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WOUND HEALING POTENTIAL OF *CARALLUMA ADSCENDENS*

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Keywords:

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ABSTRACT: The purpose of this current investigation was for screening the wound healing potential of Ethanolic extract of *Caralluma adscendens* (EECA) whole plant. *Caralluma adscendens* (Apocynaceae), a common medicinal plant in traditional Indian medicine. Mentions in folk medicine and existing data raised an outlook on this plant for evaluating wound healing potential. Ethanolic extract of *Caralluma adscendens* (EECA) has been examined for its wound healing activity by utilizing couple of wound models in rats: (i) excision wound model and (ii) incision wound model following application of standard or extract topically. Animals have been categorized into 4 groups with six in every group. Group I left untreated, Group II received soframycin skin cream as standard, Group III and IV received 100mg and 200mg of Ethanolic extract of *Caralluma adscendens* (EECA) whole plant. Results obtained upon treatment with the plant extract groups shown activity close to standard drug activity, Soframycin, in terms of wound contraction ability, time taken for wound closure and tensile strength. 200mg of plant extract has produced better wound healing ability after comparing to other dose. The obtained results notified that *Caralluma adscendens* swifts the wound healing process by reducing wound's surface area and enhancing wound's tensile strength.

INTRODUCTION: Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue [1]. Wound healing is an interaction of complex cascade of cellular and biochemical actions healing to the restoration of structural and functional integrity with regain of strength of injured tissues. Wound healing involves continuous cell – cell interaction and cell - matrix interactions that allow the process to proceed in different overlapping phases and process including inflammation, wound contraction, tissue re-epithelialization, re-modeling, & formation of granulation tissue with angiogenesis [2].

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Many Ayurvedic plants have a very important role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way [3]. More than 70% of wound healing pharma products are plant based, 20% are mineral based and remaining containing animal products as their base material. The plant base materials are used as first aid – antiseptic coagulants and wound wash [4].

Caralluma is an edible succulent plant used by tribes in India to suppress hunger and enhance endurance [5]. It is a new arrival in the family of succulent plants that are becoming increasingly popular for their appetite suppressant and weight loss properties. The plant *Caralluma adscendens* has been used in different system of traditional medication for the treatment of disease and aliments of human being. It has been reported as an anti-inflammatory, antioxidant, anti-diabetic, analgesic, anti ulcers, antibacterial, hypoglycemic activities [6]. In this current study, we designed a

method to judge the wound healing property of *C. adscendens* using excision as well as incision wound models in Wistar albino rats.

MATERIALS AND METHODS:

Collection of Plant Material and Plant Extraction: The whole plant of *Caralluma adscendens* has been assembled from the roadsides of Military Diary farm Road, Trimulgherry, Secunderabad situated in the state of Telangana (India). The plant specimen was verified by the botanist of Osmania University with voucher specimen Number 203 of the plant and it has been placed in department for subsequent reference.

The dried plant was then milled to coarse powder mechanically and allowed to extract successively with ethyl acetate, chloroform, petroleum ether as well as Ethanol in Soxhlet's apparatus whereas maceration method has been allowed for water at a duration of 72 hours. The crude extracts were evaporated to dryness under vacuum and allowed for drying using vacuum desiccators. Later stored in refrigerator. Preliminary phytochemical investigation was performed. Based on the presence of phytoconstituents, ethanolic extract of *Caralluma adscendens* (EECA) was selected for screening the wound healing potential in experimental animals.

Animals: An ethical approval of this experimental work was gained from the Institutional Animal Ethical Committee with an Approval no: CPCSEA/IAEC/JLS/11/11/19/14. Albino rats with average body weight from 150 to 250 g were utilized in this study. They were procured from Sanzyme Bio-analytical lab, Plot no. 8 Sys. No.542, Kothur(V), Shameerpet, R.R. dist. The rats were housed in polypropylene cages as well as perpetuated under standard conditions (35-60 % humidity, dark cycles at 25± 3°C, 12h light). Standard pellet feed as well as tap water have been allowed *ad-libitum*.

EXPERIMENTAL DESIGN:

Wound Models: The animals had been allowed to starve prior to wounding for 12 h. All the rats had been anaesthetized utilizing pentobarbitone (30mg/kg). The rats have been categorized into 4 main groups (*n*=6). Animals have been bared before wounding at dorsal thoracic areas.

Group I: Control group left untreated.

Group II: Standard drug (Soframycin skin cream) topically once a day for 16 days

Group III: Ethanolic extract of *Caralluma adscendens* (EECA-100mg/kg), topically once a day for 16 days.

