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EVALUATION OF WOUND HEALING ACTIVITY OF DIFFERENT EXTRACT OF *SCOPARIA DULCIS*

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ABSTRACT

The objective of the present study is to carry out the photochemical investigation and wound healing activity study of different extracts of *Scoparia dulcis*. *Scoparia dulcis* Linn belongs to the family Scrophulariaceae. The different extracts of this plant were prepared by successive extraction with petroleum ether, chloroform and ethanol. These extracts) were then taken for preliminary phytochemical screening using standard methods. The wound healing activity of the different extracts of *Scoparia dulcis* was evaluated by using excision wound model in experimental rats. The petroleum ether extract (PEESD) was found to be having potent wound healing activity on comparison to chloroform (CESD) and ethanol (EESD) extract. Result shows PEESD have significant wound healing activity on comparison with standard Nitrofurazone ointment. The wound healing potential may be due to the presence of triterpinoids and flavonoids phytoconstituents in the petroleum ether extract of the studied plant.

KEYWORDS

Scoparia dulcis, Nitrofurazone, Petroleum Ether, Chloroform and Ethanol.

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INTRODUCTION

Wound may be described as a debt or breaking of cellular and anatomic/functional continuity of living tissue[1]. Normal wound healing response begins the moment the tissue is injured. Wound healing is the process of restoration that follows injury to the skin and other soft tissues. It is a complex episode involving a number of processes, including introduction of an acute inflammatory process, reconstruction of parenchymal inflammatory process[2], migration and propagation of both parenchymal and connective tissue cells, fusion of

extracellular matrix (ECM) proteins, revamping of connective tissue and parenchymal components, and procurement of wound strength[3]. Every steps of this process are arranged in a controlled way by an assortment of cytokines including growth factors[4]. Some of these growth factors like platelet-derived growth factor B (PDGF), transforming growth factor B (TGF-B), fibroblast growth factor (FGF) and epidermal growth factor (EGF) have been identified in self-healing wound[2]. In chronic case the normal wound healing is disrupted due to the some unknown reasons, and in such cases, external application of certain growth developing agents or compounds which can boosting the in situ generation of these growth factors is required to enhance the healing process. A few components setback wound healing, including necrotic tissue, bacterial disease, obstruction with blood supply, lymphatic blockage and diabetes mellitus. Generally if the above factor could be inhibited/ controlled by any agent, increasing healing rate could be achieved[5].

Scoparia dulcis Linn generally known as *Mithi Patti* (Hindi) is found in the tropical region of India. It grows as a wasteland herb. *Scoparia dulcis* is a small much branched, glabrous, leafy, annual herb or under shrub with erects or ascending branches. Leaves inverse and 3-notely whorled, rhomboid, elliptic or elliptic lanceolate, obtuse at apex, tapering base, serrate margins; Flowers numerous, in terminal panicles, pedicelate, pedicels slim, inflexible: Calyx flaps 4, elongated; Corolla white, tube short, Capsule globose; seeds minute, numerous.

The traditional healers have developed its many promising traditional uses. Traditionally the leaves have been used for abortion, menstrual irregularities as female contraceptive. It also used against stomach aches, injuries, wounds, bronchitis, coughs, diarrhoea, eye infection, fever, and kidney failure and liver diseases. This has been used in case of infections such as gonorrhoea, skin infections and warts[10-13].

MATERIAL AND METHODS

Drug used: Nitrofurazone ointment

Animals

Healthy Wistar rats (150-180 g) of both sex were selected were obtained from the animal house of our college. The rats were maintained at a well ventilated, temperature-controlled (30ºC) animal room for 7 days prior to the experimental period. The animals were provided with normal food and water ad libitum. The rats were periodically weighted before and after experiments. The rats were anaesthetized prior to infliction of the experimental wound. The surgical interventions were carried out under sterile conditions ketamine anaesthesia (10 mg/kg). Animals were closely observed for any infections; those which showed any sign of infection were separated and excluded from the study. This study was approved by the Ethics Committee of Faculty of our college.

Acute toxicity studies on PEESD, CESD and EESD were carried out following OECD guidelines (423). Albino rats of either sex of 8-12 weeks old weighing 170 to 200g was used.

METHODOLOGY

Preparation of extract

The powder plant material was extracted with petroleum ether (60-80°C) and then extracted with chloroform and then reflux with dehydrated ethanol. Then the solvent was removed under reduced pressure. The dried extract was used for experiment and converted to the solution as per the procedure.

Preliminary Phytochemical Screening

After extraction each extract was subjected for phytochemical screening using analytical grade reagents as per standard methods described by Brain and Turner and Evans[6,7].

Detection of Alkaloids

Solutions of the extracts were prepared individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Mayer s test

Filtrates were treated with Mayer s reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner s test

Filtrates were treated with Wagner s reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

Lead acetate test

To the Extracts few drops of lead acetate solution was added. Appearance of yellow colour precipitate indicates that the presence of flavonoids.

H2SO4 test

Extracts were treated with few drops of H2SO4. Formation of orange colour indicates that the presence of flavonoids.

Detection of Steroids

Two ml of acetic anhydride was added to five mg of the extracts, each with two ml of H2SO4. The colour was changed from violet to blue or green in some samples indicate that the presence of steroids.

