



EVALUATION OF WOUND HEALING ACTIVITY OF ETHANOLIC EXTRACT OF *TERMINALIA PALLIDA* LEAVES

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ABSTRACT

Terminalia pallida L., belongs to the family Combretaceae, it is a folkloric medicine for a variety of uses as antipyretic, purgative, diuretic and as past, mixed with turmeric and applied externally to the toes and feet cure fissures and cracks in feet and in veterinary medicine. Excision wound is made in the sterile area of 7x7 mm excision wound was created by a surgical blade. Group I served as control and received simple ointment base topically. Group II served as standard and treated and treated externally with povidone- iodine ointment. Group III treated with ethanolic extract of *Terminalia pallida* L. (EETP) ointment, the ointment was topically applied daily till the complete epitheliazation starting from the day of operation. The parameters studies on alternative days until healing were complete. The present work indicates that the ethanolic extract of *Terminalia pallida*, leaves extract shows significant wound healing activity. Acute toxicity studies were conducted for the ethanolic extract of *Terminalia pallida* Leaves. The maximum tolerated dose was found to be 5000mg/kg b.w when the extract was administered orally.

Key words: *Terminalia pallida* L., Combretaceae, Povidone Iodine, Wound Healing, EETP.

INTRODUCTION

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects. *Terminalia pallida* is a taxonomically and phylogenetically complex group, consisting of 20 genera and 500 species of trees, shrubs and linans distributed mainly in tropical and subtropical countries (Vedavathy S, 1998). The Genus *Terminalia* Linn includes about 200 species of Trees and shrubs and distributed throughout the tropical and subtropical regions of the world. Besides yielding high value of timber, many *Terminalia* Species are the source of various non-wood forest products' Fruits are used as antipyretic, as a purgative, diuretic and against cold, cough. As decoction it is orally used to prevent diarrhea (Pallani *et al.*, 2009).

As powder applied externally on affected part and given orally with water to control diabetic and fruits are also consumed as dry pickles (Savithamma N, (FAO):29). As paste, mixed with turmeric and applied externally to the toes and feet to cure fissures and cracks in feet and in veterinary medicine. Bark and leaves are used as anti-inflammatory, analgesic, fever, cold, cough, diabetic, dysentery and diarrhea. Fruit decoction is used to cure piles and diabetic (Jean-Phillippe Meay *et al.*, 2007).

MATERIALS AND METHODS

Plant Materials

Fresh leaves were collected from Tirumala hills, Chittoor district, Andhra Pradesh, India and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany S.V. University, Tirupati, and Andhra Pradesh, India. Voucher Specimen No. 1295 is kept for further future reference at S.V. University, Andhra Pradesh, India.

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Extraction of Plant Materials

The leaves were washed with fresh water to remove dirt and foreign particles and are washed with absolute ethanol to avoid the microbial growth, and were dried under the shade. The dried leaves were crushed and grinded to get powder and weighed. The powdered material of leaves of *Terminalia pallida* L., was refluxed successively with ethanol in soxhlet extractor for 72 hrs. the solution so obtained was transferred to china dish and then allowed for drying. The extract so obtained was thoroughly washed with Ethyl acetate so as to remove the chlorophyll and was dried kept in a desiccators for further use.

Ointment preparation for topical application

An alcohol free extract of *Terminalia pallida* leaf was used for the preparation of the ointment for topical application. A 1%, 1.5% and 3.0% EETP of extract ointment was formulated using soft paraffin base.

Experimental animals

Albino rats (Wistar) weighing 180-220g either sex were used in this study. They were housed in polypropylene cages under standard laboratory conditions (12-h light/ 12-h dark cycle, 21 ± 2 °C, and relative humidity 55 %). The animals were given standard rodent pellets and tap water. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. Ethical clearance for handling the animals is obtained from the Institutional animal ethical committee prior to the beginning of the project work from Institutional Animal Ethical Committee (IAEC) of (IAEC/ACP/1220/a/08/CPCSE 08).

Excision wound model

Five groups of six animals in each group. Animals were anaesthetized with 0.3 ml of Lignocaine hydrochloride (local anesthetic) to prevent any movement of animals for at least two hours after administration and animals were left without being restrained.

Group I: Control (wound treated with paraffin base).

Group II: Standard (wound treated with povidone iodine).

