WOUND HEALING ACTIVITY OF METHANOL EXTRACT OF *MURRAYA KOENIGII* LEAVES

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Summary

The wound healing efficacy of methanol extract of *Murraya koenigii* was evaluated in excision and incision wound models. The parameters studied include rate of wound contraction, period of complete epithelialization and tensile strength of incision wound. The methanol extract of *Murraya koenigii* leaves was found to possess significant wound healing activity, which was evidenced by decrease in the period of epithelialization, increase in the rate of wound contraction and skin breaking strength.

Key Words: *Murraya koenigii*, wound healing, excision wound model, incision wound model.

Introduction

The therapeutic efficacies of many indigenous plants, for various diseases have been described by traditional herbal medicine practitioners [1]. Natural products are rich source of synthetic and traditional herbal medicines which are still the primary health care system in some parts of the world [2]. The past decade has seen considerable change in opinion regarding ethnopharmacological therapeutic applications. The presence of various life sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential wound healing properties. Efforts are being made all over the world to discover agents that can promote wound healing and thereby reduce the cost of hospitalization and save the patient from amputation or other severe complications [3].

The *Murraya koenigii* (L) Spreng, commonly known as *curry leaf-tree* [4], is a small tree with dark grey bark, and belongs to family Rutaceae [5]. It is widely used as a spice and condiment in India and other tropical countries [6]. The green leaves are used in bursied and applied externally to cure eruptions. The leaves and roots are bitter, acrid, cooling, alexetric, anthelmintic, relieves from pain, cure piles, allay heat of body, thirst, itching, useful in leucoderma and blood disorders [5]. In light of above and looking at the beneficial properties of this plant the present study was undertaken to investigate the wound healing activity of *Murraya koenigii* leaves.

Materials and Methods

Plant material

Leaves of *Murraya koenigii* were collected from the University of Rajasthan, Jaipur (Rajasthan, India) in the month of March 2009 and were authenticated by P.J. Parmar, Joint Director in Botanical Survey of India (BSI), Jodhpur (Rajasthan), India. Voucher specimen **(no.JNU/JPR/PC/SG-1)** has been kept in Herbarium of BSI, Jodhpur for future reference.

Extraction
The leaves were dried under shade, reduced to moderately coarse powder, loaded into Soxhlet extractor and were subjected to successive extraction with Petroleum Ether, Benzene, Chloroform and Methanol to get different extracts.

Preliminary Phytochemical Studies
The different extracts were then subjected to qualitative phytochemical screening for the identification of the phytoconstituents.

Experimental Animals
The Institutional Animal Ethics Committee, (IAEC) approved the use of animals for the present study**, (Ethical clearance number: 001/2009/IAEC/JNU).**
Healthy Wistar albino rats of both sexes 200–220 g was used for the study. They were individually housed and were allowed free access to standard pellet diet (Mahavir industries, Delhi) and water *ad libitum*. Animals were periodically weighed before and after the experiment. The rats were anaesthetized prior to and during infliction of the experimental wounds.
The surgical interventions were carried out under sterile conditions using ketamine anaesthesia (120 mg/kg). Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and were replaced.

Wound-healing activity
Excision and incision wound models were used to evaluate the wound-healing activity of methanol extract of *Murraya koenigii.*

Excision wound model
Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by Morton and Malon [7]. The dorsal fur of the animals was shaved with an electric clipper and the anticipated 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 circular area 500mm^2 and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open [8]. The animals were divided into two groups of 6 each. Group 1 animals were topically treated with the simple ointment base I.P. (Indian Pharmacopoeia 1966) as a placebo control. The animals of group 2 were topically treated with the 10% ointment of the methanol extract of *Murraya koenigii* leaves (meMKL) formulated in simple ointment base I.P. (Indian Pharmacopoeia 1966) till complete epithelization [9]. The wound closure rate was assessed by tracing the wound on days 2, 4, 8, 12 and 16 post-wounding using transparency papers and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of eschar without any residual raw wound gave the period of epithelization.

Incision wound model

As with the above model rats were anaesthetized prior to and during creation of the wound. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision, six centimeters in length was made through the skin and cutaneous muscle on the back as described by Ehrlich and Hunt et al. [10]. After the incision, surgical sutures were applied to the parted skin at intervals of one centimeter. The wounds were left undressed. The rats were given meMKL (dissolved in distilled water) orally at a dose of 400 mg/kg/day. The controls were given with distilled water only. The sutures were removed on the 8th post wound day and the treatment was continued. The skin-breaking strength was measured on the 10th day by the method described by Lee [11]. The anesthetized animal was secured to the table, and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposed to each other. One of the forceps was supported firmly, whereas the other was connected to a freely suspended light weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wound edges.

As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound, and the procedure was repeated on the contralateral wound. The mean reading for the group was taken as an individual value of breaking strength. The mean value gives the breaking strength for a given group.

Statistical Analysis [12]
All the results obtained from various activities, as described above, were analyzed statistically by using one-way ANOVA followed by Tukey's post hoc test and $p<0.05$ were considered significant.

Results

Preliminary Phytochemical Studies
Petroleum Ether, Benzene, Chloroform does not show any appreciable tests for the presence of different phytoconstituents, Methanolic extract showed positive tests for the presence of glycosides, flavonoids and alkaloids.

