

EVALUATION OF WOUND HEALING ACTIVITY OF METHANOLIC EXTRACT OF *SMILAX WIGHTII* (A. DC.) IN WISTAR ALBINO RATS.

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ABSTRACT

The present experimental study was designed to evaluate the wound healing activity of methanolic extract of *Smilax wightii* A. DC. on incision and excision wound models in Wistar albino rats. The parameters studied were wound breaking strength, wound contraction area, epithelialization period, granulation tissue wet, dry weight and hydroxyproline content in incision wound model, percentage of wound contraction and period of epithelialization in excision wound model. The rats were administered topically with 100mg/kg b.wt. (low dosage), 200mg/kg b.wt. (moderate dosage) and 500mg/kg b.wt. (high dosage) of methanolic extract of *Smilax wightii* (MESW). The activity of the extract treated groups were compared with that of the control 1% Spirit. Framycetin sulphate 0.2% w/w was used as the standard drug. In incision wound model, there was a significant increase in the wound breaking strength in all the experimental groups treated with MESW than that of the control. Similarly, significant ($P < 0.001$) decrease in wound contraction area and period of epithelialization were also observed in the test group animals treated with MESW and the standard drug treated groups when compared to that of the control. , a significant increase was observed in granulation tissue wet and dry weight and hydroxyproline content in the test groups treated with MESW compared to the control. In excision wound model, there was a significant increase ($P < 0.01$) in the percentage of wound contraction and decrease in period of epithelialization in the experimental groups treated with 200mg/kg b.wt. (moderate dosage) and 500mg/kg b.wt. (high dosage) of MESW. The extract treated groups showed significant improvement in all the wound healing parameters of incision, and excision wound models.

Keywords: *Smilax wightii* extract, wound healing, incision and excision wound models.

1. INTRODUCTION

The genus *Smilax* comprises more than 300 species which are distributed on temperate, tropic and subtropic zones worldwide (Fnaec, 2002). About 24 species of *Smilax* genus are found in India (Saldhana and Wilson, 1976, Ramaswamy, *et al.*, 2001). In South India, the genus is represented by 4 species viz., *Smilax zeylanica* Linn., *Smilax aspera* Linn., *Smilax perfoliata* Roxb. and *Smilax wightii* A. DC. (Gamble, 2004). The rare endemic plant, *Smilax wightii* A. DC. is generally distributed in Shola forests at high altitudes in Nilgiri Biosphere Reserve, the Western Ghats, Southern India (Paulsamy *et al.*, 2009). The roots of *Smilax wightii* A. DC. have been reported to cure dysentery, amoebiasis, venereal diseases, urinary complaints, fever, antifertility, anaemia, rheumatic-arthritis, veterinary amoebiasis and gastric complaints (Adhikari *et al.*, 2010). *Smilax* rhizomes have different kinds of pharmacological behaviours such as antibacterial, antifungal, antioxidant and other activities (Ozoy *et al.*, 2008).

Wounds are physical injuries which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodelling of connective tissue, collagenization and aquisition of wound

strength (Suresh Reddy *et al.*, 2002). The wound healing process consists of four steps. The first stage is the inflammatory stage which is directed at preventing further loss of blood by platelet accumulation at the site leading to coagulation those results to the formation of thrombus. The debridement stage occurs from the third to the sixth day after injury and involves the appearance of neutrophils to clear contaminating organisms. The proliferation or repair stage is characterized by endothelial budding in the nearby blood vessels forming new capillaries that penetrate and nourish the injured tissue. The maturation stage commences from tenth day to several months depending on wound severity during which the number of capillaries decreases and wound changes from pink or white (Thakare *et al.*, 2011).

Some of the synthetic drugs currently used for the treatment of wounds are not only expensive but also cause problems such as allergy, drug resistance etc and this situation has forced scientists to seek alternative drugs (Sai and Babu, 1998). A large number of plants are used by tribal and folklore in many countries for the treatment of wounds and burns. These phytomedicine are not only cheap and affordable but are also safe. The presence of various

life-sustaining constituents in plants has urged scientist to examine these medicinal plants with a view to determine potential wound healing properties (Nayak and Pinto Pereira, 2006).

A survey of literature revealed that no scientific study on the wound healing activity of this plant has been carried out. Hence the present study was undertaken to assess the wound healing activity of methanolic extract of *Smilax wightii* (MESW) on incision and excision wound models in Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Plant material

The plant *Smilax wightii* was collected from Uthagamandalam, the Nilgiri Hills, Western Ghats, Southern India, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist, M. Murugesan, Scientist, SACON, Coimbatore.

