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Research Article

WOUND HEALING ACTIVITY OF KIGELIA PINNATA BARK EXTRACT

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ABSTRACT

Purpose: The aim of the present study was to investigate the wound healing activity of the selected Indian medicinal plant Kigelia pinnata.
Method: Aqueous extract of the shade-dried bark of Kigelia pinnata was studied for its effect on wound healing in rats, using incision, excision and dead space wound models, at two different dose levels of 250 and 500 mg/kg.
Result: The plant showed a definite, positive effect on wound healing.
Conclusion: The efficacy of this plant in wound healing may be due to its epithelization, thereby justifying the traditional claim.

Key words: Kigelia pinnata , wound healing, sausage tree, cucumber.

INTRODUCTION

Kigelia pinnata (Bignoniaceae) is a small tree found in south, central and west africa and also in India[1,6]. The bark arises on stem of this tree. In Asian countries, the bark of *K. pinnata has* been used for centuries in oriental traditional medicines for treating inflammatory and malaria diseases. The bark of *K. pinnata* have also been pharmacologically documented to possess antiamoebic, antifungal, antiulcer, antibacterial, antioxidant activities and wound healing[2].

The main constituents found in the bark of *K. pinnata* are naphthoquinone lapachol, phenyl propanoid, stigmasterol, β sitosterol and small amounts of free ferulic acid, p- coumaric acid and 6 methoxymelein,. The various chemical constituents such as naphthaquinones, iridoids, fatty acids, norviburtinal, sterols, lignans, terpenoid, and flavonoids are the essential building block responsible for its wide range of activities[3].

We are unable to find any information on the wound healing properties of this plant. The present study is therefore an attempt to assess the efficacy of the bark using different parameters of wound healing in rats.

MATERIALS AND METHODS

Plant materials

The bark of *K. pinnata* used in this study were obtained from the local market and were identified based on its physical characteristics. The bark were crushed to small pieces using pestle and mortar and powdered in an electric grinder [4].

Phytochemical Screening

The powder of the bark of *K. pinnata* (500g) was subjected to successive extraction with different solvents like ethanol, methanol and water. The dry extracts were subjected to various chemical tests in order to detect the presence of different phytoconstituents.

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, saponins and glycosides were carried out on the bark powdered using standard procedures [7,8].

Preparation of Aqouos Extract

The shade-dried, powdered bark (1 kg) were extracted exhaustively using water on a Soxhlet apparatus. The total aquous extract was concentrated in vacuo to a syrupy consistency (yield 10.52%)[5].

Animals

Wistar albino rats of either sex, weighing about 150–250 each, were used for the study. They were fed with standard food and water *ad libitum*. They were housed in polypropylene cages maintained under standard conditions (12 hour light - dark cycle;25 ± 3 °C; 35–60% humidity). The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, and was cleared by same before beginning the experiment.

Acute Toxicity Studies

Healthy adult albino rats of either sex, fasted overnight, were divided into 6 groups (n = 6 per cage) and were fed with increasing doses (1, 2, 4, and 5 g/kg body wt.) of the aquous extract. The total aquous extract, administered orally in doses of up to 5000mg/kg body wt., did not produce any evident sign of toxicity or mortality in rats up to 14 days after administration.

Wound models

The studies were carried out using ether-anesthetized rats and their back was shaved, in three different wound models, at three different dose levels of 250 and 500 mg/kg body wt.

Incision wounds

Two, 6-cm long paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column of the rat[10]. Wounds were closed with interrupted sutures,1 cm apart. The sutures were removed on the seventh day. Wound-breaking strength was measured in anesthetized rats on the tenth day after wounding[11].

Excision wounds

A circular skin piece of full thickness (approximately 500 mm^2) was removed from a predetermined dorsal area[12]. The wounds were traced on 1- mm^2 graph paper on the day of wounding and subsequently on alternate days until healing was complete. Changes in the wound area were calculated, giving an indication of the rate of wound contraction. The number of days required for falling of the eschar without any residual raw wound was determined as the period of epithelization.

