Epidermal Wound Healing Potentials of Methanolic Extract of Tetracapidium Conophorum Leaf On the Limb of West African Dwarf Goats

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ABSTRACT

A wound is a disruption to the anatomic structure and the functional continuity of living tissues and wound healing is a survival mechanism and represents an attempt to maintain the normal structure and function. The aim of this study is to evaluate the healing properties of methanol extract of tetracapidium conophorum leaf on epidermal wound in West African dwarf (WAD) goat. Eight adult West African Dwarf (12-15kg) goats grouped into control and experimental of four animals each were used. Epidermal wounds were created on the trunk of all the goats using a square stencil of dimension 1cm by 1cm after shaving. Each wound was measured (in centimetre^2) daily using the length of the mid-horizontal and mid-vertical sides of the wound with the aid of a vernier calliper. Epidermal skin biopsies were taken also on days 0, 5, 10 and 20 for histology.The study demonstrated that wound contraction was much faster in treated groups compared with the control group indicating the wound healing properties of T. conophorum leaf extract. The histopathological examination showed observable granulation tissue on day 20 in the treated group while no granulation tissue in the control group. The quantification results revealed an increased fibroblast, neutrophil in the treated group as compared to untreated group which indicated healing.The extract of the leaf showed remarkable wound healing activity and it may be used for treating various types of wounds and injuries in animals and humans.

Keywords: Tetracapidium conophorum, wound, WAD, epidermal

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1. INTRODUCTION

A wound is a disruption to the anatomic structure and the functional continuity of living tissues (Robson *et al*, 2001) or a breakdown in the protective function of the skin; loss of continuity of epithelium, with or without loss of underlying connective tissue - muscle, bone, nerves following injury to the skin or underlying tissues/ organs caused by surgery, a blow, a cut, chemicals, heat/ cold, friction/shear force, pressure or as a result of disease, such as leg ulcers or carcinomas (Velnar *et al*, 2009; Clinimed dictionary, 2015). It may be described by aetiology, anatomical location, whether acute or chronic, method of closure, presenting symptoms or the appearance of the predominant tissue types in the wound bed. All definitions serve a critical purpose in the assessment and appropriate management of the wound through to symptom resolution or, if viable, healing.

Wound healing is a survival mechanism and represents an attempt to maintain the normal structure and function. The capacity of a wound to heal depends partly on its depth, overall health and nutritional status of the individual (Atiyeh *et al*, 2005). To heal a wound, the body undertakes a series of actions collectively known as the wound healing process. Wound healing is a dynamic and complex process having a series of coordinated events. These include bleeding, coagulation, acute inflammatory response, regeneration, migration and proliferation of connective tissue and parenchyma cells; synthesis of extracellular matrix proteins, remodeling of new parenchyma, connective tissue and collagen deposition. Increasing the wound strength occurs in an ordered manner and results in the repair of severed tissues (Labler *et al*, 2006; Rivera and Spencer, 2007; Strecker-McGraw *et al*, 2007). The mechanisms involved in wound healing include: inflammation; epithelialization, fibroplasias, angiogenesis, wound contraction; and remodeling. These mechanisms are initiated at the time of physical injury and proceed continuously throughout the repair process (Labler *et al*, 2006; Broughton *et al*, 2006; Velnar *et al*, 2009).

The plant *Tetracarpidium conophorum* (Mull. Arg) Hutch & Dalziel Syn. commonly called African Walnut belongs to the family Euphorbiaceae. It is a climber found in the wet part of Southern Nigeria and West Africa in general. Its habitat is usually large trees; the fruits are greenish with four round seeds in each fruit. The seed testa is hard, and the cotyledons are white in colour (Ehiagbanare and Onyibe, 2007). The fruits are edible; the plant is medicinal and used for various purposes (Burkill, 1984). The leaves, bark, and fruit of *T. conophorum* are used medicinally, and their uses include masticatory, giddiness, thrush, antihelminthic, toothache, syphilis, dysentery, and as an antidote to snakebite (Odugbemi and Akinsulire, 2008). In the Southern Nigeria ethnomedicine, African walnut is used as a male fertility agent and in the treatment of dysentery (Ajaiyeoba and Fadare, 2006). The methanol and ethylacetate extracts of *T. conophorum* leaves have been shown to possess good antibacterial activities especially against Gram +ve organisms (Ajaiyeoba and Fadare, 2006).

