



ISSN: 0975-833X

RESEARCH ARTICLE

CATALYTIC EFFECT OF MURRAYA KOENIGII IN WOUND THERAPY

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ARTICLE INFO

Article History:

Received 14th September, 2011
Received in revised form
24th October, 2011
Accepted 27th November, 2011
Published online 31st December, 2011

Key words:

Murraya koenigii;
Excision model;
Incision model;
Wound-healing activity.

ABSTRACT

Murraya koenigii is an ancient Indian medicinal plant. This native is Indo-China but grown mostly in the tropics for the medicinal and flavourant properties of the leaves. Wound-healing activity of ethanolic extract of *M. koenigii* was studied by excision and incision wound models in male Albino rats (*in vivo*). In excision model, compared to the control group, percent concentration of wound was significantly higher in *M. koenigii* (5% w/w ointment)-treated group. In incision model, tensile strength of the healing tissue after treatment with *M. koenigii* was found to be significantly higher compared to the control group, indicating better wound-healing activity of the test plant. These results suggest that ethanolic extract of *M. koenigii* possesses significant wound-healing potential in normal wound.

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INTRODUCTION

The process of wound healing involves various biological processes, such as acute inflammation, cellular proliferation and contraction of the collagen lattice formed [Bennet, 1988; Bodeker and Hughe, 1998]. Wound may extend from epidermis deep into the muscles depending on the severity of damage. Thus, wound is caused by spontaneous process in the organism through cascade of events, which starts by switching of various chemical signals in the body [Lawrence, 1994]. This facilitates the restoration of anatomical continuity and function. While partial-thickness wound heals by mere epithelialization, the healing of full-thickness wound that extends through the entire dermis involves more complex well-regulated biological events resulting in the formation of hypertrophic scars. Wound may be produced by chemical, physical, thermal, microbial or immunological insult to the tissue [Clark, 1993; Clark, 2001]. Wound causes discomfort and is more prone to infection and other troublesome complications. Many of the synthetic drugs currently used for the treatment of wounds are not only expensive but also posing problems such as allergy and drug resistance, and this situation has forced the scientists to seek alternative drugs [Ehrlich *et al.*, 1994; Ingold, 1993]. More than 80% of the world population still depends upon traditional medicines for their ailments, especially for wound management, as they provide a moist environment to encourage the establishment of the suitable environment [Bowler *et al.*, 2001; Sai and Babu, 1998; Kumara *et al.*, 2001]. Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine, though their mechanism of action and

efficacy have not been evaluated scientifically [Purna and Babu, 2000]. The present study was conducted to evaluate the excision and incision wound-healing capacity of *Murraya koenigii* leaf.

MATERIAL AND METHODS

Soxhlet extraction method

The leaves of *M. koenigii* were collected from local vegetable market, and then they were powdered. The powdered material and the solvent (ethanol or water) were taken in the Soxhlet apparatus in the ratio 1:4. The collected extracts were concentrated by using distillation unit and stored in the refrigerator.

Excision model

Excisions of wounds were made as described by Morton and Malone in 1972. Animals were anaesthetized with anaesthetic ether and placed in operation table in its natural position. Male albino rats of 150–200 g of body weight were selected. Animals were divided individually with free access of food and water. The basal food intake and weight to nearest gm were noted. The animals were starved for 12 h prior to wounding. Wounding was performed under light ether anaesthesia. For the excision wound, each group containing six animals was selected. A circular wound of about 2.5 cm diameter was made on depilated thoracic of rats under light ether anaesthesia in aseptic conditions and observed throughout the study. The animals were housed individually. The test sample (herbal extract) was formulated as an ointment in simple ointment base. Formulate ointment (0.5 g) was applied on the wound once daily for 12 days starting from the day of wounding. The observations of percentage wound

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closure were made on 5th, 10th, 15th and 20th post-wounding days. (Morton and Malone, 1972)

Incision model

For the incision wound model, study was adapted under light ether anaesthesia, rats were wounded, and two paravertebral incisions of 6 cm were made through the entire thickness of the skin on the other side of the vertical column with of a sharp blade. The incisions were sutured using 4-0 silk thread with the help of straight rounded needle. The condition threads on both wound edges were tightened for good closure of the wound. The wound was left undressed, and sample drugs along with simple ointment (control) and standard drug were administered once daily for 12 days. When wounds were cured thoroughly, the sutures were removed on 12th post-wounding day and tensile strength was determined on 15th wounding day by adopting continuous constant water-flow technique. The tensile strength was measured with a tensiometer.

Tensiometer

The instrument used for measurement is called a tensiometer. It consists of a 6 × 12 inch wooden board with one arm of 4 inch long, fixed on each side of the possible longest distance of the board. The board was placed at the edge of a table. A pulley with bearing was mounted on the top of one arm. An alligator clamp of 1 cm width was tied on the tip of the other arm by a fishing line (20 lb test monofilament) in such a way that the clamp could reach the middle of the board. Another alligator clamp was tied on a longer fishing line with a 1-L polyethylene bottle on the other end. The tensile strength of a wound represents the degree of wound healing. Usually, wound-healing agents promote a gain in tensile strength.

