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The effect of ethanol extract of *Wrightia tinctoria* bark on wound healing in rats.

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Abstract

Objectives: To screen the wound healing activity of ethanol extract of *Wrightia tinctoria* bark using incision, excision and dead space wound models and evaluate histopathological and biochemical changes of granuloma tissue. Material and method: The bark powder of *W. tinctoria* was extracted with 95% ethanol by continuous heat extraction and was subjected for phytochemical investigation and screened for wound healing activity in the incision, excision and dead space wound models in rats. A supportive study made on granuloma tissue to estimate the hydroxyproline content and histopathological examination to determine the pattern of lay-down for collagen using Masson Trichrome stain. Results: Triterpenoids, steroids and saponins were found to be present in ethanol extract of the barks of *W. tinctoria*. In resutured incision wound model, ethanol extract showed significant breaking strength ($P<0.01$) compared to control. The ethanol extract promotes better wound healing by increasing the percentage wound closure and decreasing epithelization time ($P<0.001$) compared to control. Statistically significant increase ($P<0.001$) was observed in breaking strength and hydroxyproline content of ten day old granuloma of drug treated animals compared to control animals in dead space wound model. Conclusion: The results of the present study reveal that ethanol extract of bark of *W. tinctoria* have significant wound healing activity. The pro-healing action seems to be due to the increased synthesis of collagen, it's cross-linking as well as better alignment and maturation. This may be attributed to the presence of triterpinoids in the title plant.

Key words: *Wrightia tinctoria,* Wound healing activity, Hydroxyproline, Histopathology.

1. Introduction

Wrightia tinctoria (Roxb.) R.Br. (Sans:Asita-Kutaja) belonging to family Apocynaceae is a small deciduous tree with pale grey, smooth bark, distributed in tropical Africa and Asia. It is reported to possess aphrodisiac, anthelmintic, anti-inflammatory, astringent and antimicrobial properties. The species has traditionally been used for the treatment of psoriasis, leprosy and

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infections of chest in asthma [1,2]. The major active constituents of the title plant are saponins, β-sitosterol, triterpenoids such as wrightial [3], ursolic acid, lupeol and α and β amyrins [4].

The private communications from villagers in the surroundings of Dharwad and Manipal (Karnataka) revealed that poultice prepared from the plant to treat Psoriasis, skin disease and wounds with promising results. In the Ayurvedic literature, it was mentioned that decoction and poultices prepared from the bark were used for washing wounds [5].

Therefore, it was thought worthwhile assessing the efficacy of this indigenous plant on varying parameters related to wound healing in rats.

2. Materials and methods

2.1 Plant material

The plant material of *Wrightia tinctoria* (Roxb.) R.Br. was collected from in and around Dharwad (Karnataka), in the month of May 2000. The plant was authenticated by Dr. Gopalakrishna Bhat, Professor, Department of Botany, Sri Poorna Prajna College, Udupi, Karnataka. The specimens were preserved in the herbarium of our Department.

2.2 Preparation of the plant extract

The shade dried and powdered bark of *W. tinctoria* (4 kg) was extracted exhaustively with 95% ethanol in a soxhlet apparatus by continuous heat extraction. The ethanol extract was concentrated to a small volume and then evaporated to dryness (Yield-9% w/w). Two triterpenoids, α- and β-amyrin acetates were isolated from the title plant in our laboratory.

2.3 Phytochemical screening

The coarse powder of the bark of *W. tinctoria* (300g) was subjected to successive extraction with different solvents in their increasing order of polarity from petroleum ether (60-80°C), diethyl ether, ethyl acetate, 20% methanol in ethyl acetate to finally alcohol [6, 7]. The dry extracts were subjected to various chemical tests to detect presence of different phytoconstituents.

2.4 Ethics

Before starting the experiment on animals, the experimental protocol was subjected to the scrutiny of the institutional animal ethics committee. Clearance was procured before starting the experiments (No. IAEC/KMC/11/2001).

2.5 Animals

Male Albino rats (Wistar strain) weighing 150-200 g were used for the study. Experimental animals were individually housed in polypropylene cages and well ventilated rooms. They were maintained on standard animal chow (Hindustan lever, rat pellet) and water *ad libitum* throughout the course of the study.

2.6 Pharmacological screening

2.6.1 Acute toxicity study

Healthy adult albino rats of either sex, starved overnight, were divided in to six groups (n = 6) and were orally fed with increasing doses (10, 30, 100, 300, 1000 and 3000mg/kg b.w) of ethanol extract to determine the safer dose by an up and down staircase method [8]. The animals were observed continuously for one hour, frequently for the next four hours and there after for 24 h. After administration of the drug, Irwin test was conducted, where the animals were observed for behavioral changes.

2.6.2 Wound healing studies

Albino rats were divided in to two groups (8-10 rats in each group). Group-I (control) was treated with 1ml of 2% tragacanth orally. Group-II was administered orally the extract at a dose of 375 mg/kg body weight. The animals were starved for 12 h prior to wounding. Under

light ether anaesthesia, wounding was performed after sterilizing the area with ethanol. Dried extract (6 g) was incorporated in a 60ml of 2% w/v solution of tragacanth in water and was administered for excision, incision and dead space wound models, depending upon the body weight of the each animal. Animals showing infection during the experimentation were excluded from the study. No local or systemic chemotherapy was provided.