Wound is a common threat to the life of ruminants and other animals at large which can reduce their effectiveness, productivity and economic important. Injuries to the limb usually has a slow healing rate due to poor circulation to the limb; constant and regular joint movement; minimal soft tissue between the skin and the bone of the limb; greater risk of limb contamination as it is closer to the ground (Olaifa *et al.,* 2017). Therefore, proper wound care and management needs to be ensured for wound complications not to set in which could be life threatening. Over the years, there have always been problems with antibiotics resistance, affordability of choice of drug needed with regards to wound healing. In recent times, researchers have been exploring the composition of several herbs that will give answers to all these threats.

The leaves, bark, root, seeds etc of several plants have been used also to treat different disease conditions. Therefore, medicinal plant derived drugs is under great demand due to a common belief that they are safe, reliable, clinically effective, low cost, globally competitive and better tolerated by patients (Balekar *et al.,* 2012). Since ancient times, human beings have been using many plant resources based on empirical observations without any scientific knowledge for the treatment of wounds, cuts, and burns (Wang *et al.,* 2011). The woods, roots, barks, seed, shells and kernels of *tetracarpidium conophorum* have been explored for nutritional and therapeutic functions. The plant has been used in fish wound healing (Bello *et al.,* 2013). However, there is dearth of information on the wound healing activity of this plant in ruminants. Therefore, the need to investigate its healing and morphometric activity in wound using methanolic extraction.

2. MATERIALS AND METHODS

Experimental animals

Eight adult West African Dwarf goats grouped into control and experimental of four animals each were put in stalls. The animals were housed in individual pens three weeks for stabilization before commencement of the experiment. Well-balanced diet consisting of concentrate, grass and cassava peels were fed to the animals and water provided *ad libitum*. The animals were dewormed with levamisole (10%) I/M at the dose rate of 10mg/kg body weight and also given penicillin-streptomycin premptively to take care of possible bacterial infections.

Plant extraction

Air-dried leaves of the plant were ground into powder. The powdered leaves were then soaked in methanol (analar grade) for 72hours. The extracts were filtered respectively first through a piece of satin cloth, then Whatman filter paper no. 42 (125 mm). The filtrate was completely removed by rotary evaporator and further removal of water was carried out by freeze drying. The dry extracts were weighed, respectively, stored in clean sample bottles and kept at 4^0C.

Wound creation

Using a square stencil of dimension 1cm by 1cm, the portion of the epidermis to be surgically removed which is the right lateral side of the animal just ventral to the vertebrae column was marked using an ink marker. Three mg/kg of 2% lignocaine was used in caudal epidural block and local infiltration (inverted L-Block) to desensitize the skin in order to ensure complete desensitization of nerves that might escape epidural block and provide the required anaesthesia. Booster injections of up to one-half of the initial dose were administered as needed in order to ensure that the goats were pain-free during the skin excision procedure. Each marked portion was blocked individually before surgery was done. Epidermal wounds were created on the trunk of all the goats. A sharp sterilized scalpel was used and bleeding reduced by the use of pressure gauze and shortening of surgery duration. The full thickness of the skin within the incision was then carefully stripped away by sharp dissection from its underlying muscle. All excisions were made using a scalpel blade and forceps; with particular care taken that wound edges were sharply defined (Olaifa, 2016).

Wound contraction measurement

Each wound was measured (in centimetre2) daily using the length of the mid-horizontal and mid-vertical sides of the wound with the aid of a vernier calliper. Error due to parallax was reduced by ensuring that wounds were measured under adequate illumination using the same blind observer all through the experiment. The length (L) and breadth (B) were then used to calculate the wound area in cm^2 (Olaifa, 2016).

Epidermal skin histology (H & E staining)

Skin tissue of 1cm area was harvested from the leg as wounds were created in these parts of the body on day 0. Skin biopsies were taken also on days 5 and 9 respectively. Tissues were preserved/fixed using formalin (10%) before arrival at the laboratory for histology. The staining method involves application of hematoxylin, which is a complex formed from aluminium ions and oxidized hematoxylin. This colors nucleus of cells (and a few other objects, such as keratohyalin granules) blue. The nuclear staining is followed by counterstaining with eosin, which colors eosinophilic other structures in various shades of red and pink.

