Wound Healing Activity of *Oxalis corniculata* Whole Plant Extract in Rats.

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The alcohol and petroleum ether extract of whole plant of *Oxalis corniculata* (Oxalidaceae) has been evaluated for its wound healing activity by using excision, resutured incision and dead space wound models in rats. Both the extracts at the dose of 300 and 500 mg/kg, p.o. showed significant wound healing activity by producing an increase in wound contraction rate, wound breaking strength, granuloma breaking strength and hydroxyproline content and significant decrease in epithelization period in the wound models studied.

Oxalis Corniculata Linn, belonging to family Oxalidaceae is a very common weed found throughout the warmer parts of India. It is a small annual or perennial creeping herb with yellow flowers[1]. The plant is traditionally used for the treatment of convulsions, fever, dyspepsia, stomach ache, piles, anaemia, chest complaints, tympanitis, snake and scorpion bites and inflammatory tumours. The leaves of the plant are used as antidiarrhoeal, antipyretic, astringent, appetizing, digestive, refrigerant and as a household remedy[2,3]. The plant has been reported to have smooth muscle relaxant, cardiorelaxant and hypotensive activity[4]. It is also reported to have antiinflammatory activity in carrageenan-induced paw oedema in rats[5]. However, no detailed scientific reports are available on its wound healing activity. Hence the present study was carried out to evaluate wound healing activity of alcohol and petroleum ether extracts of the whole plant of *Oxalis corniculata* in different models of experimental wounds.

The dried plant material of *Oxalis corniculata* was purchased from Genuine Company, Mumbai. Further taxonomic identification was conducted in the Department of Botany, R. L. Science institute, Belgaum. A voucher specimen has been deposited in Department of Pharmacognosy, K.L.E.S's College of Pharmacy, Belgaum. The dried material was powdered and subjected to successive extraction by soxhlet using petroleum ether (40-60°) 21/24 h and ethanol (95%) 21/24 h. The solvent was removed by distillation and evaporation to obtain a semisolid mass. Both alcohol and petroleum ether extracts were insoluble in water, hence they were suspended in 2% w/v solution of Tween 80, to obtain an aqueous suspension.

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Wistar rats and Swiss mice of either sex weighing 150-200 g and 20-25 g, respectively obtained from animal house of Shri Venkateshwara Enterprises, Bangalore were used for the study. They were housed in clean polypropylene cages at standard environmental conditions of temperature (23±1°), 12 h light/dark cycle and were provided with standard pellet chow and water *ad libitum*. Acute toxicity studies were performed on mice by administering both the extracts orally to different groups of mice in doses ranging from 1000-2500 mg/kg for LD_{50} study[6]. Rats were divided into five groups of 6 animals each. The group 1 served as control (received 0.5 ml of 2% Tween 80), group 2 and 3 received 300 and 500 mg/kg of alcohol extract and group 4 and 5 received 300 and 500 mg/kg of petroleum ether extract respectively. Wound healing activity was evaluated using excision, incision and dead space wound method in rats. Before performing these experiments, ethical clearance was obtained from Institutional Animal Ethics Committee (CPCSEA Registration No. 221).

Excision wound were induced by excising the skin of the impressed area to full thickness to obtain a wound area

of about 500 mm^2 in anaesthetized rats[8]. The parameters studied were wound closure, epithelization time and scar area. The number of days required for falling of the eschar without any residual raw wound gave the period of epithelization. The % wound closure was evaluated on d 0, d 4, d 8, d 12 and d 16. The scar shape and area were traced and measured planimetrically. Incision wound were inflicted by making two 5 cm long paravertebral incisions through full thickness of the skin on either side of the vertebral column of the anaesthetized rats[9]. Wounds were closed with interrupted sutures, which were removed on 8th post wounding d and wound breaking strength of 10 d old wound was measured[10].

Dead space wound was introduced by implanting sterilized grass piths (25x3 mm) subcutaneously in the rat groin[7]. The 10 d old granuloma were carefully dissected and cleared off adventitious tissues. The rectangular strip obtained by slitting tubular granulomatous growth on grass pith was tested for tensile strength[10]. One part of granuloma tissue was used for the determination of hydroxyproline[11]. The other part of granuloma tissue was subjected to histopathological studies by staining with haematoxylin and eosin so as to enable the assessment of fibroblast population, collagen content and thickness of tissue under light microscope. Results are expressed as mean±SEM. Statistical analysis was performed by using one way analysis of variance (ANOVA) followed by Dunnett's 't' test for comparison of test groups with control. P-values <0.05 were considered statistically significant.

