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Wound Healing Potentiality Of Methanolic Extract Of Aerial Parts Of Cleome viscosa

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Abstract: The leaves and the whole plant of *Cleome viscosa* are used as folklore remedy to cure wound, ulcers, inflammation and skin infections. The present investigation was carried out to evaluate the wound healing property of the aerial parts of the plant on experimentally induce excision and incision wound models in rats. It was an attempt to justify the traditional use of the plant. The studies of the methanolic extract of the aerial parts showed significant wound healing properties compared to the standard drug neosporin (0.2%)
Keywords: *Cleome viscosa*, skin infection, excision wound models, wound, Neosporin.

1. Introduction

Cleome viscosa Linn belonging to the family Capparidaceae is commonly known as Tickweed, wild mustard or Spider plant (English), *Hurhur* (Hindi) and *Hurhuria* (Bengali). It occurs in woodland and grassland, and is a weed of fallow land, fields, roadsides and wasteland, often occurring on sandy soils, but sometimes on calcareous and rocky soils. It is a widely distributed herb with yellow flowers and long slender pods containing seeds. The whole plant is sticky in nature and has a strong odour[1]. Traditionally the leaves, bark, root and seeds of the plants of Cleome genus are used as stimulant, ant scorbutic, anthelmintic, rubifacient, vesicant, carminative, stomachic, laxative, diuretic, anti inflammatory, anti tumour, antiseptic, anti leprosy[2,3,4,5,5] . The analgesic, anti microbial, anti diarrhoeal, anti pyretic, hepatoprotective, anti hyper lipidemic and anti ulcer[7-18] activities of the leaves have already been reported by the researchers in the past.

Wound in a normal state of body get healed by various processes which is fundamentally a connective tissue response, initial stage of this process involves an acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules which are later remodelled to form a scar.[19] A wound is a type of injury in which skin is torn, cut, or punctured (an *open* wound), or where blunt force trauma causes a contusion (closed wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin. Wounds are physical injuries that result in an opening or breaking of the skin. Two important pathological manifestations in wounds are fibrotic wounds, and keloid scar formation and ulcers.

2. Experimental

Materials and Methods

2.1.1: Plant Material

The aerial parts of *Cleome viscosa* were collected from Greater Noida. The plant was authenticated and identified at NBPGR, Pusa Campus, New Delhi and a voucher was specimen preserved in our laboratory for

future reference .Aerial parts were shade dried, coarsely powdered, passed through 40 mesh sieve and stored in air tight container.

2.1.2: Preparation of the Extracts

92 gm of the coarsely powdered aerial parts of the plant was passed through 40 mesh sieve and then subjected to extraction with methanol in Soxhlet apparatus. The methanolic extracts were dried and incorporated in to simple ointment base. Preliminary phytochemical analysis was carried out for the extracts according to the standard procedures[7,9,17,18] . The solvent was removed under vacuum and the extract was concentrated to dryness in vacuum and a solid mass (18.70 % w/w) with respect to dry starting material was obtained. The methanol extract was stored in a dessicator and used for further experimental studies.

2.1.3: Animals

Albino wistar rats weighing between 130-180 g of either sex were used for wound healing activity. All experimental animals were obtained from the animal house, Department of Pharmaceutical Technology, Noida Institute of Engineering and Technology, Greater Noida, and were maintained in 25 ± 1°C, with 55 ± 5 % humidity with 12 hr light/dark cycle. The animals were housed in the standard polypropylene cages and provided with food and water ad libitum. The experimental protocols were approved by Institutional Animal Ethics Committee (Regn No: 1121/ac/CPCSEA/07, 26/11/2007). Ethical clearance for handling the animals was obtained from the Institutional Animal Ethical Committee prior to the beginning of the project work bearing the protocol number NIET/IAEC/2013/44

2.1.4: Chemicals

Petroleum jelly, chemicals and reagents used were of analytical grade.

