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Evaluation of Wound Healing Activity of *Syzygium cumini* root extract in rats

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Abstract

Plants and their extracts have immense potential for the management and treatment of wounds. The phyto-medicines for wound healing are not only cheap and affordable but are also safe as hypersensitive reactions are rarely encountered with the use of these agents 2. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. However, there is a need for scientific validation, standardization and safety evaluation of plants of the traditional medicine before these could be recommended for healing of the wounds. In ayurvedic treatment, a medicine consists of plant products, either single drug or in combination with others (polyherbal formulation), which are considered to be less toxic and free from side effects are used as wound healing agents. On review of literature it was found that the plant *Syzygium cumini,* Linn is traditionally being used in treatment of wound. Hence, the present work was attempted to scientifically validate the traditional claim, by using experimental animal models. The aim of present study was to evaluate the wound healing activity of *Syzygium cumini,* Linn root extract in Excision, Incision and dead space wound models in rats.

Key words: Wound, Extract, Root

Introduction

Syzygium cumini Linn. It consists of the dried root of *Syzygium cumini* Linn. Belonging to family Myrtaceae, an evergreen tropical tree, is native to Indian subcontinent and naturalized in America, Africa, and Australia. It grows commonly along streams and damp places and in evergreen forests. The tree is planted as an ornamental in gardens and at roadsides. It is a large evergreen tree up to 30 meters height and girth of 3.6 meters with a bole up to 15meters. Fruit of *Syzygium cumini* contains malic acid and a small quantity of oxalic acid as its acid constituent. Gallic acid and tannins present in the fruit account for its astringency. The presence of Cyanidine and diglycoside imparts purple color to the fruit. It further contains glucose, fructose, mannose, and galactose as the principal sugar moieties. The mineral constituents are also reported to present which includes Ca, Mg, Na, K, Cu and vitamins such as thiamine, riboflavin, nicotinic acid etc. The entire plant is used in various traditional system of medicine like Siddha, Ayurveda and Unani in India. However, of all, the leaves and bark are regarded as most significant part. In Ayurveda, the bark is acrid, sweet, digestive and astringent to the bowels, anti-helminthes. Besides it is used to cure sore throat, bronchitis, asthma, thirst, biliousness, dysentery, blood impurities and ulcer. [1-2]

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In Unani, leaf ash is used to strengthen teeth and gums, seeds are used as astringent, diuretic, stop urinary discharge and remedy for diabetes and the bark is known for its wound healing properties. In Siddha, Jamun is considered to be a haematinic, semen promoting besides thermo-regulant. Traditional medical healers in Madagascar use seeds of jambolan to debilitate the complications in diabetes. [3-4]

Material and Methods

Purchase of sample

Sample of *Syzygium cumini,* Linn root were purchased from local market, Maula Enterprises Plasia, Indore, Madhya Pradesh, during the month of July 2015. The collected plant part was identified and authenticated on the basis of macroscopic and microscopic characters at Department of Botany, Govt. Agriculture College, Indore. The voucher specimen has been deposited at Department of Pharmacognosy of our college for further reference. The plant specimen was washed with distilled water to discard dust and other unwanted materials, dried under shade with casual sun drying.

Preparation of Extract

The air dried root were ground into coarse powder. The powdered was passed through sieve no #40 and stored in air-tight containers at 25°C for further use. The ethanolic extract of dried powder drug was prepared as follows:

The 100 gm air dried coarse powder of *Syzygium cumini,* Linn. root was well packed in soxhlet apparatus and subjected for continuous hot extraction with 70% ethanol. The extract was filtered while hot and the filtrate was evaporated under reduced pressure in order to remove solvent completely. The residue was dried and weighed. The dried weight of the extract was found to be 15 gm. The dried extract was stored in desiccators for further use. [5]

Animals

Healthy wistar rats of either sex and of approximately the same age, weighing about 150-250 g were used for the study. The animals were maintained at a well ventilated, temperature controlled (25±10 C) animal room for 7 day prior to the experimental period. The animals were housed in clean polypropylene cages under standard conditions (12 h light; 12 h dark cycle; 35-60% humidity). They were fed with standard rat pellet diet and water *ad libitum*. All the experimental protocols were approved by the Institutional Animal Ethical Committee. (Approval No: 1888/PO/Re/S/16/CPCSEA/2020/03) and were strictly in accordance with the norms of CPCSEA. New Delhi.

Pharmacological Activity [5-8]

Acute Toxicity Studies

Female Albino Wistar rats weighing 200 to 220 g were used in the study. Acute oral toxicity was performed as per OECD-423 guidelines. The animals were fasted overnight with water ad libitum. The starting dose of 5 mg/kg of both ethanolic and aqueous extracts was administered orally to three animals in each group. If mortality was observed in two or three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again in three animals to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 100, 200 and 400 mg/kg body weight. Animals were observed individually after dosing for the symptoms related to toxicity such as convulsions, ataxia, diarrhea, dermal irritation etc. at least once in 30 minutes with special attention during first 4 hours on the day of dosing and daily thereafter, for a total of 14 days.

