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Efficacy of Wound Healing Activity of *Terminalia catappa* in Swiss Albino Mice

*Santhi. R[1] and S. Annapoorani[2]
[1]Lecturer, Department of Biochemistry, Vivekanandha College of Arts and Sciences for Women, Elayampalayam, Tiruchengode – 637 205, Tamil Nadu
[2]Professor, Department of Biochemistry, Avinashilingam University, Coimbatore – 641 043, Tamil Nadu
**Corresponding Author:* E-mail-santhiakash@yahoo.com

Abstract

A study was conducted to evaluate the wound healing activity of *Terminalia catappa* on Swiss albino mice using the excision wound model. There was a significant increase in wound closure rate, total collagen content, and hexosamine content in the granuloma tissues of the *Terminalia catappa*-treated group when compared to the control and commercial ointment-treated groups. From the results, it may be concluded that *Terminalia catappa* has greater wound healing capacity than commercial Povdine-Iodine ointment.

Keywords: *Terminalia catappa,* Swiss albino mice, Granuloma, Excision wound model, Crude extract.

:oduction

Wound healing is a complex and complicated process :e the stages of which are interlinked by various :hemical and chemical pathways. The initial stage of rocess involves acute inflammation followed by the liferation of fibroblasts and collagen deposition which s remodeled to form a scar (Robert, 2004). The main s which have been implicated in the contraction and sure of wounds are the myofibroblasts (Gabbiani *et* 1972). Wound healing studies mainly aim to detect ious factors influencing the healing process, so that y could be used in clinical practice to alter the healing cess. The process of wound healing involves four ases: (i) Hemostasis (Immediate), which prevents blood s, (ii) Inflammation and Angiogenesis (1-4 days), (iii) Repair, including granulation and cellular proliferation (4-21 days) and (iv) Tissue remodeling and collagen deposition (21-2 years) (Douglas and Alan, 2003). A partial thickness wound involves the deeper dermal layer and includes vessel damage. A full thickness wound affects the subcutaneous fat beyond. Its healing will require the synthesis of new connective tissue and it takes longer to heal because it contracts, whereas partial thickness wounds do not (Sarah Cockbill, 2002).

The plant under study, namely *Terminalia catappa* (Combretaceae) contains alkaloids, flavonoids, phenols, lignins, protein and amino acids. A survey of literature revealed that not much work has been done to study the wound-healing activity of this plant. Hence, it was thought

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worthwhile to investigate the wound-healing activity of *Terminalia catappa* extracts in experimental models of wounds in mice.

Materials and Methods

Preparation of Leaf Extract

The fresh leaves of *Terminalia catappa* were collected from the surrounding areas of Tiruchengode. The fresh leaves were shade-dried at room temperature and made into powder form. 1.5g of *Terminalia catappa* was weighed and mixed with 20% of sodium alginate. This paste was stored at 4°C in cold room.

Phytochemical Analysis

The plant extract was subjected to phytochemical analysis for the identification of the phytoconstituents present in it such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, flavonoids, lignins and saponins (Vogel, 1971). The results are presented in Table 1.

Animals

Swiss albino mice weighing (25-50g) were used for this study. They were housed in cages and fed with pellets and water. The mice were acclimatized for a period of 2 weeks to the laboratory environment before the start of experiment. They were divided into four groups with 6 mice in each as follows

Group 1 - control mice

Group 2 - sodium alginate-applied mice

Group 3 - *Terminalia catappa* leaf extract-applied mice

Group 4 - commercial ointment-applied mice

Excision Wound Model

A standard full thickness (0.5 cm) of wound w created on the back of mice using diethyl ether anesthetic for all the four groups of mice (Morton, 197; The control group was left untreated and the wound w left undressed to the open environment. The 2nd group of mice was treated with a small amount of sodiu alginate once daily for about 8 days. The 3rd group mice was treated with *Terminalia catappa* leaf extra once daily for 8 days. The 4th group was treated wi commercial (Povdine-Iodine) ointment once for 8 day These animal models were used to monitor wour contraction rate by planimetric measurement of wour area once in 4 days. The reduction in wound area wa expressed as percentage of the original wound are epithelization time was noted as the number of days afte wounding required for the scar to fall off leaving no woun behind.

Assessment of Wound Healing

The granulation tissue formed on days 4 and 8 c wound creation were used for biochemical studies. Th amounts of total collagen, hexosamine, protein, DNA an lipid peroxides were assessed.

