Research Article

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WOUND HEALING ACTIVITY OF ALCOHOLIC EXTRACT OF *SOLANUM ERIANTHUM* D.DON IN EXCISION AND INCISION METHOD

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ABSTRACT
Solanum erianthum D. Don is an unarmed shrub or small tree with dense indumentums of soft stellate hairs. Leaves are simple, ovate-elliptical; margin entire or slight wavy, base rounded to cuneate, and apex acute to acuminate. The leaves have been extensively used for leucorrhoea, piles, hemorrhoids, scrofula, headache, vertigo, digestive troubles and for wound healing purposes. Wistar albino rats of either sex weighing between 200 and 220g were topically treated with extract formulated in gel (10% and 20%) which was applied once daily in excision and incision wound model. Rats of standard groups were treated with 5% w/w Povidone-Iodine ointment topically. The percentage of wound contraction was increased, epithelization period was decreased and wound breaking strength was increased with topical application of AESE (Alcoholic Extract of *Solanum erianthum*) gel in excision and incision wound model. The experimental data revealed that the AESE displayed remarkable wound healing activity thus supports its traditional use.
Key words: *Solanum erianthum* D.Don, wound healing, AESE gel

INTRODUCTION

Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine. These plant remedies are used since ancient time even if the mechanism of action and efficacy of very few of them have been evaluated scientifically[1]. *Solanum erianthum* D.Don Family-Solanaceae is an unarmed shrub or small tree up to 4-10m tall with a dense of soft stellate hairs, stem up to 20 cm in diameter leaves simple, ovate-elliptical, margin in entire or slightly wavy, base rounded to cuneate, apex acute to acuminate. Adding to the taxonomic confusion is the fact that *S.erianthum* has been extensively referred to as *S.verbascifolium* L. which actually proved to be identical with a South American species[2]. *S.erianthum* is a small tree. *S.erianthum* has been used as a traditional medicine for treating inflammatory diseases, burns and wounds. Decoction of the root were used for body pains, vertigo and urinary troubles[3-5]. Leaves were given in vaginal discharges. The leaves of *S.erianthum* have been reported as anti-malarial, anti-cholinergic[6, 7] and burn wound healing[19]. The present study was carried out to determine effect of alcoholic extract of *Solanum erianthum* leaves on wound healing activity in rats by excision and incision wound healing models.

Discontinuity or break in the surface of the epithelium is called wound. Wound and its management is one of the major problems in the world. The wounds can be caused due to physical, chemical and biological agents. Wound can be classified based on their etiology, lasting period, morphological characters etc. Later the color code concept was used where in the wounds were classified as red yellow or black. Based on the nature and depth wounds can be classified as:

- Closed wounds: i.e. contusions, abrasions, and hematoma
- Open wounds: i.e. incised, lacerated, penetrating and crushed

Depending on the intensity of the wound they can be termed as (a) simple wound: here the damage is only to the skin. (b) Complex wound: the wound involves the underlying tissues, tendons etc. Wound healing is a complex and dynamic process with the wound environment and keep changing with the changing health status of the individual.[8]Wound healing is a complex phenomenon, involving a number of well-orchestrated processes, including regeneration of parenchyma cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of ECM (extracellular matrix) proteins, remodeling of connective tissue (C.T.) and parenchymatous components and collagenisation and acquisition of wound strength.[9]

Healing of wound

Healing of wound can occur by

First or Primary Intention: This is seen when the wound is a clean incised wound. Healing proceeds rapidly with early closure of wound. There are several overlapping stages in the repair process.

Inflammation: The cut surfaces become inflamed, blood clot and cell debris fill the gap between them in the first few hours. Phagocytes and fibroblasts migrate in to the blood clot:

- Phagocytes begin to remove the clot and cell debris stimulating fibroblast activity
- Fibroblasts secrete collagen fibers which begin to bind the surface together. [10]

Proliferation: There is proliferation of epithelial cells across the wound, through the clot. The epidermis meets and grows upwards until the full thickness is restored. The

clot above the new tissue becomes the scab and separates after 3 to 10 days. Granulation tissue, consisting of new capillary buds, phagocytes and fibroblasts, develops, invading the clot and restoring the blood supply to the wound. Fibroblasts continue to secrete collagen fibers as the clot and any bacteria are removed by phagocytosis.

Maturation: The granulation tissue is replaced by fibrous scar tissue. Rearrangement of collagen fibers occurs and the strength of the wound increases. In time, the scar becomes less vascular, appearing after a few months as a fine line. The channels left when stitches are removed, heal by the same process.

