

Evaluation of wound-healing effect of *Ziziphus mauritiana* L. leaf extract in rats

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Background: *Ziziphus mauritiana* L. leaves are used traditionally for burns, wounds, inflamed parts, hypertension and as a diuretic. **Objective:** To evaluate the wound-healing property of ethanol extract of *Ziziphus mauritiana* L. leaf (EEZM) on rats using excision, incision and dead space wound models. **Materials and Methods:** Wistar albino rats (150 – 200 gm) were divided into four groups ($n = 6$). Group I (control) animals received no treatment, group II animals were given EEZM 300 mg/kg orally, group III animals were applied EEZM gel, 100 mg/cm² and group IV animals were applied Framycetin skin cream, 100 mg/cm² topically. In incision and dead space wound models, the treatment was given for 10 days only from the day of wound, skin-breaking strength and dry granulation tissue weight were estimated in incision wound model. Hydroxyproline content was estimated in dead space wound model. In excision wound model, treatment continued till the complete healing of the wound. Rate of wound contraction and period of epithelisation was evaluated thereafter. The data was analyzed by one-way ANOVA, followed by Dunnett's test. $P < 0.05$ is considered significant. **Results:** EEZM increased the rate of wound contraction, decreased period of epithelisation, increased skin-breaking strength and dry granulation tissue weight, elevated hydroxyproline content. **Conclusion:** *Ziziphus mauritiana* possesses wound healing activity.

Key words: Period of epithelisation, skin breaking strength, wound healing, wound contraction, *Ziziphus mauritiana*

INTRODUCTION

A wound is a disruption of tissue integrity that results in damage and is typically associated with loss of function. Wound healing can be defined as a complex dynamic process that results in the restoration of anatomic continuity and function. It is a finely orchestrated and overlapping sequence of events involving – control of infection, resolution of functional connective matrix, contraction, resurfacing, differentiation and remodelling. Wounds are generally classified as wounds without tissue loss (e.g. in surgery) and wounds with tissue loss, such as burn, wound caused as a result of trauma, abrasions or as secondary events in chronic ailments e.g.: venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and derma abrasions.^[1] Wound healing involves complex series of interactions between different cell types, cytokine mediators and the extracellular matrix. The phases of normal wound healing include haemostasis, inflammation, proliferation and remodelling.^[2]

Many medicinal plants have a very important role in the process of wound healing. Plants are potent healers because they promote the repair mechanisms in the natural way. Plant-based therapy not only accelerates healing process, but also maintains the aesthetics. More than 70% of wound-healing pharma products are plant based, 20% are mineral based and remaining contains animal products as their base material. The plant base materials are used as first aid – antiseptic coagulants and wound wash. In recent times, focus on plant research has increased all over the world and large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5-year period.^[3]

Plants have been used for medicinal purposes for as long as history has been recorded. India is inhabited by a wide variety of tribal populations who dwell in forested areas and depend on surrounding resources for their livelihood. Various tribes used *Ziziphus mauritiana* L. leaves for many of their ailments such as burns, wounds, inflamed parts, hypertension and as diuretic.^[4-6] Several studies have indicated antioxidant and free radical scavenging activity,^[7] antimicrobial activity,^[8,9] hepatoprotective activity^[10,11] and anthelmintic activity^[12] of *Ziziphus mauritiana* L. leaf extract. *Ziziphus mauritiana* L, family *Rhamnaceae*, is commonly known as BER in Hindi and BERIKAI in Kannada. The present study is taken up to evaluate

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wound-healing effect of ethanolic extract of *Ziziphus mauritiana* L leaf (EEZM) in rats using excision, incision and dead space wound model.

MATERIALS AND METHODS

Drugs and Chemicals

Ketamine was procured from Neon Laboratories Limited, Mumbai, Diazepam was procured from Ranbaxy Diagnostics and Framycetin skin cream was procured from Aventis Pharma Limited, Goa.

Plant Material and Preparation of Topical Formulation

Standardised ethanolic extract of *Ziziphus mauritiana* L leaves procured from Green Chem Herbal Extract and Formulations, Bangalore, India was used for the study. The topical gel (10% w/v) was prepared by soaking sodium CMC in water for 30 min. Ten grams of *Ziziphus mauritiana* leaf extract was incorporated in 100 gm of sodium carboxymethyl cellulose gel by mixing so as to get 10% w/w of EEZM gel. Methyl paraben was incorporated in the EEZM gel as preservative.

Experimental Animals

Inbred albino rats of Wistar strain of either sex, weighing 150 - 200 g, maintained on a 12 ± 1 h day and night schedule, fed with standard diet (Kohinoor Feeds, Bangalore) and water *ad libitum* were used in the present study. The study was approved by the Institutional Animal Ethical Committee of Visveswarapura Institute of Pharmaceutical Sciences, Bangalore and experiments were conducted as per the guidelines of CPCSEA.

For each of the three models viz., excision, incision and dead space wound models, the rats were divided into four groups ($n = 6$), Group I animals served as normal control and received no treatment; group II animals were given EEZM 300 mg/kg orally, group III animals were applied EEZM gel, 100 mg/cm² and group IV animals were applied Framycetin skin cream 100 mg/cm² topically. Doses selected are based on literature studies.^[10,11] The animals were starved for 12 hrs prior to wounding. Wounded animals were kept separately, one in each cage.

