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Wound healing profile of *Sauropus androgynus* in Wistar rats

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Abstract

Objective: To evaluate the effect of *Sauropus androgynus* on wound healing. Materials and Methods: Excision and incision wound models were used to evaluate the wound-healing activity of 5% *Sauropus androgynus* on Wistar rats of either sex. Results: *Sauropus androgynus* extract promoted the wound healing activity significantly in both wound models studied, while no action was seen with ointment base. It augmented wound contraction significantly ($P<0.001$), re-epithelization very significantly ($P<0.0001$) and increase in wound breaking strength ($P<0.001$). Histological evaluation of wound tissue showed abundant collegenation, fibroblast and lesser macrophages in animal treated with 5% *Sauropus androgynus* when compared to control and ointment base. Conclusion: Extract of *Sauropus androgynus* promotes wound-healing activity.

Key words: *Sauropus androgynus*; wound healing; wound re-epithelization; wound contraction.

1. Introduction

Sauropus androgynus is a widespread perennial shrub belonging to the family Euphorbiaceae. It was introduced in India in 1950s. The plant has many medicinal properties *viz.* leaves, young shoots, dried and crushed roots are used to treat fever, headache, hyperlipidemia, hyperuricemia, worm infestation, bronchiolitis obliterans syndrome and constipation [1]. Leaf juice was also widely advertised as "natural diet" good for weight reduction. It contains high level of provitamin A, vitamin B, vitamin C, proteins and minerals [2]. The objective of the present study was to evaluate action of *Sauropus androgynus* on wound repair, to provide scientific basis for medicinal value in the management of wound and to validate the traditional claim that *Sauropus androgynus* is a promoter of wound healing.

2. Material and Methods

Extraction of active constituent

Leaves of *Sauropus androgynus* were collected and shade dried and sent to Natural Remedies

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Pvt. Ltd and 5% cream was prepared by the following process:

Step I: White soft paraffin (20% w/w), Ceto stearyl alcohol (6.5% w/w) Cresmer 1000 (1.5% w/w) mixed together and heated at 60 – 65ºC on a water bath (Ointment base).

Step II: *Sauropus androgynus* water extract (5%w/w) was dissolved in 60% of water and heated upto 50ºC separately (extract solution).

Step III: The extract solution obtained in step II was added under stirring to step I.

Step IV: Dissolve preservatives Bronopol (0.1% w/w) in quantity sufficient to 100 ml water and add to the above mix of step III under stirring.

Step V: Add Imid urea (30%) solution (1% w/w) and 2-phenoxyethanol (1% w/w) under stirring to step IV (preservatives) Continue stirring for 5 to 10 min. Allow the final product for cooling to get the final product in the form of cream. The final product was filled in 100g tubes.

Ointment base was prepared without the active *Sauropus androgynus* water extract.

Animals

Singly housed healthy male Wistar rats weighing 150-250g were used in this study. The animals were fed with standard pellet diet with water ad libitum. Following an overnight starving, animals were anaesthetized with Pentobarbitone 30mg/kg/i.p and suitably wounded after shaving the area to be operated to bear excision or incision wounds. The study was approved by the Institutional Animal Ethics Committee.

2.1 *Wound models*

2.1.1 Incision wounds

Full skin thickness incision of 6 cm lengths were made as described by Ehrlich and Hunt [3] on the dorsum of rat on paravertebral plane. Wound edges were approximated by interrupted sutures. Sutures were removed on the 7th day; on the 10th post-wounding day, rats were sacrificed by euthanasia and breaking strength was measured by simple distracting tensiometry device. The gaping of wound was taken as measure of wound breaking strength expressed in gramme [4]. For Histological studies, the granulation tissues were fixed in 10% formalin, the materials were infiltered and embedded with paraffin, microtome section were taken at 10 µ thickness. The sections were processed and then stained with Haematoxylin - Eosin dye, and observed under the microscope of 100 x for the presence of inflammatory cells, collagen texture and nature of fibroblast.

2.1.2 Excision wounds

Excision wounds were made as described by Morton and Malone [5] by excising the full thickness circular skin (500 mm² in area) from the dorsal interscapular region. Wound contraction was monitored by measuring wound area, plannimetrically, on alternate days till the wound was completely healed. This was expressed as percentage of wound contraction. Time taken for complete epithelization was noted by recording the days required for fall of scab leaving no raw wound behind.

2.2 Drug administration

Animals bearing inflicted wound were divided into five groups each containing seven animals for each of excision and incision wound models. First group of animals received no treatment and served as control, second and third group received ointment base of 0.25 cm and 0.5 cm (approximately 0.2 g and 0.4 g) and fourth and fifth groups received the test drug of 5% *Sauropus androgynus* of 0.25 cm and 0.5 cm. These drugs were applied topically once a day.

2.3 *Statistical analysis*

Results were expressed as mean ± SEM. The data were analyzed by using Kruskal Wallis test with Chi square test for comparison between the different groups. $P<0.05$ was considered significant.