Secondary Intention: This is in the case where the wound edges are separate and there is tissue loss and sometimes the wound may be infected. Rapid closure of the wound is not possible. Therefore this leads to an ugly scar and sometimes may cause limitation of movement.

Inflammation: This develops on the surface of the healthy tissue and separation of necrotic tissue begins, mainly due to the action of phagocytes in the inflammatory exudate.

Proliferation: This begins as granulation tissue, consisting of capillary buds, phagocytes and fibroblasts, develops at the base of the cavity. It grows toward the surface, probably stimulated by macrophages. Phagocytes in the plentiful blood supply tend to prevent infection of the wound by ingestion of bacteria after separation of the slough. Some fibroblasts in the wound develop a limited ability to contract, reducing the size of the wound and healing time. When granulation tissue reaches the level of the dermis, epithelial cells at the edges proliferate towards centre.

Maturation: This occurs as scar tissue replaces granulation tissue, usually over several months until the full thickness of the skin is restored. The fibrous scar tissue is shiny and does not contain sweat glands, hair follicles or sebaceous glands.

MATERIALS AND METHODS

Plant material

Leaves of *solanum erianthum*.D.Don were collected from the local area of Bangalore, Karnataka, India during November 2010 and were authenticated by Dr. Shiddamallaya N, botanist of National Ayurveda Dietetics Research Institute, Bangalore, Karnataka, India. The voucher No: Drug authentication/ SMPU/ NADRI/ BNG/ 2010-11/840 (RRCBI-Mus/06).

Preparation of the extract

Leaves of *solanum erianthum*.D.Don were dried in shade and powdered. The powdered materials were extracted with 70% ethanol using soxhelt extraction apparatus. This ethanol extract was then concentrated and dried under reduced pressure. The semisolid mass (ethanol free) thus obtained was used for the experiment.

The preliminary phytochemical analysis

The alcoholic extract was tested qualitatively for various plant constituents according to standard methods.[11]

Animals

Wistar albino rats of either sex weighing 150-200 g and female albino mice weighing 20-25 g were used in this study. They were procured from Sri Venkateshwara Enterprises, Bangalore. The animals were acclimatized for ten days under laboratory conditions. They were housed in polypropylene cages and maintained $27^0C \pm 2^0C$, relative humidity 65 ± 10% under 12 hours light / dark cycle. The animals were fed with rodent pellet diet (Amrut Laboratory animal feeds, Pranav Agro Industries Ltd, Sangli, India) and water *ad libitum.* All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Gautham College of Pharmacy, Bangalore, India (GCP-IAEC/006/5/2010) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg No: 491/01/c/CPCSEA), Govt. of India.

Selection of doses

For assessment of excision and incision[12] wound healing activity extracts were formulated as a gel. Ethanol extract of *S.erianthum* (1g) was mixed with 9g of sodium alginate with 100 ml of distilled water to get 10 % (w /w) gel. Same procedure was followed to prepare 20 % (w /w) gel.

Skin Irritation Studies

The rabbit[13] was shaved the skin in three different position of dorsal side, each about $500mm^2$. The rabbit was kept in rabbit holder and the 1st area was kept as control, to which vehicle was applied. 2nd area was applied with 10% AESE gel. The 3rd area treated with 20% AESE gel. (Table 3)

In Vivo Wound Healing Activity

Excision wound model

Animals were divided in to four groups, each group consisting of 6 rats.[14, 15] (Table 4, 5)

Group I: Received no treatment and served as control

Group II: Received application of standard drug ointment i.e. Povidone-Iodine (5%w/w)

Group III: Received application of 10% w/w AESE which considered as low dose.

Group IV: Received application of 20% w/w AESE which considered as high dose.

AESE: Alcoholic Extract of *Solanum erianthum*

Under light ether anaesthesia an impression of 500 sq mm was made on the shaved back of the rat as described in Morton and Malone. The skin of the impressed area was excised carefully. Animals are kept in separate cages. The day on which wound was made consider as day'0' (Zero). Drugs were topically applied once a day till complete of epitheliazation, starting from day of excision.

The progressive changes in wound area were monitored metrically by tracing the wound margin on graph paper on day of creating wound and subsequently by 2nd, 4th, 6th ,8th, 10th, 12th, 14th, 16th, 18th, 20th and 22nd days post wounding. Number of days required for falling of scab without any residual raw wound, gave the period of epitheliazation.

