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Evaluation of Wound Healing Activity of an Ethanolic extract of Anogeissus Latifolia leaves in Albino rats.

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ABSTRACT

Background: The skin has been described as the largest organ in the body. **Wound**, a break in the continuity of any bodily tissue due to violence, where violence is understood to encompass any action of external agency, including, for example, surgery. **An** injury to the body (as from violence, accident, or surgery) that typically involves laceration or breaking of a membrane (such as the skin) and usually damage to underlying tissues.

The Healing is a complex process of restoration of cell structure and layers of tissue, which may occur in two ways:

a) Regeneration – It is the means of renewal of the extinct tissue by a like tissue.

b) Repair – It's the process of replacing the lost tissue by granulation tissue.

This granulation tissue fully develops to form the scar tissue.

Objective : The purpose of this study was to determine the effect of wound healing activity of herbal plant Selection Collection and Authentication of Plant Material. Extraction of plant material with Ethanol Preliminary Phytochemical analysis Evaluation of wound healing activity of *Anogeissus Latifolia* leaves in albino rats Histopathological analysis Statistical analysis.

Methods:This experimental study examined 12 animals each group containing 4 randomly divided into treatment anagoiessus latifolia ethanolic extract of oinment and control group wound healing activity was measured by using control and standard group .

Result: Extract treated excision wounds showed an increased rate of wound contraction, leading to faster healing as confirmed by the increased healed area when compared to the control untreated group. The ALLE was recorded similar effectiveness when compared to the group treated with a commercial brand of 5% povidone iodine ointment in but the magnitude was lesser than standard.

Conclusion: The study thus demonstrated the wound healing activity of ethanloic leaf extract of .*Anogeissus latifolia*,and found to be effective in the functional recovery of the wound. The result may be attributed to the phytoconstituents such as flavonoids and phenolics present in it which may be due to their individual or cumulative effect that enhanced wound healing and provided scientific evidence to the ethnomedicinal futures of *Anogeissus latifolia.*

Keywords: *wound healing , anogeissus latifolia, povidone oinment, excision wound model*

1. INTRODUCTION

WOUNDS

The skin has been described as the largest organ in the body. Wound, a break in the continuity of any bodily tissue due to violence, where violence is understood to encompass any action of external agency, including, for example, surgery. Within this general definition many subdivisions are possible, taking into account and grouping together the various forms of violence or tissue damage. An injury to the body (as from violence, accident, or surgery) that typically involves laceration or breaking of a membrane (such as the skin) and usually damage to underlying tissues.[1]

THE HEALING OF THE WOUND

The Healing is a complex process of restoration of cell structure and layers of tissue, which may occur in two ways:

a) Regeneration – It is the means of renewal of the extinct tissue by a like tissue.

b) Repair – It's the process of replacing the lost tissue by granulation tissue.

This granulation tissue fully develops to form the scar tissue.

The healing of wound occurs in the following phases:

- Haemostasis,
- Inflammatory Phase
- Proliferation or Granulation,
- Remodelling
- Scar Formation

HAEMOSTASIS

During this first phase of wound healing, the platelets seal off the ruptured blood vessels to heal the wounds. The platelets secrete vasoconstrictive substances to help in the sealing process by forming a stable clot, which seal off the affected blood vessel. The platelets aggregate in the presence of Adenosine Di Phosphate (ADP) and adhere to the collagen exposed. Formation of fibrin from fibrinogen is initiated by Thrombin. A stable hemostatic plug is formed as the platelet aggregate are strengthened by fibrin mesh. Platelet-derived growth factor (PDGF), which is secreted by platelets, initiates the following steps. Except in case of any clotting disorders, hemostasis occurs immediately after the initial injury.

INFLAMMATION PHASE [2]

It is the second phase of wound healing. It is the body's natural reaction to wound. The blood vessel in the wound area contracts and a thick clot is formed after initial wounding. Dilatation of blood vessels after hemostasis permits the antibodies, growth factors, essential cells, WBC to reach the wounded area. This results in increased exudate levels. Hence the neighboring skin should be examined for the signs of softening and breaking down. The typical manifestations of inflammation like abnormal redness of the skin, heat, swelling, pain and functional disorder are seen during this phase. The Neutrophils and Macrophages are the major phagocytic cells that play an indispensable role in the autolysis of necrotic tissue. The secretion of growth factors like FGF, EGF and IL-1 also occur during this stage.