Evaluation of Wound Healing Activity

Preparation of ointment by fusion method

(a) Preparation of simple ointment: The various ingredients used for the preparation of simple ointment are, Wool fat - 2 gm, Hard Paraffin-2 gm, Cetostearyl alcohol -2gm, White Soft Paraffin-34 gm. Each ingredient was mixed and heated gently with stirring then cooled. The base was then packed in a wide mouth container.

(b) Preparation of 5% and 10% ointment: 5 gm and 10gm extract of *Syzygium cumini,* Linn. Root, was added slowly to the above melted ingredients i.e. simple ointment to get 5% and 10% ointment respectively. The mass of the ointment was adjusted to 100 gm and stirred thoroughly until the mass cools down and a homogeneous product is formed. The ointment was then packed in a wide mouth container.

Methodology

Excision, incision and dead space wound models were used to evaluate the wound healing activity.

Adults Wistar albino rats of either sex weighing180-200 g were used for the study. Total 24 animals were numbered, weighed and divided into four groups, each containing six animals, for excision, incision and Dead space wound models. 50 mg of formulated ointments were applied topically to each animal once a day for the period of 10 days. All the animals were closely observed for any infection so that the infected animals could be excluded from the study.

The effect of the extract was evaluated on excision, incision and dead space granuloma wound models in rats. The wound-healing activity was assessed by the rate of period of wound contraction and skin-breaking strength i.e. tensile strength. Study of the granulation tissue was carried out to know the extent of collagen formation in the wound tissue.

Excision wound model

Excision wounds were used for the study of rate of contraction of wound. Animals were anaesthetized with diethyl ether and the hairs on the skin of the back, shaved with sterilized razor blades and marked. A full thickness circular excision wound of about 300 mm2 area and 2 mm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors on depilated dorsal thoracic region of excised rats, 5 cm away from ear. After skin excision, the entire wound was left open to the environment. The area of wounds was measured immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on that paper by permanent marker. This area was considered as initial wound area. The treatment was given topically as mentioned below for the period of 10 days

Group I : Control group treated with simple ointment base

Group II : Reference standard group treated with 5% (m/m) Povidone-Iodine ointment.

Group III : Test group treated with 5%, w/w Syzygium cumini, Linn. Root.

Group IV : Test group treated with 10%, w/w, of Syzygium cumini, Linn. Root.

Again the wounds were traced on transparent tracing paper on the day of wounding and subsequently on alternate day until healing were complete. Wound healing potential was monitored by wound contraction. Wound contraction was calculated as percentage reduction in wound area. Wound areas were measured on days 2, 4, 6, 8 and 10 for all groups with the help of following formula.

Incision wound model

For this study all the animals in each group were anaesthetized with anesthetic ether. Two 6cm long paravertebral, full thickness incisions were made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the depilated back of the rats. After the incision was made, the parted skin was kept together and stitched at 0.5 cm intervals continuously and tightly using surgical thread (No. 000) and a curved needle (No.11). All the groups were treated in the same manner as mentioned in the case of excision wound model. Extract ointments, simple ointment base (control), and standard drug i.e. Povidone-Iodine ointment was applied on the wounds once daily for 9 days. When the wounds were cured thoroughly, the sutures were removed on day 9 and the wound breaking strength i.e. tensile strength of the healed wound was measured on day 10 by continuous and constant water flow technique.

Dead Space Wound Model

Dead space wounds were created under light ether anesthesia, by subcutaneous implantation of two sterilized cotton pellets (10 mg), one on either side in the lumber region on the ventral surface of each rat.(9,10)

The animals were divided into 4 groups containing 6animals in each group and given the following drug treatment.

Group I : Control group treated with simple ointment base

Group II : Reference standard group treated with 5% (m/m) Povidone-Iodine ointment.

Group III : Test group treated with 5%, w/w Syzygium cumini, Linn. Root.

Group IV : Test group treated with 10%, w/w, of Syzygium cumini, Linn. Root.

Animal received test extract from 0 day to 9th post-wounding day. On the 10thpostwounding day, the granulation tissue formed on the implanted cotton pellet was carefully removed and employed for estimation of hydroxyproline content.

Wound Healing Evaluation Parameters

Measurement of Wound Contraction

An excision wound margin was traced after wound creation by using transparent paper and area measured by graph paper. Wound contraction was measured on every 2 days interval, until complete wound healing and expressed in percentage of healed wound area.[19] The evaluated surface area was then employed to calculate the percentage of wound contraction, taking initial size of wound, 300 mm2, as 100%, by using the following formula.

Epithelialization period

The period of epithelialization was calculated as the number of days required for falling of the dead tissue remnants from the wound surface without any residual raw wound.