Percentage wound closure

$$\text{Percentage Wound Closure} = \frac{(\text{Initial area of Wound} - N^{th}\text{ day area of wound})}{(\text{Initial area of Wounds})} \times 100$$

Incision wound model

Animals were divided in to four groups, each group consisting of 6 rats. [16, 17] (Table 6)

Group I: Received no treatment and served as control

Group II: Received application of standard drug ointment i.e. Povidone-Iodine (5%w/w)

Group III: Received application of 10% w/w AESE which considered as low dose.
Group IV: Received application of 20% w/w AESE which considered as high dose.
AESE: Alcoholic Extract of *Solanum erianthum*
The incision wound model was studied. Under light ether anesthesia the animal was secured to operation table in its natural position. One paravertebral straight incision of 6 cm was made on either side of the vertebral column with the help of scalpel blade. Wounds were cleaned with 70% alcohol soaked with cotton swabs. They were kept in separate cages. The extract was applied at a dose of 10% and 20% w/w gel for 10 days. All the sutures were removed on the 9th post wounding day. On tenth day the tensile strength was measured by continuous constant water supply technique.

Measurement of Wound Breaking Strength of incised wounds

Measurement of wound breaking strength was performed with certain modifications. A board was placed on the table, on which the anaesthetized animal was made to lie on its abdomen. Two clamps were clamped on either sides of healed wound at a distance 0.5 cm. The left clamp was fastened tightly to stand by means of thread. The right clamp was connected to a leak proof polythene container through a pulley, by means of a thread. A reservoir containing water was placed at a suitable height and connected to a polythene bag by means of a rubber tube. The position of the board was adjusted so that, the polythene bag was hanging freely. Water was added to polythene bag rapidly at constant rate from the reservoir until the wound opened. Amount of water in polythene bag was measured (in ml) and was considered as tensile strength of the wound.

Statistical analysis

The relative wound area results were compared using one way analysis of variance (ANOVA) followed by Dunnet's tests.[18] *P*-values less than 0.05 were considered as indicative of significance.

RESULTS

Table 1: Percentage yield of AESE

Solvent	Colour and Consistency	Percentage yield
70% hydro alcohol	Green and sticky	13.30%

Table 2: Preliminary Phytochemical Screening of AESE

Phytochemical constituents	70% hydro alcoholic extract
Carbohydrates	_
Steroids	+
Glycosides	+
Flavonoids	+
Alkaloids	+
Tannins	+
Triterpinoids	_
Saponins	
Proteins	+

Both the l0% and 20% doses of AESE gel does not showed any type of irritation, inflammation and redness.

Table 3: Results of Skin Irritation Study

Group	Sign	Score
Control	_	0
10% AESE	Not noticeable redness and inflammation	0
20% AESE	Not noticeable redness and inflammation	0

A significant decrease in period of epitheliazation was observed after 10% AESE and 20% AESE application. Treatment with Povidone-Iodine also significantly reduced period of epitheliazation as compared with control group. At the same time 10% and 20% AESE and Povidone-Iodine also decreased the wound contraction (50%) as compared with control.

Table 4: Effect of leaf extract of *S.erianthum* on excision wound [Wound Area (mm^2)]

Group	wound contraction on											Epitheliazation time (Days)
	2nd Day	4th Day	6th Day	8th Day	10th Day	12th Day	14th Day	16th Day	18th Day	20th Day	21st Day	
A Control	385.0± 6.19	330.0± 5.77	260± 7.30	210.8± 8.20	138.5± 12.07	96.5± 5.22	69±13. 12	50.83± 12.67	28.33± 9.7	10.5 ±4.7	00	9.167±0.40
B Standard Povidine-Iodine	356.7± 10.5	288.8± 8.90	213.7± 2.66	125± 6.19	48.67± 5.01	36.5± 4.63	22.67± 4.31	13.17± 2.37	3.66± 1.68	00	00	8.33±0.21
C 10% AESE	316.7± 4.21	231.7± 13.02	161.7± 13.27	88.33± 11.08	37.33± 9.26	29.67± 7.72	19.83± 6.86	8.66± 2.91	00	00	00	7.50±0.22
D 20% AESE	321.0± 5.13	263.8± 8.44	164.8± 9.44	90.17± 9.57	37.83± 5.9	29.17± 4.48	12.33± 2.89	4.16± 1.61	00	00	00	7.33±0.21

Values are mean ± SEM (n-6) one way ANOVA followed by Dunnet's test where * represents significant at < 0.05, ** represents highly significant at p < 0.01, *** represents very significant at p< 0.001.

