

Wound healing activity of ethanolic extract of *Shorea robusta* Gaertn. f. resin

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The ethanolic extract of *S. robusta* resin (10 and 30 % w/w applied locally in excised and incised wounds) produced a dose-dependent acceleration in wound contraction and increased hydroxyproline content and tensile strength of wounds in rats. The results demonstrate wound healing activity of ethanolic extract of *S. robusta* resin.

Keywords: Excised wound, Hydroxyproline, Incised wound, *Shorea robusta*

Wound healing is the process of repair that follows injury to the skin and other soft tissues. It is fundamentally a connective tissue response. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remodeled to form scar¹.

Shorea robusta Gaertn. f. (Sal) is most commonly found in Indonesia, but can also be seen in Malaysia, the Philippines and certain parts of Northern India. Different parts of the plant are traditionally used for the treatment of diverse purposes. The leaves are used to treat wounds, ulcers, itching, leprosy, gonorrhoea, cough, earache and headache. The oleoresin exuded from the cut bark has astringent and detergent properties. The bark is also used to treat diarrhoea, dysentery, wounds, ulcers, itching and vaginal discharges. In Unani system of medicine, the resin is used for treating menorrhagia, enlargement of the spleen and for relieving eye irritations. In Ayurveda the leaves are used as anthelmintic and alexiteric. The powdered stem, bark 'or' bark paste is applied to stop bleeding and promote healing of cuts among the tribal inhabitants of southern Bihar and the Kondhs of southwestern Odisha². The resin obtained from the plant is considered as astringent and detergent and is used in dysentery and for fumigating the rooms of sick persons. Its resin with honey or sugar is given in dysentery and bleeding piles. This is also given in gonorrhoea and for weak digestion. Its bark decoction

is used as drops in ear problems. Besides, its fruits are also used in diarrhoea³.

In view of the importance of medicinal plants as a potential source of cheaper, safer and effective remedies for treating diseases in animals, the present study has been undertaken on *S. robusta* with special emphasis to wound healing activities. *S. robusta* resin has been reported to possess wound healing activity^{4,5}. Moreover, resin was found to possess excellent burn-wound healing property in the experimental trials (unpublished data). *S. robusta* leaf extract has been found to possess significant anti-inflammatory activity⁶. A combination of cow ghee, flax seed oil, *Phyllanthus emblica* fruits, *S. robusta* resin and Yashada bhasma has also been demonstrated to possess wound healing activity⁷. However to the best of our knowledge, no scientific report is available on the use of ethanolic extract of *S. robusta* (SRE) resin in wound healing. Hence, the present study has been undertaken with special emphasis to investigate wound healing activity of SRE.

Materials and Methods

Plant material—Pure *S. robusta* resin was obtained from Odisha State of India where the plants grow naturally. *S. robusta* resin was identified by Departments of Botany and Chemistry, College of Basic Sciences and Humanities, Odisha University of Agriculture and Technology, Bhubaneswar, India.

Preparation of extract—Resin powder (250 g) was extracted in 1L of 70 % ethanol in a soxhlet apparatus at 60°-75 °C. Extract was concentrated by evaporation. The yield was about 16.80 %. The solid

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extract (SRE) was finely powdered and properly mixed @ 3, 10 and 30% using soft white petroleum jelly as vehicle for topical application.

Phytochemical study for qualitative analysis of active principles—Qualitative analysis of SRE for the presence of various medicinally important active phytochemicals such as, alkaloids, anthraquinones, flavonoids, saponins, tannins, sterols, reducing sugars, glycosides, resins and triterpenes was carried out as per the methods described earlier^{8,9}.

Animals—Healthy Wistar albino rats (150-250 g)/Swiss mice (18-20 g) of approximately the same age were used for the study. They were grouped into 5 groups of five animals each, in clean polypropylene cages and maintained (as per guidelines provided by IAEC and CPCSEA) on a balanced ration obtained from the Feed Technology Unit of the Institute. Fresh drinking water was offered to the animals daily *ad libitum*. The experiments were carried out in accordance with the guidelines of Animal Ethics Committee, IVRI, Izatnagar.

The rats were divided into following five groups of five animals each for excised and incised wound healing models. SRE was locally applied, in excised and incised wound models.

Group I: Control group rats with excised and incised wound were treated with soft white petroleum jelly.

Group II: Test group treated with SRE 3 % w/w in soft white petroleum jelly applied locally in excised and incised wounds.

Group III: Test group treated with SRE 10 % w/w in soft white petroleum jelly applied locally in excised and incised wounds.

Group IV: Test group treated with SRE 30 % w/w in soft white petroleum jelly applied locally in excised and incised wounds.

Group V: Standard group treated with framycetin 1 % w/w in base applied locally in excised and incised wounds.

Experimental procedure—Excision and incision wounds were created in rats under the anesthesia induced by ketamine hydrochloride (100 mg/kg body weight, ip) and xylazine (15 mg/kg body weight, im). The extract was applied locally once daily on the excision and incision wounds. In case of incisional wound the treatment was continued till 9th day post-wounding, while in case of excisional wound it was continued till 13th day post-wounding.