2.2 Preparation of methanolic extract

500 g of the whole plant powder of *Smilax wightii* was shade dried and used to extract with methanol by using soxhlet apparatus. This extract was stored at 4°C and used for further studies.

2.3 Animals

Wistar albino rats of either sex and of the same age weighing between 150 – 250 g were used for the study. They were individually housed, maintained in clean polypropylene cages and fed with commercially pelleted rat chow (M/s Hindustan Lever Ltd. Mumbai) and Water *ad libitum*. The experimental protocol was subjected to the scrutiny of Institutional Animal Ethical Committee for experimental clearance (Reg No. 659/02/a/CPCSEA).

2.4 Toxicity studies

The acute toxicity studies were carried out in adult male Wistar Albino rats weighing 180-250g. The animals were fasted overnight and 100 -1000 mg/kgb.wt of the test extract was given to various groups containing 6 animals in each group. The treated animals were monitored for 14 days for their behaviour, general health and mortality.

2.5 Experiment

The surgical interventions in incision and excision wound models were carried out under sterile conditions using ketamine anaesthesia (120mg/kgb.wt). The experimental animals were divided in to five groups of six animals in each group and received the following treatments. The first group were treated with 1% spirit topically and

considered as untreated control. The second group were treated with 100mg/kgb.wt (low dosage) of MESW, the third group with 200mg/kgb.wt (moderate dosage) of MESW, the fourth group with 500mg/kg b.wt(high dosage) of MESW and the fifth group were treated with 0.2% w/w Framycetin Sulphate (FSC) topically (standard drug). The MESW was applied on the wound for the test groups from the first day till the day of healing. The level of dosage was 2ml for all the three test groups.

2.6 Incision wound

The rats were anaesthetized prior to and during creation of the wound. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision, six centimeters in length was made through the skin and cutaneous muscle on the back (Ehrlich and Hunt, 1968). After the incision, surgical sutures were applied to the parted skin at intervals of one centimetre. The wounds were left undressed. The sutures were removed on the 8th post wound day and the treatment was continued. The wound-breaking strength was measured on the 10th day by the method of (Lee, 1968).

2.7 Determination of 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 opposite to each other. One 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 wounded 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, and the procedure was repeated on the contra lateral 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.

2.8 Determination of granulation tissue dry weight and wet weight

In the healed tissues excised from incision wound model rats, the wet weight of the granulation tissue was noted. These granulation tissues were dried at 60°C for 12 hours, and weighed, and the dry weight was recorded. To the dried tissue added 5 ml 6 N HCl and kept at 110°C for 24 hours. The neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyproline (Neuman and Logan, 1950).

2.9 Estimation of Hydroxyproline

Hydroxyproline present in the acid hydrolysate of granulation tissue oxidized by sodium peroxide in the presence of copper sulfate, when complexed with para-dimethylaminobenzaldehyde, develops a pink color that was measured at 540 nm using colorimetry.

2.10 Excision wound model

Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by (Morton and Malon, 1972). The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area 500mm² and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade or pointed scissors. The entire wound was left open (Diwan and Tiloo, 1982). The wound closure rate was assessed by tracing the wound on days 2, 4, 6, 8, 10, 12 and 14 post-wounding using transparency papers and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of eschar without any residual raw wound gave the period of epithelialization.

2.11 Statistical analysis

The data is expressed as mean \pm SEM and subjected to one way ANOVA and the level of significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

No sign of toxicity was noticed on the behaviour and general health of the animals when exposed to the extract at a dosage of 100-1000mg/kgb.wt. No deaths were also observed.

The wound contraction area and the period of epithelialization were observed at an interval of four days till the sixteenth day of wound healing. A rapid decrease in both the parameters were observed in the experimental groups treated with (MESW) and also in framycetin sulphate treated groups when compared to that of the control group in incision wound model (Table-2).

The granulation tissue wet and dry weight and the hydroxyproline content showed significant ($P < 0.01$) increase in the extract treated groups when compared to the control. (Table-3). The increase in dry granulation tissue weight in the test group animals suggests higher protein content. The

methanolic extract of *Smilax wightii* demonstrated a significant increase in the hydroxyproline content of the granulation tissue indicating increased collagen turnover. Collagen, the major component which strengthens and supports extra cellular tissue is composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen (Kumar *et al.*, 2006).