Dead-space wounds

These wounds were created by implanting two polypropylene tubes (0.5 cm × 2.5 cm each),one on either side, in the lumbar region on the dorsal surface of each rat. On the tenth postwounding day, the granuloma tissue formed on the implanted tubes was dissected out carefully. Granuloma tissue from one tube was maintained (at −64 °C) for the estimation of the determination of tensile strength[12], after which it was dried in an oven at 60 °C for 24 h and the dry weight noted. The acid hydrolysate of the dry tissue was used for the estimation of hydroxyproline[9] content in the tissue[13].

Table 1: Effect of the aqueous extract of *K. pinnata* on wound healing in the incision wound models

Wound parameter Studies	Incision model		Dead space model	
	Breakingstrength(g)	Granuloma weight (g/100g)	Breaking strength(g)	Hydroxyproline (mg/100g)
Control	211.333±4.780	10.537±0.515	139.920±0.297	840.645±0.445
Aqueous extract of bark of kigelia pinnata 250mg/kg body weight	415.15±2.140***	42.690±0.585**	339.898±1.023***	1647.330±1.532
Aqueous extract of bark of kigelia pinnata 500mg/kg body weight	327.983±1.654***	48.105±0.056***	359.785±12.294***	1928.218±18.202***

*** P < 0.0001 compared to Control. Values are mean ± S.E.M. (n = 6)

Table 2: Effect of the aquous extract of *kigelia pinnata* on the excision wound model

Wound model		Excision % of wound contraction						
Parameter Studies	Epithelization period (Days)	2	4	6	8	10	12	14
Control	21.633 ± 0.139	14.995 ± 0.139	33.547 ±0.163	38.465 ±0.129	46.473 ±0.099	77.492 ±0.105	86.667 ±0.103	91.48 ±0.54
Aqueous extract of bark of kigelia pinnata 250mg/kg	14.545 ± 0.377***	24.838 ± 0.134***	44.323 ±0.163***	59.268 ±0.055**	79.537 ±0.099***	87.410 ±0.036***	97.347 ±0.075***	92.21 ±0.12***
Aqueous extract of bark of kigelia pinnata 500mg/kg body weight	13.275 ± 0.150***	24.247 ± 0.162***	29.480 ±0.320***	54.440 ±0.128***	72.757 ±0.094***	91.648 ±0.102***	95.503 ±0.074***	98.58 ±0.42***

*** P < 0.0001 compared to control, *** P < 0.0001 compared to 500 mg. Values are mean ± S.E.M. (n = 6)

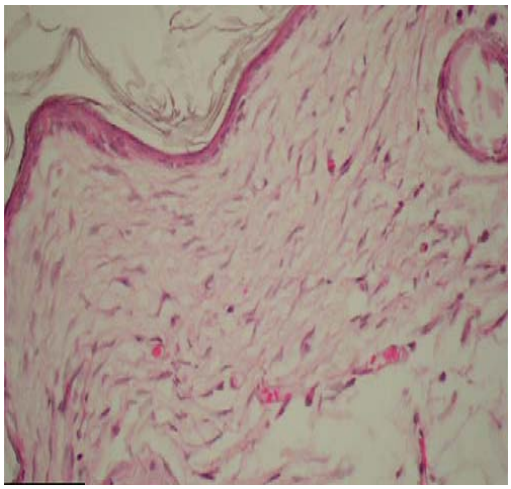

Fig. 1: Control (H&E 400×) showing well formed but thick granular cell layer, theunderlying dermis contains deposited collagen fibers with minimal inflammation