Group IV: Ethanolic extract of *Caralluma adscendens* (EECA-200mg/kg), topically once a day for16 days.

Excision Wound Model [7]: An imprint has beenallowed to made at dorsal thoracic region 1cm far from vertebral column whereas 5cm far from ear utilizing round seal with 2.5cm diameter on desensitized rat. An ablation at the imprinted skin area has been allowed to thickness for attaining about 500 mm^2 diameters of wound area. Hemostasis has been attained by staining wound with cotton swab immersed in normal saline. At Initial, for couple of weeks, contractions which are necessary for wound closure were examined by utilizing transparent paper to trace wound. With the aid of millimeter scale graph paper, the impression was measured. After complete epithelialization as well as time required for completing epithelialization Scar area in days were taken into consideration for evaluating the degree of wound healing. The following parameters i.e., percentage of wound closure, period of re-epithelialization was studied. The observation of the percentage wound closure, re- epithelialization and size and shape of scar area were documented on 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th}, 12^{th}, 14^{th} and 16^{th} day of post wounding day.

Measurement of Wound Closure: The rate of wound closure has been analyzed by tracing the wound on 2, 4, 6, 8, 10, 12, 14 as well as 16 post wounding days utilizing permanent marker and transparent paper and noticed wound regions were calculated utilizing $1mm^2$ scale of graph paper. The wound contraction progress and epithelialization period were evaluated by taking wound area into consideration. The figured-out surface area was taken into consideration in order to estimate the wound percentage by taking wound's initial size as 100 %[8]as shown below:

% wound closure = (Initial wound size - nth days of wound size) / (wound's initial size) ×100

Epithelialization Period Measurement: Wound falling with no raw scab behind has been considered as perfect epithelialization and the days in number needed for was noticed as period of epithelialization [9]. The results of epithelialization period were tabulated in **Table 4.**

Incision Wound Model [10]: The experimental rats in the incision model have been subject to anesthesia with pentobarbitone and at 1.5cm distance from the medial line on either side of the paved spine, two longitudinal para vertebral incisions of 6cm in length had been made by skin and skin muscles.

Utilizing No. 000surgical thread as well as No. 11 curved needle, the partial skin has been allowed to suture after this incision. The wounds have been left undressed. All rats except Group I have been given standard /extracts topically once a day till complete healing and on eighth post-wound day, sutures had been removed. Tensile strength on 10^{th} day has been assessed using tensiometer [11].

Tensile Strength: Wound healing agents plays vital rolein promoting an attain in tensile strength. The tensile strength is the parameter that reflects how well the wound healed. In order to measure the tensile strength, sutures were allowed to remove on ninth post wound day and allowed for measurement of strength on tenth day [12].

The mean tensile strength for each experimental animal has been known as an indicator of the wound's tensile strength. In comparison with the control sample, the extract strength treated and standard treated are tensile. Increasing tensile strength suggests improved stimulation of wound healing by the standard applied extract. Tensile strength has been determined by utilizing the given formula [13].

Tensile strength = Breaking Strength (g) / Cross-section area of skin (mm^2)

At the end of study, on tenth day, biochemical parameters like hydroxyl proline [14], hexosamine [15] and total protein contents [16] were determined from excised granulation tissue.

RESULTS AND DISCUSSION: Wound healing is a complex cellular event by which a damaged tissue restored as closely as possible to its normal stage. The healing process depends upon the reparative abilities of the tissue, the type and extent of damage and general state of health of the tissue.

The standard drug Soframycin is used as a standard reference to assess the healing potency of the crude drug and the constituent against the control. Significant decrease in the period of epithelialization and increase in wound contraction rate were observed in extract and standard treated groups of animals.

Wound Contraction: Topical application of Ethanolic extract of *Caralluma adscendens* (EECA) (100mg/kg and 200 mg/kg) whole plant showed effect on the process of wound healing on the rats. The progress of wound area **Table 1**, wound contraction induced by treatment of 100mg/kg and 200 mg/kg) whole plant extract of *Caralluma adscendens*, untreated and Soframycin skin cream were shown in **Table 2.**

TABLE 1: EFFECT OF ETHANOLIC EXTRACT OF WHOLE PLANT OF *CARALLUMA ADSCENDENS* ON WOUND INDUCED" IN RATS (EXCISION MODEL)