Data analysis

Data obtained during the experiment were subjected to student T-test. All data processing, charts and analysis were carried out using SPSS version 15 and Microsoft Office Excel 2010 (Microsoft Corporation).

3. RESULTS

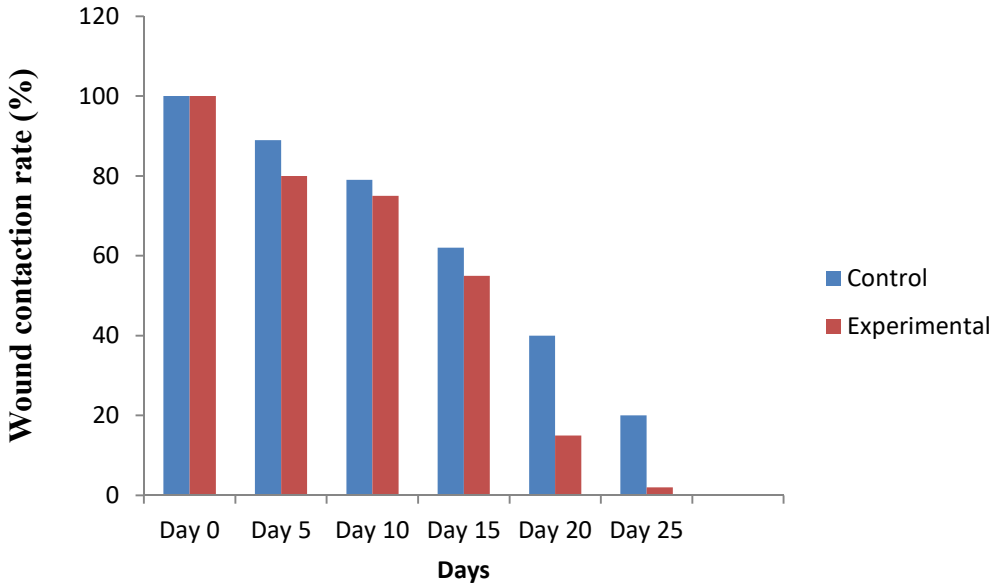


Fig 1 Wound contraction rate in control and methanolic extract *tetracapidium conophorum* treated groups

From the figure above, the wound in the experimental animal showed a higher level contraction compared (80%) with the control (90%). This pattern continued up until the 25th day when the experiment was terminated with the control animal showing consistently slower wound contraction rates than the *tetracapidium conophorum* treated animals (fig 1).

Table 1: Quantification of leucocytes, collagen fibre arrangement, degree of angiogenesis and epithelialization

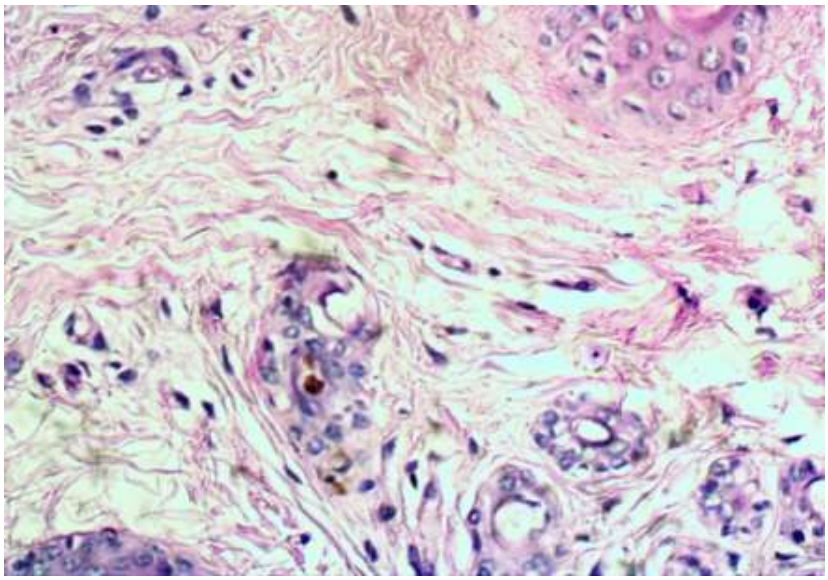
	Code	MQ	LC	FIBRO	NEUT	EOS	MC	PLAT	C	A	E
METHANOLIC EXTRACT											
DAY 0		28	28	4	0	0	2	0	1p	1	1
DAY 5	C	88± 0.4	68±0.5	24±0.41	172±4.8	0	0	0	2p	1	1
	E	40±0.7*	36±0.7*	12±1.12*	48±2.8*	0	1	6	3p	2	1
DAY 10	C	44±0.8	8±1.2	48±0.8	104±2.8	0	2	5	3h	2	1
	E	32±0.8*	44±1.6*	60±2.2	88±1.0*	0	2	12	3h	2	2
DAY 15	C	32±0.8	28±0.8	72±2.9	44±0.9	0	0	0	3h	2	2
	EX	12±1.0	8±0.5*	48±0.9*	8±0.3*	6	1	4	1p	1	1
DAY 20	C	8±1.2	8±1.2	12±1.5	4	0	1	1	2p	2	3
	EX	4±0.8*	4±0.8*	8±1.2*	0	0	0	5	3h	2	3