Incisional wound model—One mid-dorsal incision (2 cm long) was made through the full thickness of the skin on the middle of the vertebral column under anesthesia induced by ketamine hydrochloride (100 mg/kg body weight, ip) and xylazine (15 mg/kg body weight, im). Wounds were closed with interrupted sutures, 1 cm apart. The sutures were removed on 7th day. Wound breaking strength (WBS) was measured on 10th post-wounding day¹⁰.

Determination of wound breaking strength (WBS)—Rats were secured to the operation table and a line was drawn on either side of the wound 3 mm away from the wound. Two Allis forceps were firmly applied on to the line facing each other. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated container through a string running over to a pulley. Water was allowed to flow from the reservoir slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site pulling apart the wound edges. As and when the wound just opened up, the water flow was arrested and the volume of water collected in the container (approximately equal to its weight) was noted. Three readings were recorded for a given incision wound. The average reading of the group was taken as an individual value of breaking strength. Mean value gives the breaking strength for a given group.

Excisional wound model—A circular piece of full thickness (~400 mm²) was cut off from a predetermined area on the back of the rat under ketamine hydrochloride (100 mg/kg body weight, ip) and xylazine (15 mg/kg body weight, im) anesthesia. Wounds were traced on transparent polythene sheet on the day of wounding and subsequently on the alternate days, until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction¹¹.

Tissue harvesting and estimation of hydroxyproline—After the last measurement of wound area, the animals were sacrificed with an overdose of diethyl ether¹² and the healed skin was carefully lifted, freed of adhesions and excised out along with about 2 mm adjacent normal skin so as to differentiate between the healed and normal skin. The method of hydroxyproline estimation by Neuman and Logan¹³ was employed for the estimation of collagen content of the healed tissue.

Statistical analysis—The data were analysed by Graph Pad InStat Software using one way and two

way ANOVA with Bonferroni's multiple comparison test.

Results

Phytochemical analysis—Phytochemical analysis of the extract showed the presence of triterpenoids, sterols and resin.

Effect of SRE on excisional wound contraction in rats—Results of locally applied SRE on wound contraction in excised wounds are presented in Table 1. SRE (10 % w/w) on 2nd, 4th and 6th day of wound healing, caused significant ($P<0.01$, <0.001 and <0.05 , respectively) increase in wound contraction, as compared to the control group. SRE (30 % w/w) on 2nd, 4th, 6th and 8th day of wound healing also produced significant ($P<0.001$, <0.001 , <0.001 and <0.05 , respectively) increase in wound contraction, as compared to the control group. SRE showed significant ($P<0.05$) dose-dependent increase in wound contraction i.e. results were significantly different when comparison was made between 10 and 30 mg/kg SRE. However, reference drug framycetin (1 % w/w) did not show any significant increase in wound contraction, as compared to control group.

Effect on hydroxyproline content in rats—Results of locally applied SRE on hydroxylproline content in excised wounds are summarized in Table 2. SRE (10 and 30 %) significantly ($P<0.001$) increased the hydroxyproline content, as compared to the control group. SRE showed significant ($P<0.001$) dose-dependent increase in hydroxyproline content i.e. results were significant when compared to 30 mg/kg SRE. However, reference drug framycetin (1 % w/w)

showed significant ($P<0.001$) decrease in hydroxyproline content when compared to the control group.

Effect on tensile strength in incised wound in rats—Results of locally applied SRE on tensile strength in incised wounds are summarized in Table 3. SRE (10 and 30 %) significantly ($P<0.001$) increased the

Table 2—Effect of ethanolic extract of *S. robusta* resin (SRE) once daily on hydroxyproline content in excised wounds in rats [Values are mean ± SE from 5 animals in each group]

Treatment	Hydroxyproline (mg/100 mg of dry tissue)
Vehicle control	3.84±0.08
SRE 3 %	4.01±0.11
10 %	5.35±0.05 ^{ac, bc}
30 %	6.71±0.07 ^{ac, bc}
Framycetin 1 %	2.23±0.12 ^{ac, bc}

P values: ^{ac} <0.001 (compared with control); ^{bc} <0.001 (compared with SRE 30)

Table 3—Effect of ethanolic extract of *S. robusta* resin (SRE) once daily on tensile strength of incised wounds in rats [Values are mean ± SE from 5 animals in each group]

Treatment	Tensile strength (g/0.75 cm of incised wound)
Vehicle control	243.86±11.07
SRE 3 %	252.66±8.25
10 %	322.00±6.7 ^{ac, ba}
30 %	378.33±22.15 ^{ac, bc}
Framycetin 1 %	222.33±13.06

P values: ^{ac} <0.001 (compared with control); ^{ba} <0.05 , ^{bc} <0.001 (compared with SRE 30)

Table 1—Effect of ethanolic extract of *S. robusta* resin (SRE) on wound contraction in excised wounds in rats [Values are mean ± SE from 5 animals in each group]