Histopathological Study

The mice were sacrificed and tissues from the wounc site of individual animal were removed and fixed with 40% formalin, dehydrated through graded alcohol series and embedded in paraffin wax. Serial sections of 5 μm were cut and stained with hematoxylin and eosin. The sections were viewed under a light microscope and photomicrographs were taken.

Results and Discussion

The preliminary phytochemical tests of leaf extracts revealed the presence of flavonoids, tannins, saponins, glycosides, steroids, protein, phytosterols and carbohydrates (Table 1).

The effect of crude extracts of *Terminalia catappa* leaf on excision wound model is presented in Table 2. It was observed that the wound contracting ability of the extract-treated group is significantly greater than that of the control and the standard Povidine-Iodine ointment treated gruops. The extract-treated wound was found to contract faster.

Table 1. Preliminary Phytochemical Screening of the Crude Extract of *Terminalia Catappa*

Test	*Terminalia catappa* leaf extract
Alkaloids	-
Carbohydrates	+
Phytosterol	+
Fixed oil & fats	+
Phenolic compounds and tannins	+
Flavonoids	+
Saponins	+
Glycosides	+
Proteins & amino acids	+

Table 2. Effect of *Terminalia Catappa* on Excision Model in Swiss Albino Mice

Parameters	Control		Sodium alginate treated		*Terminalia catappa* treated		Ointment treated	
	4th day	8th day	4th day	8th day	4th day	8th day	4th day	8th day
Collagen (mg/100mg tissue)	1.80 ± 0.01	2.98 ± 0.03	2.61 ± 0.03	5.58 ± 0.03	5.96 ± 0.03	10.80 ± 0.03	3.74 ± 0.03	7.44 ± 0.03
Hexosamine (µg/100mg tissue)	640.8 ± 2.79	412.5 ± 3.41	700.5 ± 3.04	579.1 ± 2.31	981.0 ± 3.41	660.5 ± 3.50	800.0 ± 2.12	601.0 ± 4.06
Protein (mg/100mg tissue)	11.2 ± 0.46	13.46 ± 0.11	13.15 ± 0.33	15.38 ± 0.27	31.5 ± 0.30	35.58 ± 0.24	25.41 ± 0.31	27.9 ± 0.34
DNA (mg/100mg tissue)	1.11 ± 0.04	3.21 ± 0.05	1.51 ± 0.04	3.81 ± 0.04	2.4[illegible] ± 0.05	5.71 ± 0.05	1.88 ± 0.05	4.72 ± 0.05
Lipid peroxide (MDA/100mg tissue)	1075.5 ± 4.57	774.3 ± 3.03	875.3 ± 3.09	676.3 ± 3.44	425.5 ± 2.98	226.1 ± 3.57	624.0 ± 3.41	475.5 ± 2.81
Wound contraction (%)	37.7 ± 2.35	63.7 ± 2.74	44.0 ± 2.41	75.8 ± 3.18	61.3 ± 4.34	94.1 ± 3.91	55.1 ± 2.60	85.3 ± 7.70

Simillar results were seen with ethanolic extract of *Polygonum barbatum* by Nithya and Balasubramanian (2008). This study describes the clinical importance of *Terminalia catappa* in wound healing-tissue-repair. Wound healing is a complex process which involves inflammation, fibroblast proliferation and resurfacing of wound.

Wound healing comprises different phases such as contraction, epithelialization, granulation and collagenation. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength. The breakdown of collagen liberates free hydroxyproline. The measurement of hydroxy proline could be used as an index of collagen turn-over (Madhura and Sushma, 2003). In the present study, a significant increase in hydroxy proline content was observed in treated wounds. This indicates that a large amount of collagen helps to heal wounds quickly. There was an increase in hexosamine which showed that fibroblast activity synthesized the ground substratum. The increase in DNA content in treated wounds indicates hyperplasia of cells. The lipid peroxide content of treated wounds showed a decrease. The protein content of tissue increased (Azad, 2002). The potential decrease in lipid peroxides in granulation tissue of treated mice clearly indicates the antioxidant nature of *Terminalia catappa*. Percentage closure of wound area was significantly higher in the leaf- extract-treated group than among other groups of animals. The rate of wound contraction w less in the control group of animals whereas tl percentage of wound closure was higher in the lea extract-treated group.

