line the walls of the neo-vessels (Figure 5A).

Positive control batch

The positive control batch, which received the Elugel reference gel, revealed a fleshy bud consisting of predominantly mononuclear fibrino-leukocytic cells and a cluster of epithelial cells (Figure 5B).

Negative control batch

The negative control lot, after dental avulsion, received nothing apart from a sterile compress at the site. A fleshy bud made of very sparse cellular material was observed (Figure 5C).

- **At day 7^e** : scar tissue in which neo-vessels, different types of leukocytes, collagen fibres, marrow spaces and mesenchymal stem cells are found.

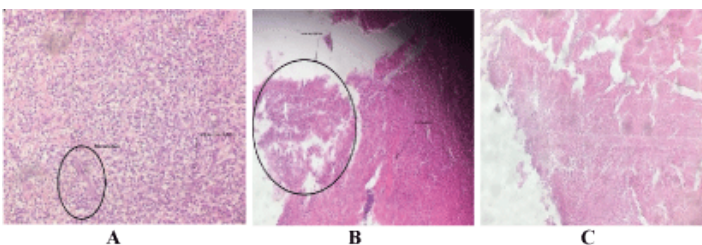


Figure 5A: Microphotograph of the gum flaps from the test batch at D3

Figure 5B: Microphotograph of the gum flaps from the positive control batch at D3

Figure 5C: Microphotograph of the gum flaps from the positive control batch at D3

Test batch

The fleshy bud consists of a base made up of more collagen fibres secreted by numerous fibroblasts dissociated by numerous

capillaries of varying diameters, all infiltrated by inflammatory cells of predominantly mononuclear origin (Figure 6A).

Positive control batch

A fibrous base of younger connective tissue secreted by numerous fibroblasts and predominantly mononuclear inflammatory cells was observed (Figure 6B).

Negative control batch

The granulation bud was more compact and consisted of dense tissue secreted by predominantly mononuclear inflammatory cells (Figure 6C).

- **At 14^e day:** calcification of osteoid tissue at the bottom and edges of the socket, anarchic distribution of collagen fibres was observed.

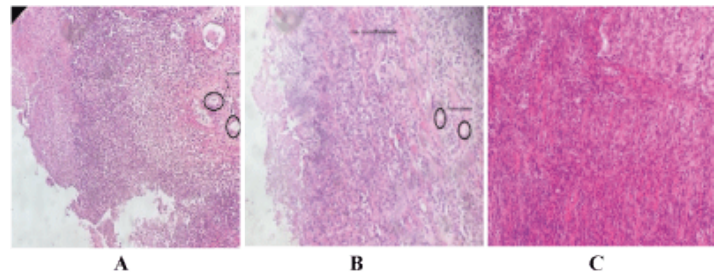


Figure 6A: Microphotograph of the gum flaps from the test batch at D7

Figure 6B: Microphotograph of the gum flaps from the positive control batch at D7

Figure 6C: Microphotograph of the gum flaps from the negative control lot at D7

Test batch

Epithelialization of the surface. There is a dense fibrous base, rich in fibroblasts, surmounted by an epithelium of normal thickness, reflecting a complete restitution of the gingiva (Figure 7A).

Positive control batch

Epithelialization of the surface. A somewhat acanthosed and keratinised epithelium, rich in fibroblast, was noted, reflecting a complete restitution of the gingiva (Figure 7B).

Negative control batch

A fibrous base with persistent inflammatory infiltrate, neo-vessels, and a leukocytic fibrous coating was observed (Figure 7C).

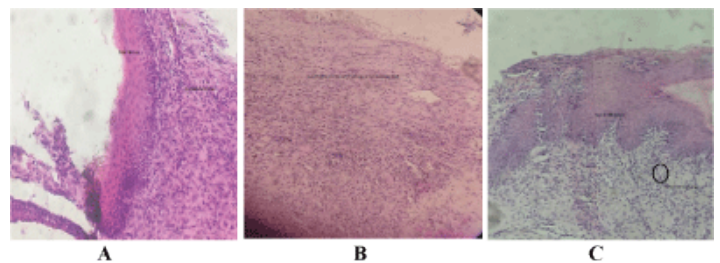


Figure 7A: Microphotograph of the gum flaps from the test batch at D14

Figure 7B: Microphotograph of the gum flaps from the positive control lot at D14

Figure 7C: Microphotograph of the gum flaps from the negative control lot at D14

Discussion

A phytochemical study was carried out to determine the secondary metabolites, which revealed the presence of alkaloids, phenols, tannins, saponins, flavonoids, polyphenols, tri terpenes, steroids, with a predominance of phenols, polyphenols, flavonoids and tannins phenols have anti-inflammatory, anti-cancer, anti-coagulant, antipyretic and analgesic properties, which justifies their great effectiveness in traditional therapeutic use. Phenolic substances are not equally distributed among plants. They are produced in a specific organ, tissue or cell type at particular stages of development of the flower, fruit, seed or seedling [10]. This could be the reason for its low presence in the bark [11].

The main function of flavonoids is to induce colouration in plants. Flavonoid plants have very important and extensive biological properties (anti-oxidant, capillary permeability reduction, antimicrobial). Plant species containing them are highly prized by scientific researchers for their antimicrobial effects, in order to alleviate problems of antibiotic resistance [12].

Tannins are secondary metabolites of higher plants found in almost all parts of plants where they act as a chemical defensive weapon against certain pests. Therapeutically, tannins have pronounced astringent properties that hasten the healing of wounds and inflamed mucous membranes. They are used externally to treat diarrhoea, hypersecretion of intestinal mucous membranes, varicose ulcers, haemorrhoids, chilblains, burns and as mouthwashes for the treatment of stomatitis and periodontal diseases [13].

The formulated gel has characteristics in accordance with good formulation methods according to the European Pharmacopoeia, with a pH close to the oral cavity, a soft consistency and dark brown colour with good adhesion compared to the Elugel reference gel.

The haemostatic activity was evaluated by taking the bleeding time after tooth extraction and application of the gel, showing a cessation of bleeding and coagulation observed within 1 to 2 minutes after application of the gel based on our *Vernonia conferta* leaf extract, in comparison with the positive control and the negative control. This faster haemostatic activity observed after application of our gel, would be due to the high presence of tannins in our aqueous extract of *Vernonia conferta* leaves Benth [11].

The healing activity of *V. conferta* trunk bark, due to its richness in secondary metabolites (tannins, flavonoids, polyphenols), could induce a stimulation of fibroblast proliferation, an acceleration of re-epithelialisation and keratinisation. The evaluation of the healing activity of our extract revealed a rapid re-epithelialisation at 14th days observed under the microscope by studying the histological sections in comparison to the results found in the positive and negative control batches. This result is similar to those found by Y. Allarem in 2020 on the evaluation of the healing activity of *V. conferta* bark on skin wounds.

Conclusion

Vernonia conferta is a plant rich in tannins, flavonoids, polyphenols and phenols, which are responsible for the haemostatic and healing activity of *Vernonia conferta* leaves, thus justifying its popular use for the dressing of incurable wounds. The 2% aqueous extract-based gel formulated had good adhesion to the oral mucosa and its haemostatic and healing action on the post-extraction wound was found to be faster.

Declarations

This study was carried out in accordance with the recommendations of the ethical approval obtained from the Institutional Ethics Committee for Research of Faculty of Medicine and Biomedical Sciences of University of Yaounde 1 under the reference N°151 for 11 May 2021.

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