2.6.2.1 Resutured incision wound [9]

Two para-vertebral straight incisions of 6 cm each were made through the entire thickness of skin on either side at least 1 cm lateral to the vertebral column. Wounds were sutured with catgut. Sutures were removed on 7th post wounding day and the breaking strength was estimated on 10th post wounding day by continuous, constant water flow technique [10].

2.6.2.2 Excision wound [11]

This model was employed to study the rate of wound contraction and epithelization. A round seal of 2.5 cm in diameter was impressed on the dorsal thoracic central region 5 cm away from the ears. The entire thickness of the skin from demarked area was excised to get a wound measuring around 500 mm^2. Animals were subjected to the treatment from '0' day till the wound completely healed or upto 21st post wounding day, whichever was earlier. The observations of percentage wound contraction were made on 2nd, 6th, 10th and 14th post wounding days.

2.6.2.3 Dead space wound [12,13]

Wound was created by implanting subcutaneously 2.5 x 0.5 cm polypropylene tube in the lumbar region on dorsal side of the animal. Animals received drugs from '0' day to 9th post wounding day. On the 10th day, granulation tissue developed around the tube was harvested. The tubular granulation was cut along its length to obtain a sheet of granulation tissue, which was further cut into approximately equal pieces. The breaking strength was measured by continuous, constant water flow technique [10]. The granuloma tissue was dried in an oven at 60°C for 24 h and the dry weight noted. Acid hydrolysate of the dry tissue was used for the determination of hydroxyproline [14].

2.6.2.4 Histopathology

Small pieces of the granuloma tissue were subjected to histopathological evaluation so as to examine the pattern of lay down for collagen using Masson Trichrome stain. Later, the microscopic slides of the granuloma tissues were photographed.

2.7 Statistical analysis

All results are presented as the mean ± s.e.m. The test of significance was statistically analyzed using Student's *t* test [15].

3. Results

Preliminary phytochemical screening revealed the presence of triterpenoids, steroids and saponins.

In the present study, animals showed good tolerance to ethanol extract of *W. tinctoria* and a dose as high as 3000mg/kg was found to be non-lethal. Hence 375mg/kg dose (1/8th of highest safer dose) was selected in the study.

In resutured incision wound model, extract showed significant increased ($P<0.01$) mean breaking strength (350.6 ± 12.6 g) of a 10 days old wound compared to control (296.7 ± 5.1) as shown in Table 1. The drug treated animals of dead space wound model showed significant increase ($P<0.001$) in dry granuloma weight, granuloma breaking strength and hydroxyproline content (Table 1).

The histo-pathological study revealed that the drug treated granuloma showed marked

Table 1.
Effects of orally administered ethanol extract of *Wrightia tinctoria* bark on resutured incision and dead space wounds in rats.

Treatment	Incision	Dead space		
	breaking strength (g)	Dry granuloma weight (mg)	Breaking strength (g)	Hydroxyproline content (μg)
Control	296.7 ± 5.1	37 ± 1.8	192.5 ± 3.1	979.4 ± 0.8
Extract 375 mg/kg	350.6 ± 12.6[b]	99.36 ± 1.8[a]	315 ± 3.7[a]	2020.17 ± 139.3[a]

Values are mean ± s. e. m. of six rats in each group. [a]P<0.001, [b]P<0.01 significantly different from control (Student's *t* test).

Table 2.
Mean percentage wound closure and period of epithelization of excision wounds following post wounding days.

Treatment	2nd day	6th day	10th day	14th day	Period of Epithelization in days
Control	19.4 ± 0.6	42.2 ± 0.82	83.4 ± 0.97	94.9 ± 0.79	19.5 ± 0.8
Extract 375 mg/kg	21.9 ± 0.7[a]	56.1 ± 2.0[a]	96.2 ± 0.7[a]	99.2 ± 0.3[a]	15.5 ± 0.4[a]

Values are mean ± s. e. m. of six rats in each group. [a]P<0.001 significantly different from control (Student's *t* test).

increase in both the number of proliferating capillaries and amount of fibroblastic collagenous connective tissue, when compared with granuloma of the control animal.

The excision wound heals by contraction and epithelization. The mean percentage wound closure (Table 2) for extract was found to increase significantly at P<0.001 on 2nd, 6th, 10th and 14th post wounding days in comparison with control. Where as, the extract showed significantly decreased period of epithelization (15.5 ± 0.4 days), when compared with control (19.5 ± 0.8 days).

4. Discussion

The wound healing activity proposed for the extract of bark of *W. tinctoria* was in line with the observations made. It has been reported that triterpenoids possess an ability to increase the collagen content, which is one of the factors promoting wound healing [16].

As the title plant is rich in triterpenoids, it may be responsible for the wound healing activity. Furthermore aforesaid activity was attributed to free radical scavenging activity of amyrins and lupeol [17]. Thus the triterpenoids, by reducing the lipid peroxidation, may not only prevent or slowdown the onset of cell necrosis, but also improve vascularity.

Lipid peroxidation is an important process in several types of injuries like burns, infected wounds, skin ulcers, etc. Hence any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils, which in turn results in increase in the strength of collagen fibres by increasing the circulation, preventing the cell damage and promoting the DNA

synthesis [18]. This is suggested by the fact that there was an increase in the wound breaking and granuloma breaking strength after the administration of the title plant extract.

Thus it may be concluded that the bark of *W. tinctoria* is endowed with significant wound healing activity, justifying its use in the traditional system of medicine. Further studies are in progress to ascertain the bioactive components of the plant extract.

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