PROLIFERATIVE PHASE

During this stage, repair of the wound tissue occurs with the formation of granulation tissue, which is primarily composed of collagen and extracellular matrix. A new 7 network of blood vessels develops into this new granulation tissue. This process is known as angiogenesis. The blood vessels supply sufficient oxygen and nutrients to fibroblast, which in turn results in the proliferation of granulation tissue. Healthy granulation tissue is granular and uneven in appearance and is pink in colour which indicates the wound is healing. If the granulation tissue is dark, it indicates that perfusion is poor and the presence of infection. Finally the epithelial cells reappear over the wound surface and this process is known as epithelialization.

REMODELLING OR MATURATION PHASE

During this phase remodelling of the dermal tissues occurs once the wound has closed. The fibroblasts play a key role in this process.Collagen type I get remodelled from type III, cellular activity reduces due to decrease in blood vessels. [3]

SCAR FORMATION

The final product of the process of healing is a scar. The inconsistency in the process of healing can lead to formation of a typical scars.

ACTIVITIES LEADING TO WOUND HEALING

Anti-inflammatory Activity:

The acute inflammatory response during the early stages of injury generates factors that are essential for tissue growth and repair. When prolonged, however, chronic inflammation can to be detrimental, preventing wound remodeling and matrix synthesis, leading to delay in wound closure and an increase in wound pain.

Antioxidant Effect:

The production of free radicals at or around the wound may contribute to delay in wound healing through the destruction of lipids, proteins, collagen, proteoglycan and hyaluronic acid. Agents that demonstrate a significant antioxidant activity may, therefore, preserve viable tissue and facilitate wound healing.

Antimicrobial activity:

Reducing the bacterial load of a wound may be necessary to facilitate wound healing as well as to reduce local inflammation and tissue destruction.

Analgesic Activity:

The open wounds can generate pain and subsequent disability, it is important that the dressing applied does not increase pain, and if possible, it should lessen the pain.

2. RESEARCH METHODS

PLANT COLLECTION AND AUTHENTICATION:The leaves part of the plant *Terminalia anogeissiana* Gere & Boatwr. [Synonym: *Anogeissus Latifolia* (Roxb. Ex DC.) Wall. ex Guill & Perr.] was collected from Virudhunagar District of Tamilnadu and authenticated from the Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu. Soon after collection, the leaves were removed from the stem and shade dried for a few days. After drying, these leaves were crushed to a coarse powder, stored in air tight plastic container for further use.

EXTRACTION OF THE PLANT MATERIAL: The coarsely powdered leaves (250gm) were taken in a round bottom flask and with Ethanol for 48 hours at room temperature. After extraction the extracts were evaporated or concentrated by using rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4^0C for the further analysis.

Animals and management:[7]

Female Wistar rats of 6-8 weeks old and 160-180g body weight were offered by SB College of Pharmacy, Sivakasi. All rats were kept at room temperature and allowed to accommodate in standard conditions at 12-hr light and 12-hr dark cycle in the animal house. Animals were fed with commercial pellet diet and water *ad libitum* freely throughout the study. The experimental procedure was approved by IAEC (Institutional animal ethical committee of SBCP), governed by CPCSEA, Government of India.

Drug:

Commercially available Povidone Iodine Ointment (5%) were used as standard drug.[4] The ointments were applied topically over the wound area.

PREPARATION OF TEST DRUG

Anogeissus Latifolia (ALLE) ointment was formulated using (1%) *Anogeissus Latifolia* leaf extract in simple base ointment.

PREPARATION OF SIMPLE OINTMENT BASE

Ingredients:

Wool fat	5.0g
Hard paraffin	5.0g
Cetostearyl alcohol	5.0g
White soft paraffin	85.0g

Type of preparation: Absorption ointment base

Procedure:

Hard paraffin and cetostearyl alcohol taken in a china dish kept on water-bath at 70^0C. Wool fat and white soft paraffin are added to this mixture and stirred until all the ingredients are melted.[4] If required decanted or strained and stirred until cold and packed in suitable container[8]. 1% of *Anogeissus Latifolia* were separately mixed with the above prepared simple ointment base.