Quantitative (relative, %) {multiply by 400} *P<0.05, MQ- macrophages, LC- Lymphocytes, Fibro- fibroblast, Neut- neutrophils, Eosin- eosinophils, MC- mast cells, Plat- platelets
Qualitative: C- collagen fibre arrangement, A-degree of angiogenesis, E- degree of epithelialization
1-mild, 2-moderate, 3- severe, h- haphazard, p- parallel arrangement

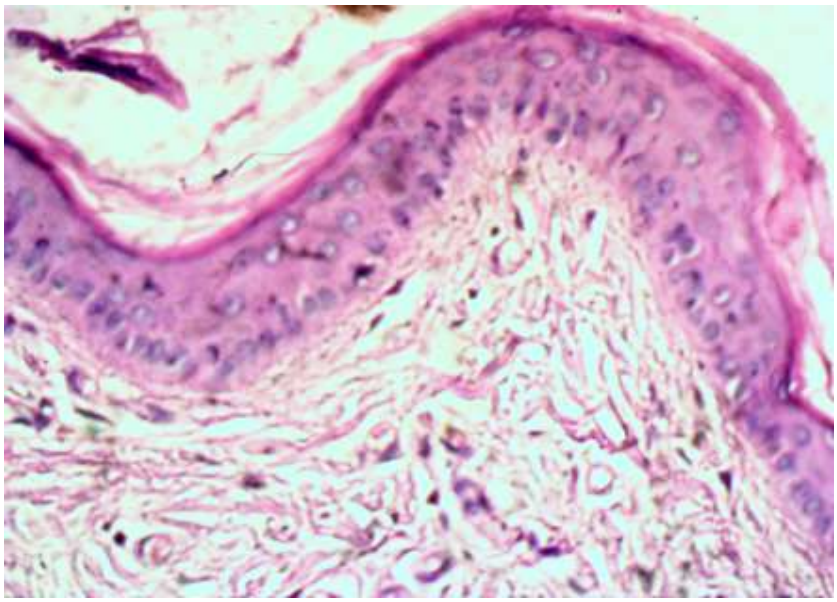
The quantification of macrophages and neutrophils in the control animals were significantly higher than in the treated animals. Lymphocytes and fibroblast were significantly higher in the control animals than the treated animals on day 5, 15 and 20