The alcohol and petroleum ether extract of *Oxalis corniculata* when given up to 2500 mg/kg by oral route did not produce any lethality in mice. Hence, the study dose selected for wound healing activity was 300 and 500 mg/kg per d. The % closure of excision wound area was significantly ($p<0.05$) increased in both alcohol and petroleum ether extract treated groups at the doses of 300 and 500 mg/kg when compared to control. The time required in days for complete epithelization of excision wound was significantly ($p<0.05$) decreased in both alcohol and petroleum ether extract treated groups at the doses of 300 and 500 mg/kg when compared to control. The scar area in mm^2 on complete epithelization was significantly ($p<0.05$) decreased in both alcohol and petroleum ether extract treated groups at both the doses studied when compared to control (Table 1). In the incision wound model, significant ($p<0.05$) increase in breaking strength of 10 d old resutured incision wound was observed in both alcohol and petroleum ether extract treated rats at doses of 300 and 500 mg/kg when compared to control (Table 1). In dead space wound model, there was significant ($p<0.05$) increase in granuloma breaking strength in both alcohol and petroleum ether extract treated rats at 300 and 500 mg/kg doses when compared to control. The histopathological study of granuloma tissue revealed that in extracts treated groups, there was increase in granulation tissue with increase in fibroblasts and collagen content as compared to control. It was also found that there was significant ($p<0.05$) increase in hydroxyproline content in both alcohol and petroleum ether extract treated rats at 300 and 500 mg/kg doses as compared to control (Table 2).

TABLE 1: EFFECT OF *OXALIS CORNICULATA* ON EXCISION WOUND

Group	Treatment (mg/kg)	% Closure of excision wound area				Epithelization (d)	Scar area (sq. mm)
		4 d	8 d	12 d	16 d		
1	Control	12.0 ±4.71	47.3 ±2.08	78.4 ±0.62	90.1 ±0.94	22.6±0.05	51.4±0.67
2	Alcohol extract (300)	40.9 ±4.86*	79.4 ±1.70*	92.8 ±0.4*	99.7 ±0.21*	16.0±0.44*	40.0±0.94*
3	Alcohol extract (500)	39.8±1.05*	78.0 ±0.53*	91.0 ±0.49*	99.7 ±0.38*	16.0±0.44*	39.2±0.66*
4	Petroleum ether extract (300)	36.9 ±3.32*	79.8 ±0.94*	93.5 ±0.56*	99.7 ±0.21*	16.2±0.58*	37.6±0.50*
5	Petroleum ether extract (500)	39.2 ±0.63*	77.0 ±0.6*	90.8 ±0.53*	99.5 ±0.9*	16.2±0.58*	37.8±0.71*

Values are mean±S.E.M. for 6 observations, *P<0.05 vs control group. Control group was administered 0.5 ml of 2% Tween 80 solution.

TABLE 2: EFEECT OF *OXALIS CORNICULATA* ON INCISION AND DEAD SPACE WOUND

Group	Treatment (mg/kg)	Incision wound	Dead space wound	
		Wound breaking strength (g)	Granuloma breaking strength (g)	Hydroxyproline content (μg/ml)
1	Control	278±9.32	250±4.04	1.98±0.11
2	Alcohol extract (300)	487±1.05*	297±4.10*	4.91±0.58*
3	Alcohol extract (500)	491±10.9*	305±5.41*	4.98±0.06*
4	Petroleum ether extract (300)	475±10.55*	287±3.10*	4.75±0.06*
5	Petroleum ether extract (500)	479±10.85*	302±6.63*	5.00±0.07*

Values are mean±S.E.M. for 6 observations, *P<0.05 vs control group. Control group was administered 0.5 ml of 2% Tween 80 solution.

Wound healing involves different phases such as contraction, epithelization, granulation and collagenation. There was significant increase in rate of wound contraction in rats treated with both the extracts of *O. corniculata* treated rats as evidenced by enhanced epithelization of excision wound. In resutured incision wound, wound breaking strength is determined, which indirectly represents collagenation phase of healing and this parameter is commonly used to assess the healing, perhaps because surgeons are specially interested and concerned with the strength of healed incision wound[12]. Breaking strength of the resutured incision wound was increased in extract treated groups as compared to control.

Dead space wound provides an opportunity to study the effect on granulation and collagenation of the healing process. Such wound models have been employed for quantitative and qualitative studies on wound healing such as granuloma breaking strength and hydroxyproline content[13]. Gain in granuloma breaking strength indicates increased collagen maturation by increased cross-linking. Hydroxyproline content estimation gives the net rate of synthesis and deposition of collagen in healing wound[14]. In this study, both the extracts of *O. corniculata* significantly increased the granuloma tissue breaking strength and hydroxyproline content as compared to control. The study confirms that *Oxalis corniculata* whole plant extract possess significant wound healing activity.

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