Experimental model:

Rats were divided into 4 groups each containing 12 animals as follows.The rats were given the following as in the table such as control, standard, simple ointment and ALLE (*Anogeissus Latifolia* Extract) Ointment.[9]

Table 1: Experimental design for excision wound model

GROUP	GROUP SPECIFICATION	INTERVENTION
Group I	Control	Untreated
Group II	Standard	Povidone iodine ointment 5%
Group III	Test	ALLE ointment 1%

Induction of wound :

On wounding day the rats were anaesthetized prior to creation of the wounds, by ether anaesthesia. The dorsal fur of the animal was shaved with an electric clipper and the area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil.A full thickness of the excision wound of 1.5cm in width (circular area 2.25cm^2) created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open. All the surgical interventions were carried out under sterile condition. After 24h of wound creation, the ointments was applied gently to cover the wounded area once daily until complete healing Wound area and wound contraction, epithelializtion period and hydroxyproline content were monitored.

Estimation of parameters

Measurement of wound contraction:

The progression of wound healing was judged by the periodic assessment of the contraction of excision wounds. Wound contraction was monitored by tracing the outline of the wound on tracing sheet and then using graph sheet to calculate the area of the wound size.[5] All animals in each group were monitored until complete healing of wounds occurred and the day at which each wound healed was recorded. Mean of all healed wounds was determined.

$$\text{Percent wound contraction} = \frac{\text{Healed area}}{\text{Total Area}} \times 100$$

$$\text{Wound percentage} = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

3. RESULT

Phytochemical screening of ALLE were performed and the results revealed the presence of alkaloids, proteins and amino acids, flavonoids, tannins, phenols, glycosides, tannins, carbohydrates. The levigation method was used to prepare ointment so that uniform mixing of the herbal extract with the ointment base was occured which was stable during the storage. The physicochemical properties were studied which shows satisfactory results for spreadability, extrudability, washability, solubility, loss on drying and others. Ethanolic leaf extract of *Anogeissus latifolia* demonstrated wound healing properties comparable with that of antibiotic standard. Animals in the untreated group showed some degree of healing. As earlier suggested, healing in this untreated group may be due to self-immunity. It is important to note that throughout the period of wound treatment, the extracts did not cause irritation or pain to the animals as the rats neither show any signs of restlessness nor scratching/biting of wound site when the extract were applied.In this investigation three models were used to assess the effect of the ALLE extracts as applied topically. The plant may have a beneficial influence on the various phases of wound healing like fibroplasias, collagen synthesis, and wound contraction, resulting in faster healing. The results of the present investigation showed that both plant extracts have definite wound healing action.

Table 2 :PhytochemicalAnalysisof*Anogeissus latifolia*leaves Extract

S.NO	**PHYTOCHEMICALCONSTITUENTS**	**ALLE**
1	Alkaloids	Positive
2	Proteins andAminoacids	Positive
3	Flavonoids	Positive
4	Phenols	Positive
5	Glycosides	Positive
6	Tannins	Positive
7	Carbohydrates	Positive

Percentage woundcontraction:

Table3: Percentagewoundcontractioninexcisionwoundmodel

COMPOUNDS	**0thDay**	**4rdDay**	**8thDay**	**12thDay**	**18thDay**	**21thDay**
	%WC	**%WC**	**%WC**	**%WC**	**%WC**	**%WC**
CONTROL	0	30.76±0.44	31.57±0.85	39.62±0.55	40.86±0.83	61.25±0.9
STANDARD	0	34.48±0.15	38.02±0.61	47.88±0.62	65.38±0.85	84.26±0.59
ALLEOINTMENT	0	32.61±0.15	36.87±0.16	41.63±0.42	59.76±0.82	79.58±0.96

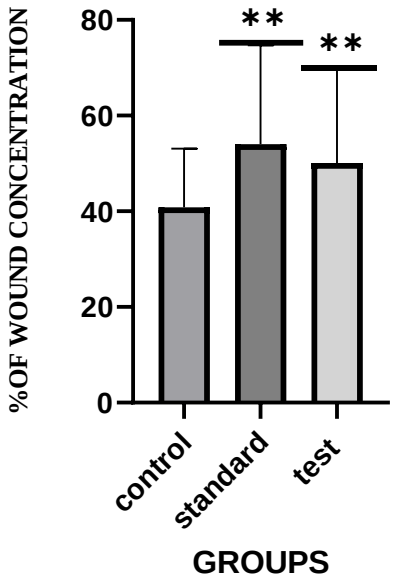

Figure 1: EffectofALLEon% woundcontractionin Excisionwoundmodel

Histopathological study:

Histopathologicalevaluationofexcisionwoundmodel[10]

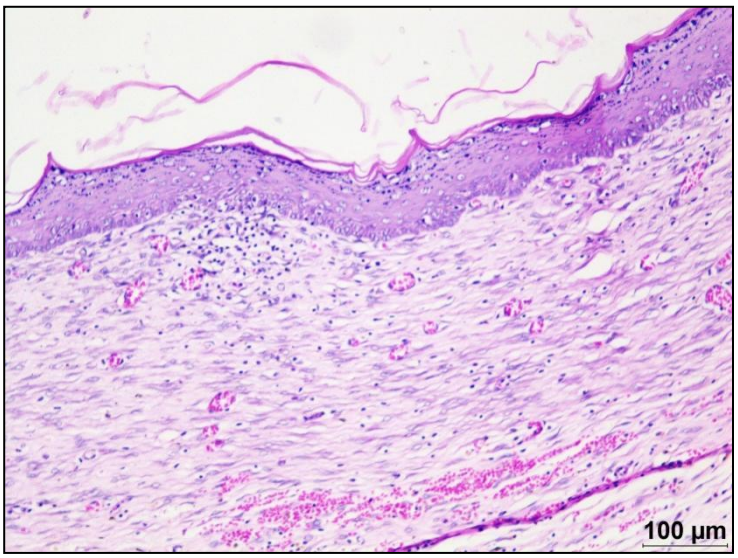

Figure 2-Group I -Control

The section of the skin and epidermis shows incomplete healing with less epithelialization and lesser collagen formation indicated the incomplete wound healing.

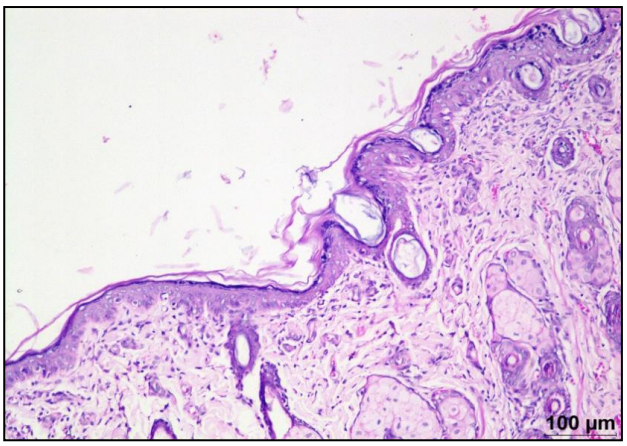

Figure 3-Group II- Standard

Section of the epidermis shows Re-epithelialization with the proliferation of fibroblasts and few lymphocytes in the sub epithelium with thin walled congested blood vessels.

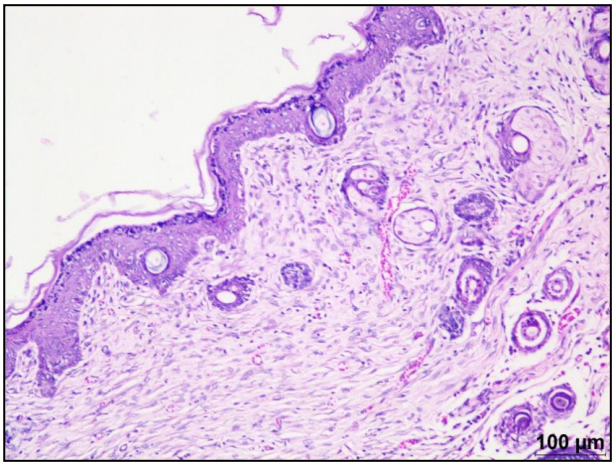

Figure 3- Group III -Test

Section of the epidermis shows Re-epithelialization with the proliferation of fibroblasts, of dense fibrous tissue and blood capillaries was observed. Few macrophages were also present.

4. CONCLUSION

Wound healing is a complex and continuous process that begins immediately after injury, followed by homeostasis, blood clotting, inflammation, proliferation and remodeling phases. All these phases can promote or prolong healing by influencing external or internal factors including infection, sex hormones and nutrition. Delay in healing process increases the possibility of getting infected, improper recovery, and formation of unpleasant scar.The result may be attributed to the phytoconstituents such as flavonoids and phenolics present in it which may be due to their individual or cumulative effect that enhanced wound healing

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