Excision Wound Model

An impression was made on the dorsal thoracic region using round seal of 2.5-cm diameter on the anaesthetised rat. The

skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm² diameters. Treatment was continued till the complete healing of wound. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. Contractions contributed for wound closure on 4th, 8th, 12th, 16th and 20th post-wounding day were observed along with period of epithelisation.^[13,14]

Incision Wound Model

Two longitudinal para-vertebral incisions of 6cm length were made through the skin and cutaneous muscle, at a distance of about 1.5 cm from the middle on each side of depilated back. After the incision, the parted skin was sutured 1-cm apart using a surgical thread and curved needle. The wound were left undressed. The sutures were removed on 9th post-wound day. The skin-breaking strength of the 10th day wound was measured by constant water flow technique.^[13]

Dead Space Wound Model

Dead space wound were made by implantation of polypropylene tubing (0.5 × 2.5cm), beneath the dorsal para vertebral skin at fore limbs and hind limbs. On 10th day the granulation tissue from the dead space wound were dissected, and cut into two approximately equal halves. One of the granulation tissue was dried in an oven 60°C and dry weight was noted. The granulation tissue so harvested was subjected to hydroxyproline estimation.^[13,14]

Statistical Analysis

All the values are expressed as mean ± SEM. The data was analyzed by one-way ANOVA, followed by Dunnett's test. $P < 0.05$ is considered significant.

RESULTS

Excision Wound Model

The rate of wound contraction was increased and period of epithelisation was reduced by treatment with EEZM gel, 100 mg/cm² applied topically [Table 1].

Incision Wound Model

EEZMgel, 100 mg/cm² applied topically, increased the wound breaking strength on day 10 [Table 2].

Table 1: Effect of ethanolic extract of *Ziziphus mauritiana* L leaf on wound contraction and period of epithelisation

Treatment	WC on day 4 in mm ²	WC on day 8 in mm ²	WC on day 12 in mm ²	WC on day 16 in mm ²	WC on day 20 in mm ²	Period of epithelisation in days
Control	475.33±1.99	382.33±1.40	287.83±1.64	194±1.75	120.16±2.34	26.16±0.30
Zm 300 mg/kg p.o.	413±1.59	254.66±3.73	181±1.91	106.83±2.77	29.33±2.49	22.33±0.21
EEZM gel 100 mg/cm ² topical application	395.33±0.9*	223.1±1.47*	142.5±1.97*	87.16±2.16*	4.33±2.76*	19.5±0.56*
Framycetin skin cream 100 mg/cm ²	385.33±1.3*	227.5±1.99*	147.66±1.58*	93.33±1.97*	2.16±1.42*	19.33±0.49*

Values are expressed as mean±SEM, $n=6$ $P<0.01$ v/s control, one-way ANOVA, followed by Dunnett's test. WC – Wound contraction

Table 2: Effect of ethanolic extract of *Ziziphus mauritiana* L leaf on wound-breaking strength, dry tissue weight and hydroxyproline content

Treatment	Wound breaking strength in grams	Dry tissue weight (mg % of body weight)	Concentration of hydroxyproline ($\mu\text{g/g}$ tissue)
Control	322.83 \pm 4.34	36.63 \pm 0.96	2154.83 \pm 69.87
Zm 300 mg/kg p.o.	407.5 \pm 5.87	54.6 \pm 0.87	5474.16 \pm 63.11
EEZMgel 100 mg/cm ² topical application	444 \pm 2.7*	64.76 \pm 1.07*	6233.33 \pm 56.19*
Framycetin skin cream 100 mg/cm ²	486.83 \pm 4.8*	75.25 \pm 1.06*	6691.33 \pm 50.96*

Values are expressed as mean \pm SEM, n=6* P<0.01 v/s control, one-way ANOVA, followed by Dunnett's test

Dead Space Wound Model

EEZMgel, 100 mg/cm² applied topically, increased dry tissue weight and hydroxyproline content [Table 2].

DISCUSSION

The present study was undertaken to evaluate wound-healing activity of *Ziziphus mauritiana* L leaf in experimentally produced wounds in rats. The study was designed to investigate the effect of the *Ziziphus mauritiana* L leaf on three phase of wound healing, namely collagenation, wound contraction and epithelisation.

In the present investigation, EEZM gel promoted wound contraction and decreased period of epithelisation in excision wound model. This may be due to presence of phytochemical constituents like saponins, flavonoids, triterpenoids^[15] and tannins.^[16] These constituents are known to promote wound contraction and decrease period of epithelisation due to their astringent, anti-oxidant and antimicrobial activity.^[17] Tannins promote the wound healing through several cellular mechanisms; chelation of free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation capillary vessels and fibroblasts and including keratinocyte proliferation.^[14]

In incision wound model, EEZMgel increased mean tensile strength, indicating better collagen synthesis. The increased tensile strength of the wound observed in the present study may have been contributed by the flavonoids present in *Ziziphus mauritiana*. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibers, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to granulation tissue.^[17]

As seen from Table 2, the hydroxyproline content of the granulation tissue of the animals treated with EEZM gel was significantly increased, indicating increased collagen

turnover. Increase in granulation dry weight is indicative of higher protein content and collagen maturation.^[18]

Topical application of *Ziziphus mauritiana* ethanolic leaf extract gel was found to possess better wound-healing property than oral administration. Topical route can be preferred as wound-healing agent, as it has minimal systemic toxicity and is convenient to use.^[19,20]

The present findings provide scientific evidence for ethanolic extract of *Ziziphus mauritiana* L leaf as potential wound healer, there by justifying its use in the indigenous system of medicine. However, confirmation of this suggestion will need well-designed clinical evaluation.

CONCLUSIONS

Ziziphus mauritiana possesses wound-healing activity.

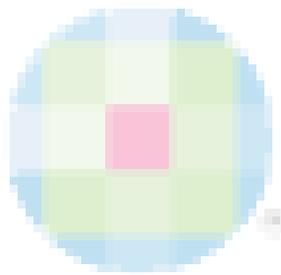
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