Table 5: Effect of leaf extract of *S.erianthum* on excision wound (% Wound closure)

Day	Group I	Group II	Group III	Group IV
0	0	0	0	0
2	13.74%	24.09%	27.98%	27.38%
4	26.03%	36.00%	42.25%	40.03%
6	41.78%	47.28%	63.17%	62.72%
8	52.80%	71.73%	79.91%	79.53%
10	69.50%	88.97%	91.51%	88.61%
12	83.97%	91.64%	93.25%	93.38%
14	84.5%	94.83%	94.59%	96.63%
16	88.58%	96.97%	97.04%	98.55%
18	93.62%	98.31%	100%	100%
20	95.26%	100%	100%	100%
21	100%	100%	100%	100%

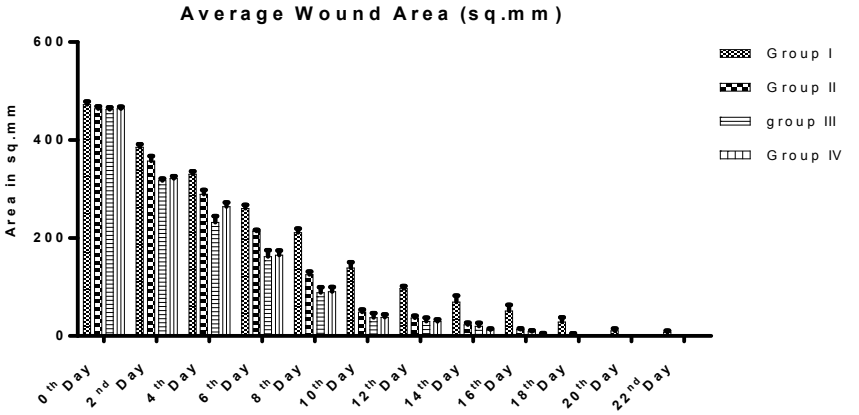

Figure 1: Effect of AESE on wound area (sq.mm) in Excision Wound Model

Table 6: Effect of leaf extract of *S.erianthum* on wound breaking strength (g) in incision wounds

Groups	Treatment	Wound Breaking Strength (g)
Group I	--	268.0±7.5
Group II	5%w/w Povidone-Iodine	368.9±14.51***
Group III	10% AESE	417.9±12.23***
Group IV	20% AESE	483.8±11.0***

Values are mean ± SEM (n=6) one way ANOVA followed by Dunnet's test where * represents significant at < 0.05, ** represents highly significant at p < 0.01, *** represents very significant at p< 0.001