Histological studies of granulation tissue of th control group showed aggregation of more number o macrophages and less collagen fibres whereas the *Terminalia catappa* leaf- extract-treated group of animals revealed a significant increase in collagen deposition with lesser macrophages (Figure 1). Many workers have studied the wound-healing properties of several plants such as *Leucas hirta* (Manjunatha *et al.*, 2006), *Datura alba* (Priya *et al.*, 2002), *Coronopus didymus* (Prabhakar *et al.*, 2002) and *Aloe vera* (Udupa *et al.*, 1994); The wound-healing potency of these medicinal plants may be attributed to the active constituents present in them. These active constituents promote the process of wound-healing by increasing the viability of collagen fibrils, by increasing the strength of collagen fibres either by increasing the circulation or by preventing cell damage or by promoting DNA synthesis (Getie *et al.*, 2002). The present study clearly showed that the leaf extract of *Terminalia catappa* has great wound healing properties. This could be a good source of wound-healing activity.

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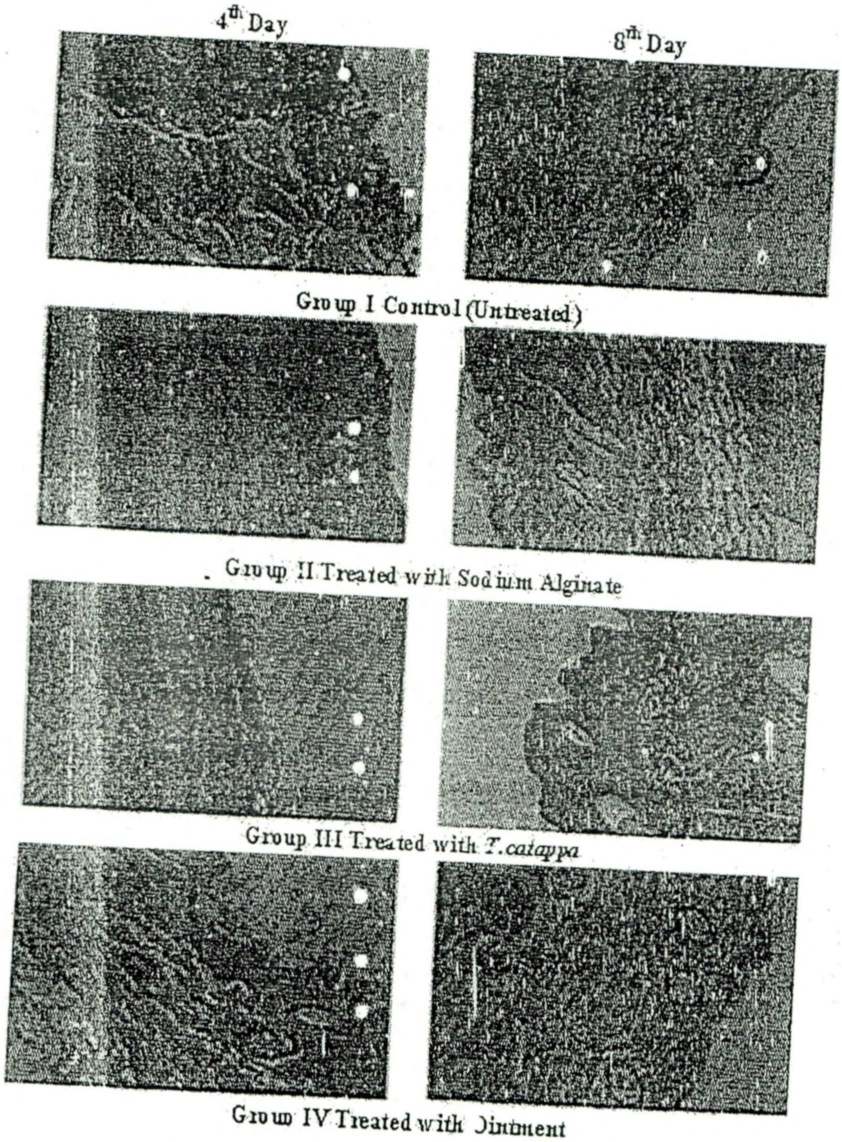
Figure 1. Histopathological Study



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"In the last century alone more than 75 per cent of all known food crops have disappeared and the world relies on just a few varieties of rice, potatoes, maize, wheat and other staples.

Unfortunately many of those lost or in decline are indigenous species that are the most nutritious, whether the green leafy vegetables of Africa or the millets of India. We need to prepare ahead of time for the shifts in plant and crop species that will survive temperatures higher than the highest average temperatures of today".

Emile Frison, Director General, Biodiversity International

(Courtesy The Hindu dated February 16th, 2010)