2.1.5: Drugs

Standard drug: Neosporin, Test drug: plant extract of *Cleome viscosa.*

3: Oral acute toxicity studies

Healthy adult wistar rats of either sex, starved overnight, were divided into groups (n=6) and were orally fed with increasing dose of ethanol extracts. Total ethanol extracts administered orally in doses of up to 2g/Kg, did not produce any sign of toxicity and mortality in rats when observed for 7 days after administration.[20]

4: Wound healing Activity by excision model

Albino wistar rats (130-180 gm) of either sex were used for evaluation of wound healing activity. The animals were divided into four groups. Four groups of animal containing six in each group were anaesthetised by open mask method with anaesthetic ether. The rats were depilated on the back and a predetermined area of 500mm^2 full thickness skin was excised from the dorsal inter scapular region. Rats wounds were left undressed in open environment to monitor wound contraction and epithelisation time. The reference standard drug (0.2% Neosporin ointment), white petroleum jelly, *Cleome viscosa* methanolic extract ointment (5% and 10% w/w of aerial parts) were applied everyday till the wound was completely healed. The progressive changes in the wound area were monitored by tracing the wound margin on a graph paper. The measurement of wound area on graph paper was expressed as unit (mm^2). Wound contraction was expressed as percentage reduction of original wound size.

Group I	Control (Petroleum jelly)
Group II	Standard (0.2%Neosporin Ointment)
Group III	5%w/w *cleome viscosa* extract ointment
Group IV	10% *cleome viscosa* extract ointment

Changes in wound area were calculated giving an indication of the rate of wound contraction. The areas of the wounds were measured by tracing the wounds on to a graph paper on the day of wounding and subsequently on 4th, 8th, 12th, 16th and 18th day post wounding. The number of days required for falling of the scar without any residual raw wound, gave the period of epithelization. The observations of the percentage

wound contraction were made on 4th, 8th, 12th, 16th and 18th day post wounding days. All the values were statistically analyzed by unpaired student t-test comparing with control.[22,23]

4.1: Wound healing Activity by incision model:

Four groups of animals containing six animals in each group were taken. These animals were anaesthesized under light ether anaesthesia. One full thickness par vertebral incision of 6 cm in length was made including subcutaneous muscles of the depilated back of each rat. After the incision was made the parted skin was kept together and stitched with sutures 1cm apart. The continuous threads on both wound edges were tightened for good adaptation of wound and it was left undressed. The ointment of the leaf extract, standard drug and white petroleum jelly was applied to the wound twice daily until complete recovery to the respective groups of animals.[24]

Group I	Control(Petroleum jelly)
Group II	Standard (0.2% Neosporin ointment)
Group III	5% CVAE
Group IV	10% CVAE

On the 9th day after wounding the sutures were removed and the tensile strength was measured on the 10th day. For measuring the tensile strength the rats were again anaesthesized and each rat was placed on the stack of towels on the middle of the board. The amount of the towels could be adjusted in such a way so that the wound was on the same level as the tips of the arms. The clamps were then carefully clamped on the skin opposite of the wound at a distance of 0.5cm away from the wound. The longer pieces of the fishing line were placed on the pulley and finally polyethylene bottle. The position of the board was adjusted so that the bottle received a rapid and constant rate of water from a large reservoir, until the wound began to open. The amount of water in polyethylene bag was weighed and equated as tensile strength of the wound. The tensile strength induce by the extract and by Neosporin ointment treated wounds were compared with control.[24]

5: Result And Discussion

5.1: Phytochemical analysis

The phytochemical investigation revealed the presence of alkaloid, tannins, saponins, flavonoids, in methanolic extracts of the aerial parts of *Cleome viscosa*. Thus the wound healing activity of the plant can be attributed to the presence of their phytoconstituents which may be active individually or it may be the synergic activity of the constituents.

5.2 Oral Acute Toxicity Studies

In the acute oral toxicity studies, no mortality and no macroscopically organ abnormality/damage were observed at the tested dose. Thus acute toxicity studies showed that *Cleome viscosa* extracts were safe up to maximum dose of 2g/Kg body weight of the animal.