Wound-healing activity
The significant increase in the wound-healing activity was observed in the animals treated with the *Murraya koenigii* extract compared with those who received the placebo control treatments. In the excision wound model, *Murraya koenigii* treated animals showed a significant reduction in the wound area ($p < 0.001$) and epithelization period (Table 1). Table 2 shows the effects of the methanol extract of *Murraya koenigii* administered orally at a dose of 400 mg/kg/day for 10 days on wound healing activity in rats inflicted with incision wound. In the incision wound model, a significant increase in the wound breaking strength (453.2 ± 0.2769g) was observed when compared with the control.

Table 1 Wound healing effect of *Murraya koenigii* in Excision wound model

Parameter	Wound area (mm2) and percentage of wound contraction	
Post Wounding Days	**Placebo control**	**Experimental**
Day 2	458.1 ± 7.97 (9.2 ± 1.89)	447.1 ± 2.85 (10.5 ± 0.57)
Day 6	262.6 ± 9.93 (47.4 ± 1.5)	221.5 ± 3.24*** (55.7 ± 0.6) ***
Day 10	223.1 ± 5.02 (55.3 ± 1.0)	142.1 ± 4.69*** (71.5 ± 0.93) ***
Day 14	110.0 ± 6.97 (78.0 ± 1.39)	92.3 ± 3.89* (81.5 ± 0.77)*
Day 18	37.1 ± 2.98 (92.5 ± 0.59)	19.6 ± 1.54*** (96.0 ± 0.30) ***
Period of epithelization (day)	19.5 ± 0.42	15.6 ± 0.55***

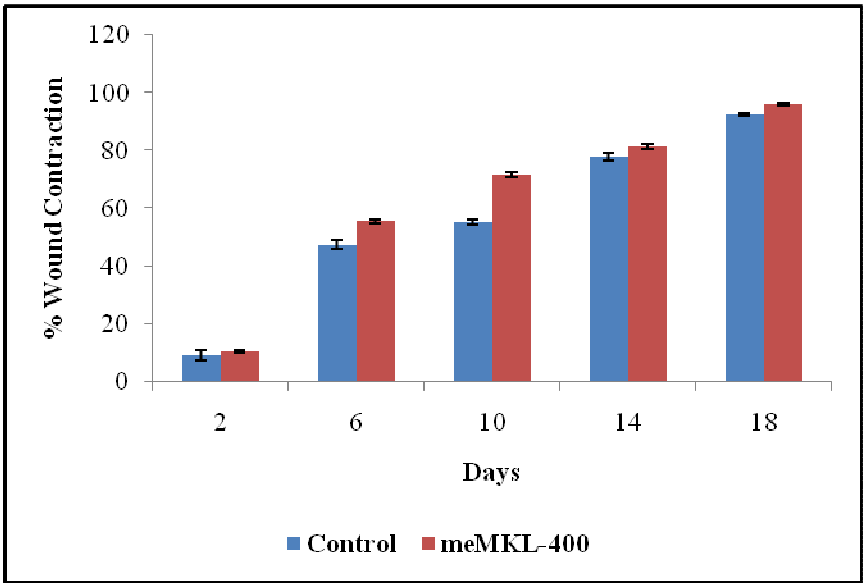
Values are mean SEM± (n=6); values in bracket indicate percentage wound contraction.
*P<0.05; ***P<0.001 when compared to control group.

Table 2 Wound healing effect of *Murraya koenigii* in Incision wound model

Parameter	Placebo control	Experimental
Skin breaking strength (g)	325.46 ± 0.206	453.2 ± 0.2769***

Values are mean SEM± (n=6)
***p<0.001 significant as compared to control group

Fig. 1 Wound healing effect of *Murraya koenigii* in Excision wound model



Discussion

Wound healing is a highly complex, but orchestrated cascade of events that can roughly be divided into three overlapping phases- inflammation, granulation tissue formation and remodeling of the extra-cellular matrix. These events involve several cellular phenomenons such as migration, proliferation, adhesion, phenotypic differentiation, etc. Immediately after injury, there is clot formation and the earlier phases of wound repair involve inflammation and synthesis of ground substance. The ground substance mainly consists of proteoglycans, which are heterogenous, non-fibrillar components of the extra-cellular matrix.

These complex macromolecules are made up of a protein core linked covalently to linear heteropolysaccharides, the glycosaminoglycans (GAGS). Proteoglycans and GAGS have been shown to play important roles in all the above-mentioned events of wound healing [17].

The wound-healing property of *Murraya koenigii* may be attributed to the phytoconstituents present in the plant, and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. The early tissue approximation and increased tensile strength of the incision wound observed in our study may have been contributed by the phytoconstituents of *Murraya koenigii.* Further phytochemical studies are in progress to isolate, characterize and identify the specific active compounds in this plant responsible for wound healing activity.

Conclusion

The present study has demonstrated that the methanol extract of *Murraya koenigii* has properties that render it capable of promoting accelerated wound healing activity compared with placebo controls. Wound contraction and increased tensile strength support further evaluation of *Murraya koenigii* in the topical treatment and management of wounds.

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