The wound contraction was assessed at an interval of two days till the fourteenth day of wound healing. It was observed that the percentage of wound contraction increased and the epithelialization period decreased significantly in excision wound model with the application of MESW as compared to the control group. The standard drug framycetin sulphate also showed profound activity in the percentage of wound contraction in excision wound model rats (Table-4). Preliminary phytochemical screening revealed the presence of tannins, alkaloids, flavonoids, terpenoids, phenols and tannins in *Smilax wightii*. Flavonoids have been documented to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important components of wound healing (Devipriya and Shyاملadevi, 1999).

4. CONCLUSION

The results of the present study has led to the conclusion that the methanolic extract of *Smilax wightii* has exhibited prominent wound healing activity in incision and excision wound models with significant results in all the parameters studied.

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Table 1. Effect of methanolic extract of *Smilax wightii* (MESW) on wound breaking strength in incision wound model.

Groups (n)	Wound breaking strength(g)
Control - I	318.26± 4.18
MESW-100mg/kg (low) - II	431.16± 4.84 **
MESW-200mg/kg - (moderate) - III	482.16 ±3.93 **
MESW-500mg/kg (high) - IV	514.1±3.84 ***
Standard drug (FSC) - V	365.16 ±3.54

* P < 0.05,** P<0.01,*** P<0.001, compared to control vs drug treated groups values are mean ± S.E.M. (n = 6)

In incision wound model, the extract treated animal (group II, III and IV) showed significant improvement in wound breaking strength when compared to the control group (Table-1).

Table 2. Effect of methanolic extract of *Smilax wightii* (MESW) on incision wound model. mean ± SE wound contraction area (mm²)

Groups (n)	4th day	8th day	12th day	16th day	Epithelization period
Group I	381.16±1.48	361.16±2.91	210.16±2.93	138.13±2.54	26.16±3.13
Group II	284.16±2.16	232.13±1.93	156.84±2.06**	30.16±1.22***	17.84±0.54***
Group III	230.16±1.84	176.84±2.04*	76.16±0.93***	22.15±1.16***	16.34±0.84***
Group IV	216.84±3.16	184.92±3.10**	31.18±0.93***	1.16±0.09***	12.28±0.85***
Group V	211.16±3.11	152.84±1.93***	54.13±0.18***	08.16±0.34***	11.65±1.13***

* P < 0.05,** P<0.01,*** P<0.001, compared to control vs drug treated groups values are mean ± S.E.M. (n = 6)

Table 3. Effect of methanolic extract of *Smilax wightii* (MESW) on granulation tissue wet, dry weight and hydroxyproline content in incision wound model.

Groups (n)	Granulation tissue wet weight (mg)	Granulation tissue dry weight (mg)	Hydroxyproline (mg/tissue)
Control - I	82.6 ±2.16	12.5± 0.91	32.4 ±1.90
MESW-100mg/kg (low) - II	131.58± 1.93**	21.13± 0.16 **	80.2± 4.16*
MESW-200mg/kg - (moderate) - III	172.46 ±3.84**	22.5± 0.34 **	85.84± 2.27**
MESW-500mg/kg (high) - IV	188.54± 4.20 **	24.16± 0.93**	95.96± 2.55**
Standard drug (FSC) - V	124.16 ±3.40*	18.4± 0.16**	92.16± 2.88*

* P < 0.05, ** P<0.01, *** P<0.001, compared to control vs drug treated groups values are mean ± S.E.M. (n = 6)

Table 4. Effect of methanolic extract of *Smilax wightii* (MESW) on percentage of wound contraction in excision wound model.

Groups	Epithelization Period (days)	Excision Wound model% of Wound contraction in different days						
		2	4	6	8	10	12	14
Group I	20.16±0.28	16.31±0.27	28.16±0.16	34.65±0.78	41.16±0.75	55.16±0.93	63.16±0.91	69.54±1.16
Group II	13.42±0.84**	21.34±1.06	43.91±0.84*	59.16±0.93	76.28±0.16*	84.24±0.16**	87.16±1.84	93.16±0.84**
Group III	15.13±0.92**	29.16±0.84*	58.31±0.96*	68.36±0.91*	82.68±1.16**	96.1±1.34*	98.54±0.91**	99.13±1.06**
Group IV	17.83±0.28*	30.84±0.91*	62.84±0.84*	75.16±10.6*	89.16±0.28*	90.33±1.81*	96.84±0.56**	98.84±0.16**
Group V	16.33±0.27	24.13±0.18*	60.33±0.16*	69.26±1.06*	85.16±0.18*	91.36±0.91*	94.32±0.84**	97.29±1.04**

* P < 0.05, ** P<0.01, *** P<0.001, compared to control vs drug treated groups Values are mean ± S.E.M. (n = 6)