5.3: Wound healing Activity

The changes in wound area were calculated giving an indication of the rate of wound contraction. The areas of the wounds were measured by tracing the wounds on to a graph paper on the day of wounding and subsequently on 4th, 8th, 12th, 16th and 18th day post wounding. The number of days required for falling of the scar without any residual raw wound, gave the period of epithelization. The observations of the percentage wound contraction were made on 4th, 8th, 12th, 16th and 18th day post wounding days (table1). All the values were statistically analyzed by unpaired student-t test comparing with control. The topical administration of methanolic extract the aerial part of the plant accelerated the process of wound healing. It was also observed that epithelization period of standard group and treated group was less in comparison with that of simple ointment base treated group. The wound closure time was also less as well as the percentage of wound contraction was much more with the 10% w/w extract was comparable with that of Neosporin treated group. However 5% w/w

treated group of animals showed significant wound contraction from the eight day onwards and achieved 100% wound closure time at 18th day 18.83±1.6 justifying the folklore claim.

Table: 1 Wound healing effect of aerial parts of *Cleome viscosa*(Excision method)

Groups	Post wounding days					Epithelization in days ±SEM
	0th	4th	8th	12th	16th	
Control	79.02±5.75 (0%)	70.06±8.18 (11%)	58.53±3.83 (25%)	50.06±4.2 (35%)	40.05±0.1 (49%)	35.06± 0.46
Standard (Neosporin ointment)	79.02±5.75 (0%)	56.91±0.41 (27%)	22.44±4.97 (71%)	2.01±1.57* (97%)	0* (100%)	16.50 ±0.23
Whole plant methanolic extracts 10% (WPME)	81.5±4.86 (0%)	54.15±7.4 (32.8%)	22.97±6.32 (71.9%)	2.61±1.54* (97.2%)	0* (100%)	16.50± 0.23
(WPME) 5%	84.77±8.08 (0%)	57.41±5.73 (32%)	32.18±4.37 (62%)	8.01±1.59 (90%)	2.64±1.56 (97%)	18.83±1.6

Wound area (mm^2 ± SEM) % contraction Student t- test * P<0.05 result is significant, n= 6

Table 2: Evaluation of *Cleome viscosa extract* on incision wound model in rats.

Treatment	Tensile strength(g)
Control	415.02±4.3
Neosporin(0.2%w/w)	585.22±3.7
CVAE(5%w/w)	505.40±3.4
CVAE(10%w/w)	574.89±1.5

Results were compared with control and p value was calculated by Dunnet't t-test,p<0.01,n=6. mean±SEM

Better healing pattern and tensile strength was observed (table 2) for the treated group on 10th day and was found to be significant (p<0.01) than control group as shown in table2. The tensile strength of the 10% extract treated group and the Neosporin ointment treated group were comparable to each other. The 5% extract ointment treated group showed lesser (550.40±3.4)g but significant increase in tensile strength compared to the control group(415.02±4.3)g. The tensile strength of 10% treated group was (580.89±1.5) g. Thus both concentrations of the extract as well as the standard drug showed a significant increase in tensile strength. The result of the present showed that aerial parts *Cleome viscosa* possesses a definite prohealing action. In excision wound healing model the methanolic extract of the aerial parts of the plant showed significant increase in the percentage closure of wounds by enhance epithelisation. Similarly, the breaking strength of the incision wounds was increased in (5% and 10 %) ointment in incision wound model.

6: Conclusion

Thus the wound healing activity of the plant can be attributed to the presence of their phytoconstituents which may be active individually or it may be the synergic activity of the constituents. High performance thin layer chromatography analysis showed the presence of quercetin and gallic acid (0.3% and 0.25% w/w, respectively) in extract. Study showed that *C. viscosa* possesses significant activity, probably due to free radical scavenging activity .The results showed that the aerial parts of the plant possess significant wound healing activity thereby justifying its use in the indigenous system of medicine.

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