Treatment	Wound area (cm^2) post-wounding days (SEM± Mean)								
	0^{th} day	2^{nd} day	4^{th} day	6^{th} day	8^{th} day	10^{th} day	12^{th} day	14^{th} day	16^{th} day
Control (Untreated)	500	486.16± 2.04	378.33 ± 2.94	342.16 ± 2.31	325.0 ± 1.78	299.50 ± 1.37	243.83 ± 3.25	189.66 ± 3.61	141.66 ± 2.94
Standard (Soframycin)	500	419.83 ± 3.18***	321.0 ± 2.09***	237.83 ± 1.16***	203.83 ± 2.31***	134.16 ± 1.72***	82.50 ± 3.33***	41.50 ± 2.81***	26.83 ± 2.13***
EECA (100mg/kg)	500	467.50 ± 2.42*	339.66 ± 1.96*	284.50 ± 1.87*	252.66 ± 3.50*	216.33 ± 1.96*	185.33 ± 2.58*	124.16 ± 2.31*	85.50 ± 2.66*
EECA (200mg/kg)	500	438.33 ± 1.75**	327.83 ± 1.16**	275.66 ± 2.87**	226.33 ± 3.77**	193.50 ± 1.37**	145.83 ± 1.72**	86.50 ± 3.93**	55.66 ± 2.42**

Values are expressed as mean ±SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism software & Data were analyzed by using one way analysis of variances (ANOVA). ***p < 0.001, **p < 0.01, *p < 0.05 when compared to control group; one way ANOVA.

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF *CARALLUMA ADSCENDENS* (EECA) WHOLE PLANT ON PROGRESS OF % WOUND CLOSURE (EXCISION MODEL)

Treatment	% of wound closure post-wounding days (SD± Mean)								
	0	2	4	6	8	10	12	14	16
Control	0	2.76	24.33	31.56	35	40.1	51.23	62.06	71.66
Standard	0	16.03	35.8	52.43	59.23	73.16	83.5	91.7	94.63
EECA (100mg/kg)	0	6.5	32.06	43.1	49.46	56.73	62.93	75.16	82.9
EECA (200mg/kg)	0	12.33	34.43	44.86	54.73	61.3	70.83	82.7	88.86

Values are expressed as mean ±SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism software & Data were analyzed by using one way analysis of variances (ANOVA). ***p < 0.001, **p < 0.01, *p < 0.05 when compared to control group; one way ANOVA.

The ethanolic extract with 200 mg/kg treated group shown noticeable wound contraction from the second day of treatment, and with highly significant difference were seen from 10th day in comparison with the control group. As shown in **Table 1**, there was no reliable difference in activity between the 200mg/kg and 100mg/kg extract was observed. But, level of wound closure was observed a little higher with 200mg/kg ethanolic extract. 200mg/kg ethanolic extract shows comparable efficacy with Soframycin skin cream. The maximum rate of wound contraction was seen on the 10th, 12th, 14th and 16thday. The animals treated with 100 mg/kg and 200mg/kg of ethanolic extract demonstrated considerable wound contraction in comparison with the control group (untreated) from the 10th day and 8th on wards respectively. Significant wound contraction have also beenseen for Soframycin skin cream treated group from 6th day onwards as compared to untreated with highly significant wound contraction on 12th, 14th and 16th days. The maximum wound contraction rate for Soframycin skin cream was seen on the 12th, 14th and 16th day.

Epithelialization Period: The time required for complete epithelialization of the excision wound is an important parameter to assess the wound healing process. Epithelialization period has been read during the study. It was found that the mean time taken for complete epithelialization of the excision wound in extract treated group was less than the animals left untreated. No significant difference of epithelialization period has been found between 200mg/kg and 100mg/kg extract and standard drug and the data are shown in **Table 3.**

TABLE 3: EFFECT OF ETHANOLIC EXTRACT OF *CARALLUMA ADSCENDENS* (EECA) WHOLE PLANT ON PERIOD OF EPITHELIALIZATION (NO. OF DAYS)

Groups	Period of epithelization No. of days (mean ± SEM)
Group I: Control (Untreated)	17.83±1.11
Group II: Standard (Soframycin)	13.00±0.45***
Group III: EECA (100mg/kg)	15.50±0.96*
Group IV: EECA (200mg/kg)	14.16±0.40**

"Values were expressed as Mean ±SD *(n=6).* Using Dunnett's -test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were analyzed by using One way ANOVA. **p<0.05, **p<0.01, and* *** *p<0.001* in comparison to control group.