Group III: Wound treated with EETP leaf extract ointment. 1%

Group IV: Wound treated with EETP leaf extract ointment. 1.5%

Group V: Wound treated with EETP fruit extract ointment. 3%

For this study the animals served for 12 hours prior to wounding and shaved with electrical clipper on dorsal area of the animal by sterilized with 70% ethanol before Single circular wound is made in the sterile area of 7×7 mm excision wound was created by a surgical blade. The experimental groups were topically applied with the extracts twice daily for consecutive 16 days. The group treated with povidone iodine drug served as a reference-standard. A progressive decrease in the wound area was periodically monitored. The wound contractions or closures were measured by a tracing paper on the wounded margin and calculated as percentage reduction in wounded area. The actual value was converted into percentage value taking the size of the wound at the time of wounding as 100%. Measurement of wound closure was taken the 4, 8, 12 and 16th day post wound creation.

Acute toxicity studies as per OECD guideline 425

An acute toxicity study was carried out to determination of LD₅₀ values by using different doses 200, 400,600 and 5000mg/kg body weight of the extract in healthy Albino rats (Wistar) weighing 180-220 g weighing between 180-220g body weights were selected for oral acute toxicity study.

This study was carried out as per the OECD guidelines Number 425. From the toxicity study, it was indicated that the extract is safe up to dose 2.0g/kg body weight. It is very safe for further studies at different doses. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days to check the mortality rate (OECD 425).

Statistical analysis

The results were subjected to statistical analysis by using ANOVA followed by Turkey Kramer multiple comparison test. The values are expressed as Mean \pm SEM, n=6 in each group.

Table 1. Effect of topical application of different gel formulations EETP in excision wound model

| Groups | Wound area in mm | | | |
|-----------|------------------|------------------|-------------------|-------------------|
| | 4 th | 8 th | 12 th | 16 th |
| Control | 6.94 \pm 0.02 | 6.81 \pm 0.02* | 6.21 \pm 0.03** | 5.24 \pm 0.17** |
| EETP 1% | 6.93 \pm 0.01 | 6.73 \pm 0.15* | 5.67 \pm 0.02** | 4.59 \pm 0.29** |
| EETP 1.5% | 6.92 \pm 0.04 | 6.59 \pm 0.23* | 4.65 \pm 0.07** | 3.42 \pm 0.25** |
| EETP 3% | 6.91 \pm 0.04 | 6.21 \pm 0.33* | 4.57 \pm 0.08** | 3.25 \pm 0.14** |
| Standard | 6.51 \pm 0.04 | 6.21 \pm 0.03 | 4.7 \pm 0.08 | 2.23 \pm 0.14 |

All expressed as mean and standard error mean (S.E.M). Mean in columns with different letters were significantly different (ns = not significant, * $P < 0.05$ ** $P < 0.01$).

RESULTS

The preliminary phytochemical investigation of the plant extract showed the presence of glycosides, flavonoids, tannins and phenolic compounds, steroids and saponins.

In excision wound model the potency of wound healing activity of the plant was found to be highly significant.

Excision wound showed that there is almost complete healing on the 16th post wounding day with ethanolic extract. The topical application of *Terminalia pallida* leaf ointment increased the percentage of wound contraction and this indicates rapid epithelization.

The administration of this extract *Terminalia pallida* leaf accelerated the progression of wound healing by 16th day i.e. $3.25 \pm 0.14^{**}$ compared with control ($5.24 \pm 0.17^{**}$)

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DISCUSSION AND CONCLUSION

Wound may be defined as break in the epithelial integrity of the skin or loss of functional continuity of living tissue (Krishna Murti *et al.*, 2011). Wound may occur in the form of burns, cuts or tissue grafting. Each type has its own mechanism of action in restoring the original skin texture (Jayaraman S *et al.*, 2011). A wound healing involves different phases such as contraction, granulation, epithelialization, and collagenation (Krishna Murti *et al.*, 2011; Purna KS *et al.*, 1995). Phytoconstituents such as tannins and flavonoids are responsible for wound healing activity by their astringent and antimicrobial property. Excision wounds heal by contraction, epithelialization and the percentage of wound closure rate includes by recording the changes in wound are at regular intervals like 4th, 8th, 12th and 16th day after treating with ethanolic extract.