Table 1. Effect of *Sauropus androgynus* and ointment base on wound breaking strength

Groups		Resutured incision wound	
n = 7	Dose	Breaking strength (g)	P Value
Control [a]	Not treated	95 ± 5	
Ointment base [b]	0.25 cm	88.3 ± 1.05	0.08
Ointment base [b]	0.5 cm	89.28 ± 1.3	0.15
*Sauropus androgynus**	0.25 cm	120.71 ± 2.3	0.02
*Sauropus androgynus**	0.5 cm	162.86 ± 6.3	<0.0001

a.*p<0.05 vs control; b *p<0.05 vs ointment base; values represent mean ± SEM

Table 2. Effect of topical application of *Sauropus androgynus* and ointment base on excision wound model.

Group n=7	Percentage of closure of excision wound area (original wound area 500 sq.mm)							
	0 Day	3rd Day	7th Day	9th Day	11th Day	13th Day	15th Day	17th Day
Control	510.01 ± 1.52	476.52 ± 1.3 (6.56)	257.33 ± 30.3 (49.5)	220 ± 7.0 (56.86)	146.67 ± 7.6 (71.24)	98.7 ± 5.6 (80.65)	60.5 ± 24.4 (88.14)	30.5 ± 2.4 (94.02)
Ointment base 0.25 cm	506 ± 15.2	484.03 ± 1.82 (4.34)	404 ± 9.8 (20.15)	266.17 ± 27.8 (47.39)	101 ± 15.4 (80.03)	50.33 ± 11.3 (90.05)	11.17 ± 3.15 (97.79)	5.00 ± 1.38 (99.01)
Ointment base 0.5 cm	502.12 ± 1.48	468.40 ± 14.4 (6.71)	421.14 ± 13.7 (16.12)	240.6 ± 15.5 (52.03)	69 ± 6.8 (86.25)	16.86 ± 3.4 (96.64)	9.33 ± 3.4 (98.14)	4.5 ± 1.56 (99.68)
**Sauropus androgynus* 0.25 cm	508.94 ± 0.48	454 ± 1.18 (10.79)	161 ± 8.5 (68.36)	69.14 ± 4.9 (86.41)	39.57 ± 2.5 (92.22)	15.71 ± 3.3 (96.91)	13.6 ± 2.6 (97.32)	0 (100)
**Sauropus androgynus* 0.5 cm	511.86 ± 1.14	403.69 ± 1.48 (21.13)	158.66 ± 10.6 (69.00)	72.5 ± 7.4 (85.83)	25 ± 3.8 (95.11)	9 ± 0.4 (98.24)	0 (100)	0

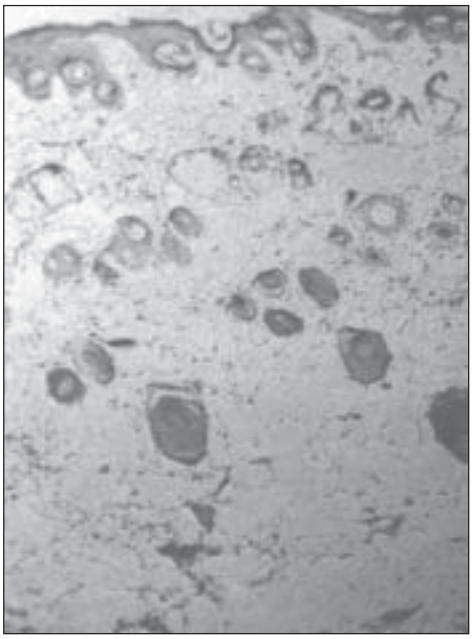
a.*p<0.05 vs control; ointment base; values are mean ± SEM

Table 3. Effect of *Sauropus androgynus* and ointment base on wound re-epithelization.

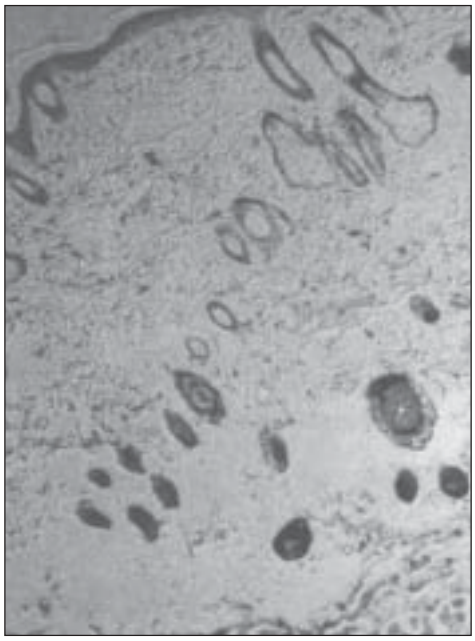
Groups (n = 7)	Dose	Re-epithelization period (days)	P-Value
Control [a]	Not treated	22 ± 0.2	
Ointment base [b]	0.25 cm	17.7 ± 0.2	0.1183
Ointment base [b]	0.5 cm	17 ± 0.3	0.0115
* *Sauropus androgynus*	0.25 cm	16.42 ± 0.3	0.0014
* *Sauropus androgynus*	0.5 cm	14.3 ± 0.4	<0.0001