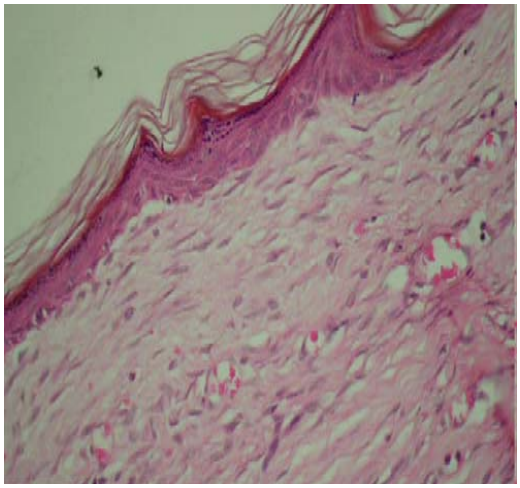

Fig. 2: Animals treated with *Kigelia pinnata* (H&E 400×) showing thin well-formed epidermis with hair follicle formation in the dermis and no inflammatory cells in a well organized dermis.

Histopathological studies

A section of the granuloma tissue was subjected to histopathological examination to determine the pattern of lay-down for collagen using two special stains i.e.Van Gieson and Masson Trichrome[14].

Statistical Analysis

Results, expressed as mean ± SE., were evaluated using one-way ANOVA with posthoc Scheffe's *post hoc* test. Values of p <0.0001 were considered statistically significant.

RESULTS

Preliminary phytochemical screening revealed the presence of naphthoquinone lapachol, stigmasterol, β sitosterol, phenyl propanoid, p-coumaric acid, ferulic acid compounds.The acute toxicity studies show that the drug was safe up to a maximum dose of 5000mg/kg body wt. of the animal.

In the incision wound model, a significant increase was observed in the skin tensile strength of the ethanol extract-treated group on the tenth post-wounding day, at both dose levels (Table 1). The drug-treated animals of the dead-space wound model showed a

significant increase in dry granuloma weight, granuloma breaking strength and the level of hydroxyproline content at both dose levels. Histological examination revealed increased collagen deposition in the drug, treated group (Fig. 2), as compared to control (1).

In studies using the excision wound model, animals treated with the aquous extract of *K. pinnata* showed a significant decrease in the epithelization period, as evidenced by the shorter period for the fall of eschar compared to control. The extract also facilitated the rate of wound contraction significantly at both dose levels (Table 2).

The results in this study are in support that the wound healing and repair is accelerated by applying *kigelia pinnata* which was high-lighted by the full thickness coverage of the wound area by an organized epidermis in the presence of mature scar tissue in the dermis.

DISCUSSION

Wound healing involves various phases which include granulation, collagenation, collagen maturation and scar maturation. Many plant extracts and medicinal herbs have shown potent antioxidant activity. naphthoquinone lapachol, stigmasterol, β sitosterol the main components of many plant extracts, act as free radical scavengers. Research into the role of antioxidants from plant extracts in wound healing has been published widely. Wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent to each other. Hence in the present study two different wound models were used. In the incision wound model, a significant increase was observed in the skin tensile strength of the aquous extract- treated group, at both dose levels (Table 1). The drug animals at both dose levels of the dead-space wound model showed a significant increase in dry granuloma weight, granuloma breaking strength and the level of hydroxyproline content (Table 1).

The histopathological study revealed increased collagen deposition in the drug, treated group (Figs. 1, 2), as compared to control. In studies using the excision wound model,animals treated with the aquous extract of *K. pinnata* showed a significant decrease in the epithelization period, as evidenced by the shorter period for the fall of eschar compared to control. The drug extract also facilitated the rate of wound contraction significantly at both dose levels (Table 2).

Phytochemical work reveals that aquous extract of bark of *K.pinnata* contains high amount of free ferulic acid and 6 methoxymelein naphthoquinone lapachol, stigmasterol, β sitosterol ,implied that β sitosterol is one of the active compounds which may be responsible for the epithelization activity. So in this study scavenging effect might be one of the most important components of wound healing which may be responsible to support wound healing property. Thus the enhanced wound healing may be due to the free radical scavenging action of the plant.

CONCLUSION

This finding provides an insight into the usage of the bark of *K.pinnata* in traditional treatment of wounds or burns associated with bacterial infections.

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