Treatment	Wound area in mm ²					
	day 2	day 4	day 6	day 8	day 10	day 12
Vehicle (control)	491.23±7.30 (1.76)	430.20±15.01 (13.96)	343.91±14.35 (31.22)	140.71±12.4 (71.86)	64.51±4.72 (87.10)	28.95±2.35 (94.20)
SRE 3 %	488.18±9.98 (2.36)	426.21±13.23 (14.76)	325.12±3.50 (343.98)	132.08±9.96 (73.60)	58.92±8.24 (88.22)	22.86±3.76 (95.44)
10 %	432.30±11.42 ^{ab, bb} (13.54)	346.45±14.40 ^{ac, bc} (30.72)	300.22±20.21 ^{aa} (39.96)	106.68±8.65 (78.66)	38.10±3.11 (92.38)	14.73±2.18 (97.06)
30 %	358.64±11.97 ^{ac, bc} (28.28)	340.36±11.69 ^{ac, bc} (31.88)	275.33±9.55 ^{ac, ba} (44.94)	94.99±14.84 ^{aa} (81.00)	28.44±2.18 (94.31)	12.19±2.18 (97.56)
Framycetin	453.64±20.39 (9.34)	428.75±15.99 (14.26)	333.75±17.53 (33.26)	140.20±4.91 (71.96)	45.72±4.75 (90.86)	15.74±2.59 (96.85)

P values: ^{aa} <0.05 , ^{ac} <0.001 (compared with control); ^{ba} <0.05 , ^{bb} <0.01 , ^{bc} <0.001 (compared with SRE 30). Figures in parenthesis are per cent value as compared to control value on '0' day in each treatment group

tensile strength, as compared to the control group. SRE showed significant ($P < 0.05$) dose-dependent increase in tensile strength i.e. results were significant when compared to 30 mg/kg SRE. However, reference drug framycetin (1 % w/w) did not show any significant increase in tensile strength.

Discussion

Wound healing has many events/phases such as granulation, collagenation, contraction, epithelialization and scar-remodeling¹⁴. All these phases, except scar remodeling run concurrently and influence each other. Therefore, it may not be possible to draw firm conclusion about the influence of a given agent on healing by studying only one phase of healing¹⁵. With this in view, the present study was designed to monitor effects SRE on different phases of wound healing in rats by employing excisional and incisional models of wound healing.

Employment of an incisional wound model allows the assessment of all the three phases using a combination of clinical and morphological end points and quantitative strength measurement¹⁶. This model analyses the biomechanical strength of wound, a critical outcome of wound repair. Wound strength helps to evaluate the efficacy of tissue healing process¹⁷. Excisional wound model results in an open wound that requires considerable fibroplasia to fill the defect and wound provides more tissue for quantification of connective tissue metabolism and collagen deposition. Excisional wounds also resemble chronic and open wound encountered in clinical situations. Inflammation is the basis for wound repair. Presence of optimal inflammation is essential for proper wound healing but pain and swelling accompanying excessive inflammation may cause agony to the patient¹⁸. One of the most important events in wound healing is the wound contraction which occurs in order to decrease the size of the wound so as to speed up the repairing process¹⁹. At first, contraction occurs without myofibroblast involvement²⁰. Later, fibroblasts, stimulated by growth factors, differentiate into myofibroblasts. Myofibroblasts are responsible for wound contraction¹⁹. Around a week after wounding, fibroblasts differentiate into myofibroblasts and the wound begins to contract rapidly²¹. In full thickness wounds, contraction peaks at 5 to 15 days post wounding²². As the actin in myofibroblasts contracts,

the wound edges are pulled together. Fibroblasts lay down collagen to reinforce the wound as myofibroblasts contract²³. The repair and tensile strength of soft tissue wounds is a function of fibroblast generation and collagen formation²⁴. The unique high but fairly constant hydroxyproline content of collagen helps in estimation of collagen in a sample of tissue by simple colorimetric estimation of hydroxyproline¹³.

In the present experiments, SRE at 10 and 30 % w/w applied locally caused significant increase in wound contraction in excisional wound model on day 2nd to 6th of wound healing, whereas 30 % SRE w/w applied locally caused significant increase in contraction on day 8th as well. Also, the hydroxyproline contents of the wounds were significantly increased by SRE 10 and 30 % w/w in excisional wounds. In incisional wounds, the tensile strength was significantly increased in SRE (10 and 30 % w/w)-treated groups. This could be related to the capability of the extract to act on proliferative events of granulation tissue formation. Hence, it is clear that SRE has marked wound healing activity, as shown in the present models of wound healing. These results are in agreement with an earlier observation⁷ demonstrating wound healing activity of topical application forms of *S. robusta* resin. Increased contraction was also observed in excision wounds in rats by *Ocimum sanctum* extract²⁵.

The present data demonstrate that the ethanolic extract of *S. robusta* may be capable of promoting wound healing activity due to its ability to accelerate wound contraction, increased tensile strength and increased hydroxyproline content and suggest its therapeutic potential in wound healing.

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