Histology

Day 0

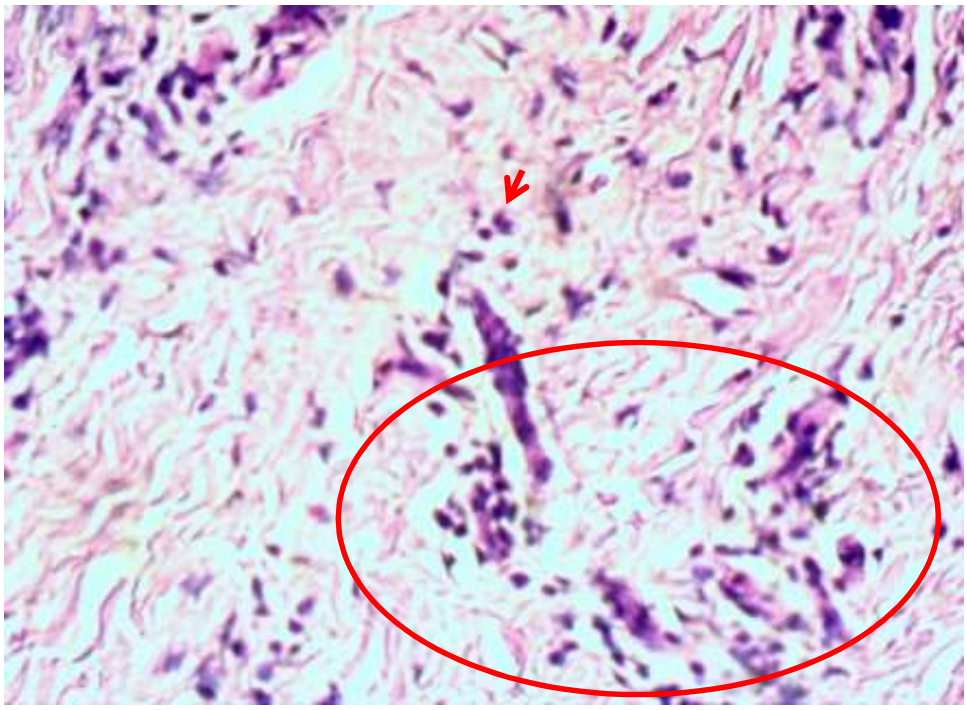


Control

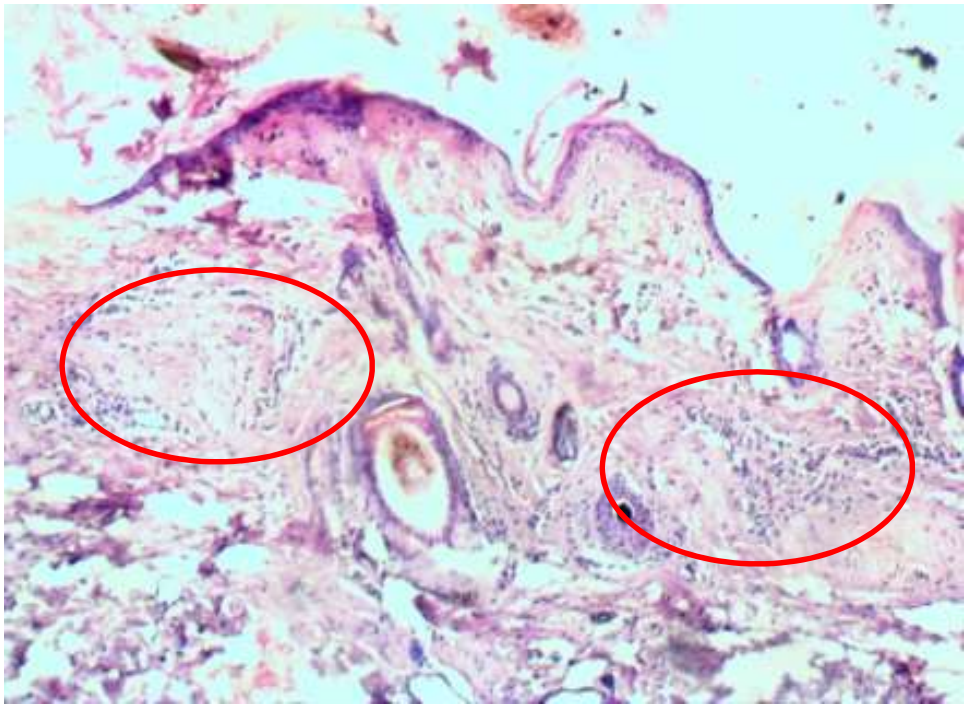


Treated

DAY 5

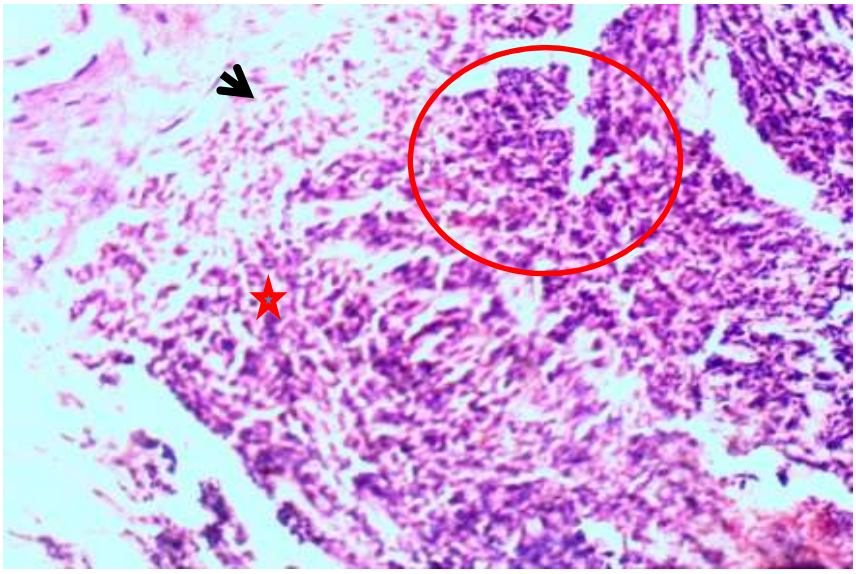


Early inflammatory phase.
Dermis contain a few inflammatory cells (red arrows) within collagen fibres. HE x400