Determination of tensile strength

The sutures were removed on the ninth day after wound, and the tensile strength was measured on the 10th day. The sample drugs along with standard and control were administered throughout the period once daily for 9 days; on the 10th day, the rats were again anaesthetized, and each rat was placed on a stack of paper towels on the middle of the board. The number of the towels could be adjusted in such a way that the wound was on the same level as the tips of the arms. The clamps were then carefully attached to the skin on the opposite sides of the wound at a distance of 0.5 cm. away from the wound. The longer pieces of the fishing line were placed on the pulley and finally on the polyethylene bottle, and the position of the board was adjusted so that the bottle receives a rapid and constant rate of water from a large reservoir until the wound began to open. The amount of water in the polyethylene bag was weighed and considered as an indirect measure of the tensile strength of the wound. The mean taken as the measures of the tensile strength of the extract-treated wounds was compared with that of the controls. The increment in tensile strength indicates better wound-healing stimulation by the applied drug.

RESULTS AND DISCUSSION

Many studies indicate that plant products are potential agents for wound healing and are largely preferred because of the absence of unwanted side effects and their effectiveness. In

Table 1. Wound-healing activity of ethanolic extracts of *Murraya koenigii*: Excision model

Drug	Wound contraction (mm ²) on day			
	5 th	10 th	15 th	20 th
Control	25.72 ± 0.72	33.44 ± 1.2	42.42 ± 0.43	50.42 ± 0.61
Test sample ointment	29.12 ± 0.41	50.15 ± 0.24	78.82 ± 0.9	84.49 ± 0.94*
Standard (nitrofurazone) ointment	21.63 ± 1.74	42.51 ± 0.51	72.48 ± 1.23	96.91 ± 0.66*

*Values are mean ± S.E.M of animals in each group ($p < 0.01$) vs. control by *t*-test.



Fig. 1. Drug-treated (test)



Fig. 2. Drug-treated (standard)



Fig. 3. Healthy (control)

In this study, we analyzed the wound-healing activity of ethanol extract of *M. koenigii* [15]. The overall performances of topical applications of plant extracts like *Centella asiatica*, *Vernonia arobroea* and *Moringa oleifera* were almost close to the effectiveness of *M. koenigii*. [Manjunatha and Krishnan, 2003]. As shown in Table 1, the studies on excision wound-healing models revealed that all the three groups showed decreased wound area from day to day. However, on 20th post wounding day, group I animals showed 50.42% healing (Fig. 1). Whereas nitrofurazone-treated animals showed 96.91% healing (Fig. 2), the test sample-treated group showed 84.49% wound healing (Fig. 3). Table 2 reveals the incision model, and the tensile strength of the extract-treated group was found to be 352.58 g 15th post-wounding day. When compared with controls, the activity of the extract was found to be highly significant ($p > 0.01$).

Table 2. Wound-healing activity of ethanolic extracts of *Murraya koenigii*: Incision model

Drug	Dose	Grams \pm S.E.
Control	1 ml of 2% acacia	192.8 \pm 8.4
Test sample	200 mg/kg	352.5 \pm 7.2
Standard (griseofulvin)	100 mg/kg	575.45 \pm 8.9

$N = 6$ Mean \pm S.E.M. $p > 0.01$ vs. control

Excision model

In the incision model, the tensile strength of the extract-treated group is found to be 193.33 g on 12th post-wounding day. When compared with the controls, the activity of the extract was found to be highly significant ($p < 0.001$). The ethanolic extracts of *M. koenigii* in the form of ointment (0.5 g) were found to be effective in this model. The wound-contracting ability of the extract ointment and standard reference drug nitrofurazone ointment (0.2 g) was significantly greater than those of the control at 84.49 ± 0.61 and 96.91 ± 0.66 days, respectively.

Incision model

In the incision wound studies, there was a significant increase in tensile strength of the 12-day-old wound due to treatment with *M. koenigii* extract ointment (test) and griseofulvin (standard) ointment when compared with respective control (Table 2). The process of wound healing occurs in four phases: (i) coagulation, which prevents blood loss, (ii) inflammation and debridement of wound, (iii) repair including cellular proliferation and (iv) tissue remodeling and collagen. Any agent that accelerates the aforementioned process is a promoter of wound healing.

CONCLUSION

In this study, the ethanolic extract of leaves of *M. koenigii* was screened for wound-healing activity on male albino rates. In the excision, wound-healing model reveals that all three groups show decreased wound area from day to day. The excision model exhibited significantly better wound-healing activity than the control ($p < 0.01$ vs. control by test). Incision model showed a significant increase in tensile strength of the 12-day-old wound due to treatment with *M. koenigii*.

The activity of the extract was found to be highly significant ($p > 0.01$). Thus, the leaves of *M. koenigii* were found to exhibit significant wound-healing activity.

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