Wound Healing (Incision Model): The breaking strength is the strength of a healing wound and is measured experimentally by the amount of force required to disrupt it. In the beginning a wound will be having little breaking strength because the clot will alone will be holding the edges together. Thereafter breaking strength increases rapidly as collagen deposition increases and crosslinkages are formed between the collagen fibers. The promotion of wound healing activity is also well gazed by its tensile strength of the incision wound. Generally wound healing agents have the properties to enhance the deposition of collagen content, which provides strength to the tissues and forms cross-linkages between collagen fibers. The tensile strength of the incision wound was significantly increased in the animals treated with Ethanolic extract as similar to that of the standard drug Soframycin. A moderate gain in tensile strength was observed in ethanol extract treated animals and it was insignificant in control groups **Table 4**. The results of these experiments are expressed as mean ± S.E. of six animals in each group. The data were evaluated by one-way ANOVA.

TABLE 4: EFFECT OF ETHANOLIC EXTRACT OF *CARALLUMA ADSCENDENS* WHOLE PLANT WHOLE PLANT ON WOUND, INDUCED IN RATS (INCISION MODEL)

Groups	Tensile strength (g) (mean ±SEM)
Group I: Control (Untreated)	164 ±0.93
Group II: Standard (Soframycin)	428.3 ±2.46***
Group III: EECA (100mg/kg)	374.6±0.99*
Group IV: EECA (200mg/kg)	394.6±1.78**

Values were expressed as Mean ±SD *(n=6).* Using Dunnett's -test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were analyzed by using One way ANOVA. **p<0.05, **p<0.01, and *** p<0.001* in comparison tocontrol group.

Biochemical Parameters: Wound healing is the physiological response to the tissue injury that results in the replacement of destroyed tissue by living tissue and thus restoration of tissue integrity. The mechanism of wound repair occurs by four basic processes such as inflammation, wound contraction, epithelialization and granulation tissue formation. Inflammation starts immediately after the disruption of tissue integrity.

The platelets became adherent with clotting factors and form haemostatic plug to stop bleeding from the vessels. The prostaglandins (PGE1 and PGE2) are released in the inflammation area and seem to be the final mediators of acute inflammation and may play a haemostatic role for white cells and fibroblasts. The active motile white cells migrate into the wound and start engulfing cellular debris, at the initial stages wound contraction begin slowly but became rapid after 3 or 4 days. The myofibroblsts present in the margin of the wound appear to constitute the machinery for the wound contraction. These are responsible for overlaying debris. The epithelialization of the wound mainly occurs by proliferation and migration of the marginal basal cells lying close to the wound margin.

The hematoma within the wound may be replaced by granulation tissue, which consists of new capillaries and fibroblasts. The fibroblasts are responsible for production of the mucopolysaccharide ground substance. The lymphatics develop new nerve fibers and there is also formation of scar tissue in which collagen turn over increases. Due to the effect of EECA and standard drug, formation of collagen fibers increases. At the later stage the breaking strength of the wound increases correspondingly to the increase of collagen content.

In incision wound model, it has been found that wound's tensile strength treated with plant extract and standard were high after comparing to control group as well as may be because of an enhancement in collagen levels and stabilization of the fibers. EECA extract at two doses showed raised collagen synthesis and cellular proliferation at wound area as it was proved by increasing total protein along with total collagen contents reflected by hydroxyproline content of granulation tissues **Table 5.**

TABLE 5: "EFFECT OF ETHANOLIC EXTRACT OF *CARALLUMA ADSCENDENS* (EECA) WHOLE PLANT ON BIOCHEMICAL PARAMETERS

Groups	Hydroxyproline content (mg/g tissue)	Hexosamine (mg/g tissue)	Total Protein(mg/g tissue)
Group I: Control (Untreated)	23.87±0.01	0.43±0.56	106.7±0.97
Group II: Standard (Soframycin)	73.46±0.04***	0.79±0.05***	121.76±0.24***
Group III: EECA (100mg/kg)	62.18±0.11*	0.57±0.06*	108.1±0.09*
Group IV: EECA (200mg/kg)	68.47±0.26**	0.61±0.07**	112.4±0.22**

Values were expressed as Mean ±SD *(n=6).* Using Dunnett's -test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were analyzed by using One way ANOVA. **p<0.05, **p<0.01, and *** p<0.001* in comparison to control group.

CONCLUSION: In this research work, Ethanolic extract of *Caralluma adscendens* whole plant was examined for its wound healing activity in two types of wound model in rats: (i) the excision wound model and (ii) the incision wound model. Ethanol extract of the plant material produced a significant response in both of the wound types tested. The results were also comparable to those of a standard drug, Soframycin, in terms of wound contracting ability, wound closure time, tensile strength and regeneration of tissues at the wound site.

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