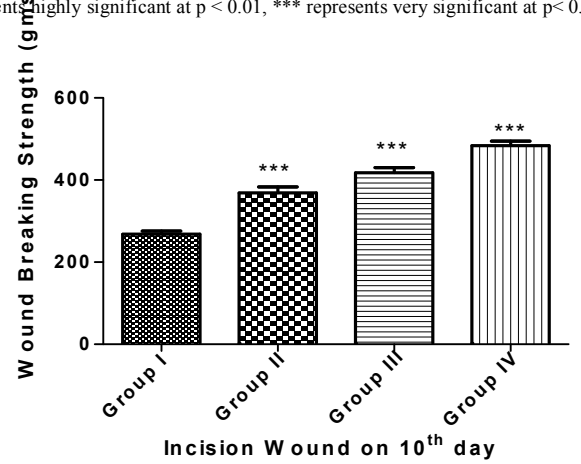

Figure 2: Effect of AESE on Breaking Strength (g) in Incision Wound Model

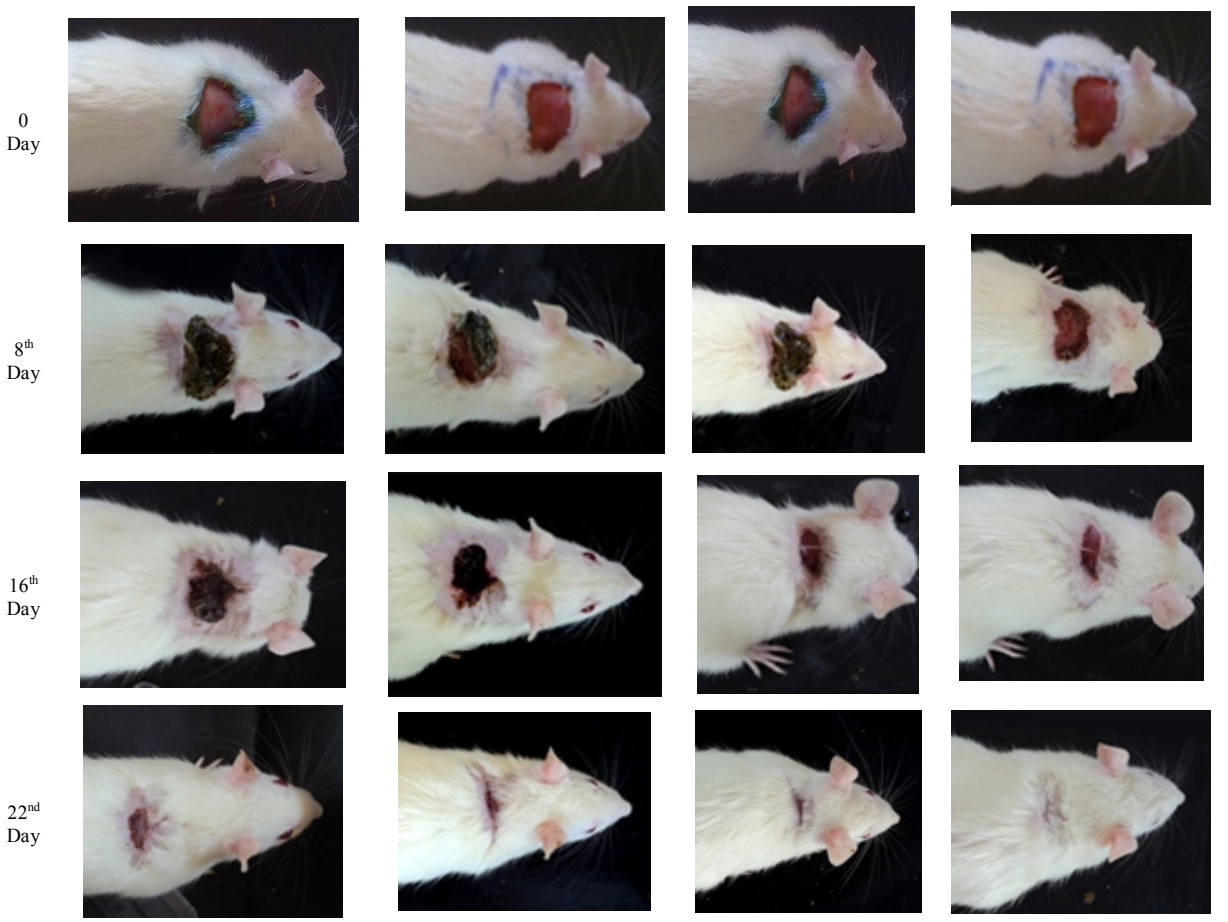

Figure 3: Photographs showing excision wound (topically treated) at different days

Resutured incision wound

Figure 4: Tensile strength measurement and setup for its measurement

DISCUSSION

The present study was undertaken to evaluate whether *Solanum erianthum* leaf extract could promote wound healing in experimentally produced wound in rats. This observation substantiates the use of *Solanum erianthum* leaves in folklore medicine for the treatment of wounds.

The preliminary phytochemical analysis of AESE revealed the presence of flavonoids, alkaloids, tannins, steroids, glycosides, carbohydrates and proteins.

In the present study, the extract applied topically promoted the breaking strength, the wound contraction and period of epitheliazation.

The skin irritation study on the rabbit skin proved that drug does not show any type of inflammation when applied to the skin. This suggests that the drug may contain some chemical constituents, which does not produce any irritation and navigate the wound healing activity of *Solanum erianthum.*

In excision wound model the AESE showed faster healing compared with control groups and wound contraction rate was faster. It may be due to stimulation of interlukin-8. The epitheliazation period was decreased may be due to enhanced collagen synthesis.

In incision model, the wound breaking strength was increased. Increase in tensile strength may be due to increase in collagen concentration and stabilization of the fibres.

Flavonoids are known to reduce lipid peroxidation, not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis.

AESE contains alkaloids, which are used internally to treat stomach-ache and is applied externally to skin irritations and rashes.

Solanum erianthum was more potent than Povidone – Iodine in excision and incision wound models.

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