Measurement of tensile strength

The tensile strength of a wound represents the degree of wound healing. It indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. The sutures were removed on the 9th day after wounding and the tensile strength of removed tissue was measured on the 10thday. In this method, wound breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen.

Wound breaking strength: The anesthetized animal was secured to the table, and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposed to each other. One side of the forceps was supported firmly, whereas the other was connected to a freely suspended light-weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wound edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound. The mean reading for the group was taken as an individual value of breaking strength. The mean value gives the breaking strength for a given group.

Results and Discussion

In the acute toxicity study with ethanolic extracts of *Syzygium cumini* Linn root no mortality was observed or during the period of 24 hours even with the higher dose tested i.e. 400 mg/kg and the none of the animals showed any symptom related to toxicity such as convulsions, ataxia, diarrhea, dermal irritation etc.

However, topical application of Syzygium cumini Linn root ointment for acute dermal toxicity testing showed the safety profile of the tested drugs, where there was no any visible sign of skin irritation, inflammation, swelling or any other change on the skin was observed. Therefore, both the oral and topical applications of Syzygium cumini Linn root were found to be safe for the present study.

The studies on excision wound healing model reveals that all the four groups showed gradual decreased wound area from day 1 today 10.

On 10th post wounding day, group II i.e. reference drug treated group showed significant decrease in wound area as compared to control group ($p< 0.01$). Similarly group III and group IV i.e. 5% extract ointment treated group and 10% extract ointment treated group respectively also showed significant decrease in wound area as compared to control group ($p< 0.01$). Table1.

However on 10thpost wounding day all the groups showed significant increase in wound contraction when compared to control group ($p< 0.01$). .

The epitheliazation time i.e. time at which complete scar formation occur, also suggest that all the groups i.e. reference drug treated group, 5% extract ointment treated group and 10% extract ointment treated group were found to be significant and comparable with control (table2).

In the incision wound model, a significant increase in mean breaking strength ($P<0.01$) was observed in rats treated with reference drug treated group, 5% extract ointment treated group and 10% extract ointment treated group when compared with control group.

The mean tensile strength in the control group was 3.48% whereas in standard ointment treated group,5% extract ointment treated group and 10% extract ointment treated group it was 5.16%, 4.28% and 4.83% respectivel. These observations of incision wound model confirms significant wound healing effect of standard ointment treated group and *Syzygium cumini* Linn root extract ointment treated group as compared to control group.

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Table 1: Effect of *Syzygium cumini* Linn root extract ointment on excision model in rats

Group	Wound area (mm^2)					
	0^{th}	2^{nd}	4^{th}	6^{th}	8^{th}	10^{th}
Group I Simple ointmen base	302±1.03	281±1.39	259±1.84	221±2.15	198±2.05	141±1.96
Group II 5% Povidone Iodine ointment	301±1.24	252±1.79**	202±1.65**	165±1.91**	81±1.65**	11±1.68*
Group III 5% Extract ointment	302±1.97	274±1.77*	224±2.78**	170±1.78*	102±1.54*	49±1.59*
Group IV 10% Extract ointment	301±1.36	262±1.52**	219±1.80**	166±1.36*	94±1.69**	23±1.87*

Values are mean ± SEM, *p< 0.05 and ** p<0.01 as compared to control group

Table 2: Effect of *Syzygium cumini* Linn root extract ointment on excision model in rats

Group	Treatment	Percentage wound contraction on post wounding days					Period of Epithelialization in days
		2^{nd}	4^{th}	6^{th}	8^{th}	10^{th}	
Group I	Simple ointment base	6.33	13.66	26.33	34.00	53.00	18
Group II	5% Povidone-Iodine ointment	16.00	32.66	45.00	73.00	96.33	9
Group III	5% Extract ointment	8.66	25.33	43.33	66.00	83.66	12
Group IV	10% Extract ointment	12.66	27.00	44.66	68.66	92.33	11

Table 3: Effect of *Syzygium cumini* Linn root extract ointment on incision model in rats i.e. measurement of wound breaking strength

Group	Treatment	Breaking strength (g)
Group I	Simple ointment base	209
Group II	5% Povidone-Iodine ointment	310±1.98*
Group III	5% Extract ointment	257±1.67*
Group IV	10% Extract ointment	290±2.18*

Values are mean ± SEM, *p< 0.05 and ** p<0.01 as compared to control group

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Table 4: Effect of *Syzygium cumini* Linn root extract ointment on incision model in rats i.e. measurement of wound tensile strength

Group	Treatment	Tensile strength (g)
Group I	Simple ointment base	**3.48**
Group II	5% Povidone-Iodine ointment	**5.16**
Group III	5% Extract ointment	**4.28**
Group IV	10% Extract ointment	**4.83**

Conclusion

In conclusion, the observations and results obtained in the present study indicated that the root extract of *Syzygium cumini* Linn significantly stimulated wound contraction. The increased wound contraction, decreased period of epithelialisation, increased breaking strength, increased tensile strength.

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