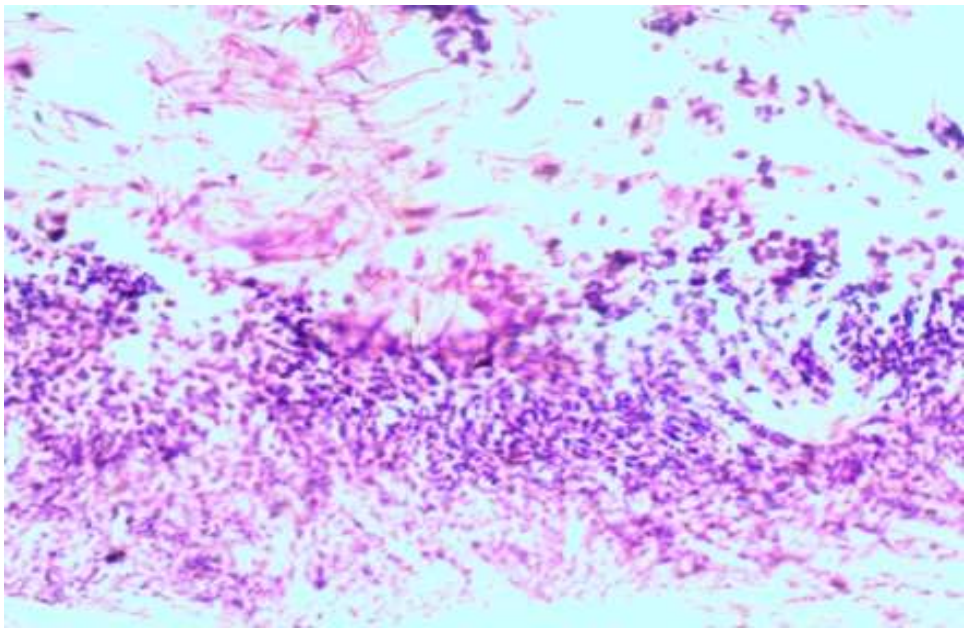


Treated
Early inflammatory phase. HE x100

DAY 10

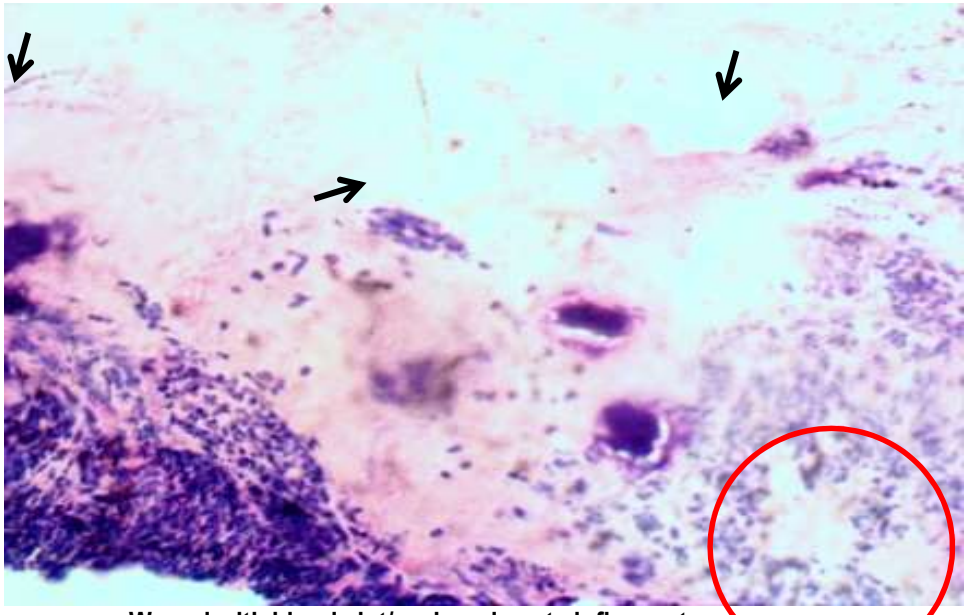


Control
Granulation tissue and inflammatory cells.Note granulation tissue (asterick) containing numerous fibroblasts on the skin. Necrotic zone (black arrow) separates granulation tissue and skin surface
HE x100, 400

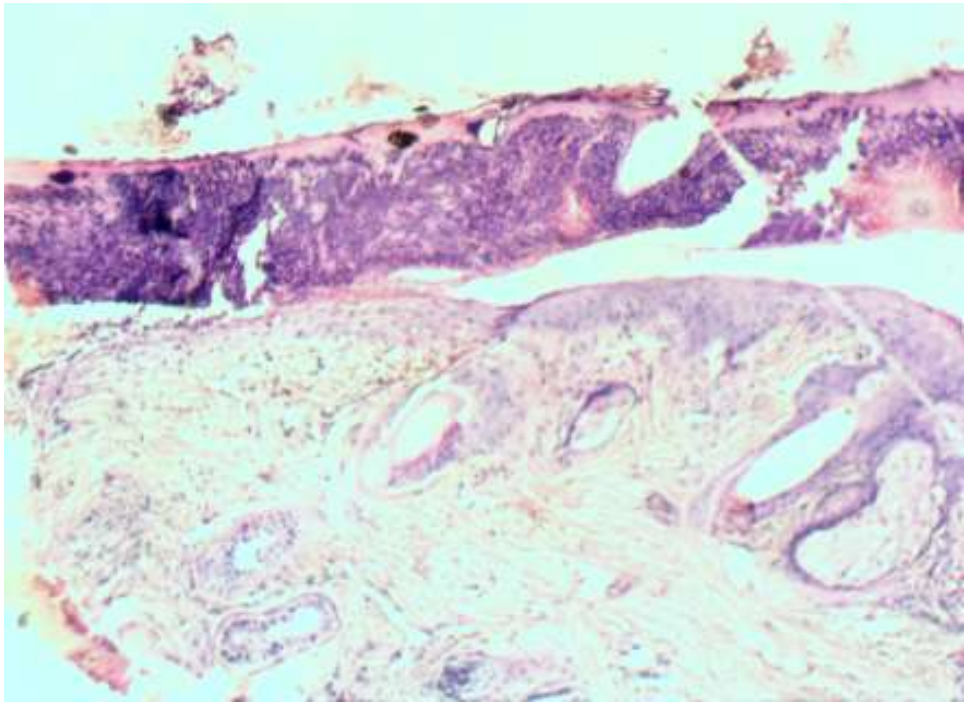


Treated
Layers of Inflammatory cells and scab (asterick)
HE x100, 400

DAY 20



Wound with blood clot/scab and acute inflammatory response
Oedema (black arrows) and some inflammatory cells
HE x100, 400



Granulation tissue (asterick) separated from underlying epidermis

4. DISCUSSION

Wound healing progression is comprised of a systematic process of events starting from the moment of injury, that is, the inflammatory phase (the establishment of homeostasis and inflammation), the proliferation phase (granulation, contraction, and epithelialisation), and finally, the remodeling phase, which determines the strength and appearance of the healed tissue (Kondo, 2007; Wasman, *et al.,* 2010). This study demonstrated that wound contraction was much faster in treated groups compared with the control group indicating the wound healing properties of *T. conophorum* leaf extract. This is in agreement with previous studies in rats (Ezealisiji *et al.,* 2014) and fish (Bello *et al.,* 2013). Phytochemical screening of the leaf of *T. conophorum* showed the presence of tannins, alkaloids, saponins, steroids, tannins and flavonoids. The presence of several secondary metabolites in the plant could possibly account for the reasons why the leaf has numerous therapeutic indications including wound healing. Studies have shown that phytochemical constituents like flavonoids (Tsuchiya *et al.,* 1996) and triterpenoids (Scortichini and Pia, 1991) are known to promote the wound healing process mainly due to their astringent and antimicrobial properties which appear to be responsible for the wound healing and increased rate of epithelialisation (Tsuchiya *et al.,* 1996). Tannins have been reported to possess wound healing action by improving the regeneration and organisation of the new tissue (Leite *et al*. 2002).