a. p<0.05 vs control; b. p<0.05 vs ointment base; values are mean ± SEM

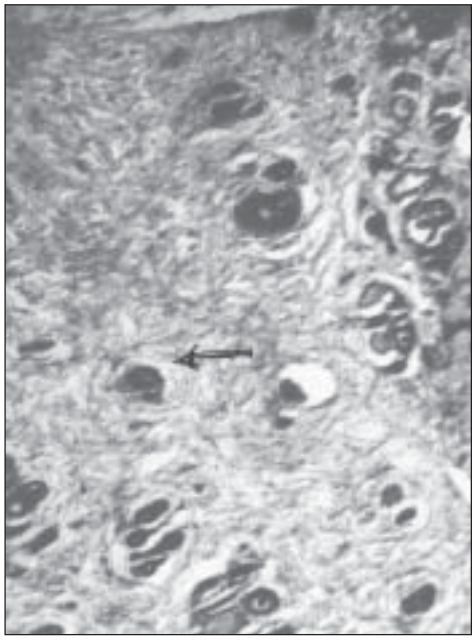
Photomicrograph of granulation tissue
(H and E stain)



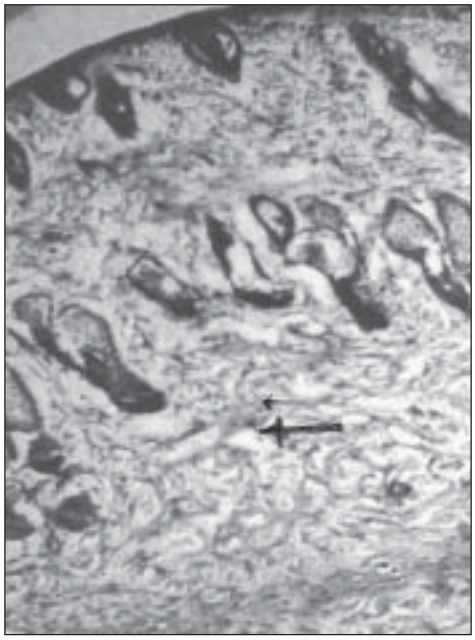
A. Control



B. Ointment base



C. 0.25 cm Sauropus androgynus



C. 0.5 cm Sauropus andrugynus

Histological section of granulation tissue of the animal treated with 0.25 & 0.5 cm of 5% sauropus androgynus shows abudant collegen (arrow), fibroblasts & scanty macrophages as compared to control and ointment base

3. Results

3.1 Incision wounds

Table 1 shows the effect of topical application of ointment base and *Sauropus androgynus* ointment of resutured incision wounds. The wound breaking strength was significant ($p<0.05$) increased in the entire drug treated group as compared to that of control and ointment base.

The histological studies of granulation tissue revealed increase number of fibroblasts and thick bundle of collagen tissues in *Sauropus androgynus* treated groups as compared to that of control and ointment base groups (Fig 1a-1d).

3.2 Excision wounds

The rate of wound contraction as compared to that of control was significantly ($p<0.05$) increased in a dose dependent manner with *Sauropus androgynus* ointment. Ointment base did not alter the pattern of wound contraction as compared to control (Table 2). The *Sauropus androgynus* extract treated animals showed faster epithelization of the wound 14.3 ± 0.4 than the control group 22 ± 0.2. The period of epithelization was 17 ± 0.3 in the case of ointment base treated animals (Table 3).

4. Discussion

Sauropus androgynus (vitamin green) is traditionally used to promote wound repair. This present study was undertaken to validate the traditional claim that *Sauropus androgynus* acts as a prohealer. Topical application of 5% *Sauropus androgynus* ointment on incision and excision wounds in Wistar rats produced a dose dependent significant action on wound tensile strength, wound contraction, and augmented reepithelization very significantly. The ingredients present in *Sauropus androgynus* ointment thus promote collagenation, fibrillogenesis and matrix production.

Sauropus androgynus extract contains carotenoids, vitamin B, ascorbic acid, proteins, minerals, toluene and thymol. It is well recognized that vitamin A have a role in maintaining epithelial integrity [6-7]. Vitamin B acts as a co-factor for collagen synthesis and cross-linking which mainly determines the wound strength [8-9]. Ascorbic acid as an antioxidant is crucial for collagen synthesis, and deficiency of vitamin C retards the wound contraction [10-11]. And thymol is a wound-healing stimulant. Hence, the wound healing effects of *Sauropus androgynus* may be attributable to the presence of vitamins, other minerals and substances present in the extract.

Our preclinical findings suggested that *Sauropus androgynus* is a useful wound healing agents. Hopefully, this will pave the way for introducing viable commercial formulations of *Sauropus androgynus* for the management of wound repair, in the days to come.

5. Acknowledgement

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