Alkaloids, a major constituent of the extract could also be responsible for the enhanced healing. The wound healing activity of the total alkaloid extract could be attributed to the fact that extract caused an increased rate of formation of epithelial cells thus speeding up the re-epithelialization process which is critical in wound healing. There is also the possibility that angiogenesis which is the formation of new blood vessels was accelerated. This will in turn increase blood supply to the newly formed epithelial cells and thus in effect cause an overall increase in the rate of wound contraction. Zahra *et al.* (2011) showed that wounds treated with some plant extracts contain more collagen deposition and fewer inflammatory cells and angiogenesis. An increase in the rate of healing activity has been attributed to angiogenesis and collagen deposition in granulation tissue (Paladini *et al.,* 1996). Acceleration of wound-healing potential of the total alkaloid extract may therefore be due to the deposition of more collagen fibres with angiogenesis and less inflammatory cells in granulation tissue of wound area. This could be achieved by the inhibition of the production of cytokines following a cutaneous injury. Inflammation results in trauma and in the presence of trauma wound healing is delayed (Hess, 2011). On the other hand, the anti-inflammatory effect of the extract may give rise to a quickening of the wound healing process. Research conducted by Kulasekaran *et al*. (2004) also revealed that alcoholic extract of Celosia argentea (*Amaranthaceae*), which contains several alkaloids, has a good wound healing activity.

Histopathological studies of the wound healing process are normally used for evaluating the efficacy of pharmacological products which promote and accelerate dermal skin substitutes. The histopathological examination provided additional evidence for the experimental wound healing studies which was based on the contraction value of wound area. The histopathological examination showed observable granulation tissue on day 20 in the treated group while no granulation tissue in the control group. Granulation tissue primarily contains fibroblasts, collagen fibers, with less oedema and newly generated blood vessels which were observed in leaf extract treated animals. Layers of inflammatory cells were also seen in the untreated groups. Inflammation is the first response during the healing period as a defence mechanism of the tissue, although a long duration in the inflammatory phase can cause a delay in the healing process (Clark, 1991).

The quantification results revealed an increased fibroblast, neutrophil in the treated group as compared to untreated group which indicated enhanced healing. Severe parallel arrangement of collagen and degree of epithelialization were also observed in the treated group as opposed to moderate arrangement of collagen and mild degree of epithelialization in untreated group. Fibroblasts produce collagen in skin, which plays an important role in preserving the anatomic integrity of wound healing. Collagen deposition increases the strength of the wound; before it is laid down, the only thing holding the wound closed is the fibrin-fibronectin clot, which does not provide much resistance to traumatic injury (Greenhalgh, 1998). During the inflammation phase of healing, neutrophils and macrophages are attracted into the injured tissue by various chemo tactic factors (Hernandez *et al.,* 2001).

They locate, identify, phagocytize, kill and digest the microbes and thus eliminate wound debris through their characteristic 'respiratory burst' activity and phagocytosis. This was seen in the quantification in day 0 and day 5 by increased number of macrophages and lymphocyte in the control group. However, suppression of inflammatory cells (macrophages, lymphocyte) in treated groups in days 10, 15, 20 could be responsible for the accelerated wound healing in the treated animals. The presence of flavonoids, alkaloids, cardenolides, saponins along with other secondary metabolites in the leaves of *Tetracapidium conophorum* with analgesic and anti-inflammatory properties has been established (Fernanda *et al*., 2002; Amaeze *et al*., 2011; Onasanwo *et al.,* 2016).

In conclusion, the present study clearly demonstrated that the methanolic extract of *tetracapidium conophorum* promoted wound healing activities in goats. The extract of the leaf showed remarkable wound healing activity and it may be suggested for treating various types of wounds and injuries in animals. The enhanced wound healing activity of *tetracapidium conophorum* could possibly be made use of clinically in the healing of open wounds, most especially on the distal aspect of the limbs where wound healing is slow due to poor vascularization and high tendencies of infection. Also, the equine species has a high tendency for the formation of excessive granulation tissue termed 'Proud flesh' and the application of this plant could offer a permanent solution to this problem.

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