Detection of Terpenoids

Salkowski s Test

Five mg of the extract of the leaves, flowers and seeds was mixed with two ml of chloroform and concentrated H2SO4 (3ml) was carefully added to form a layer. A development of reddish brown colour in the inner face was shows that the presence of terpenoids.

Detection of Anthroquinones

Borntragers Test

About five mg of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl3 was added to the filtrate. Few drops of 10% NH3 were added to the mixture and heated. Formation of pink colour indicates that the presence anthroquinones.

Detection of Phenols

Ferric chloride test

10mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol.

Lead acetate test

10mg extracts was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates that the presence of phenol.

Detection of Saponins

About 0.5mg of the extract was shaken with five ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows that the presence of saponins.

Detection of Tannins

A small quantity of extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour was formed. It indicates that the presence of tannins.

Detection of Carbohydrates

0.5mg extracts were dissolved individually in five ml distilled water and filtered. The filtrate was taken to test the presence of carbohydrates.

Detection of Protein and Amino Acids

Biuret test

To 0.5 mg of extract equal volume of 40% NaOH solution and two drops of one percent copper sulphate solution was added. The development of violet colour indicates that the presence of protein.

Ninhydrin test

About 0.5 mg of extract was taken and two drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates that the presence of proteins, peptides or amino acids.

Detection of Oils and Resins

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates that the presence of oils and resins.

Wound Healing Activity

Excision and incision model was used to evaluate the wound - healing activity of *Scopari adulcis*.

Excision wound

The rats were inflicted with excision wounds as described by Morton and Malon[8]. The rats were anaesthetized prior to creation of the wounds, with anaesthetic ether .The dorsal fur of the animal was shaved with an electric clipper and the area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless stencil. A full thickness of the excision wound of 2.5 cm in width (circular area = 4.90 cm^2) and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open[9]. The animals were divided into 5 groups 6 in each. The group I

animals were treated with simple ointment base (control). Group II were treated with a reference standard 0.2% w/w nitrofurazone (NFZ) ointment. Group III, IV and V were treated with 10% w/w of petroleum ether, chloroform and ethanol extract ointments respectively for 16 days. The extract ointments (10% w/w) at a quantity of 0.5 g were applied once daily to treat different group of animals. The normal ointment base and 0.2% w/w NFZ treatment were applied in an indistinguishable amount to serve from control and standard respectively.

Wound healing potential was monitored by wound contraction and wound closure time. The wound contraction was calculated as percentage reduction in wound area. The progressive change in wound area were monitored planimetrically using transparent paper and a permanent marker on 4^{th}, 8^{th}, 12^{th} and 16^{th} day following the initial wound. The recorded wound areas were measured with graph paper.

Percentage of wound contraction was calculated by using the formula: -

$$\frac{\text{Initialwoundarea} - \text{Woundareainpostwoundday}}{\text{Initialwoudarea}} \times 100$$

Incision wounds

The animals were randomly divided into 5 groups, each consisting of 6 rats. The group I animals were treated with simple ointment base (control). Group II were treated with a reference standard 0.2% w/w nitrofurazone (NFZ) ointment. Group III, IV and Vwere treated with 10% w/w of petroleum ether, chloroform and ethanol extract ointments respectively for 10 days. The extract ointments (10% w/w) at a quantity of 0.5 g were applied once daily to treat different group of animals. The simple ointment base and 0.2% w/w and NFZ ointment were applied in the same quantity to serve as control and standard respectively. The dorsal fur of the animals was removed with a depilator cream prior to the wounding. A longitudinal 6 cm long and 2 mm deep paravertebral incision was made with a sterile scalpel through the skin and cutaneous muscle on the dorsal surface. The wounds were closed with surgical sutures at intervals of 1 cm. The extract ointments were topically applied once daily, starting from the initial day for 10 days. Sutures were evacuated on the 8^{th}day and the tensile strength of the recuperated wound was measured on the tenth day, by the 'Constant Water Pouring Technique[10].

RESULTS AND DISCUSSION

Preliminary phytochemical studies on the extracts of *Scoparia dulcis* revealed the presence of Alkaloids, carbohydrates, glycosides (cardiac glycosides), tannins-phenolic compounds, proteins –amino acids, steroids-sterols, flavonoids, Saponin, starch and fats.

In this excision model studied, significantly improved wound-healing activity has been observed with the PESD, ESD and CSD compared to that of the reference standard and control group of animals and the healing capacity was in the order of PESD > CSD > ESD.

The effect of the extract ointments, NFZ ointment (standard) and simple ointment base (control) in the excision wound model was assayed by measuring the wound area and wound contraction respectively. The present study noticed that the test extract in various concentration in the ointment base were equipped for delivering critical wound healing action. The preliminary phytochemical analysis of the tested extract of *Scoparia dulcis* revealed the presence of flavonoids and triterpinoids and alkaloids. Triterpinoids[10] and flavonoids[11] are known to promote the wound healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelisation. Flavonoids are known to reduce lipid peroxidation not only by preventing or inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by promoting the DNA synthesis[12] Thus wound healing property of *Scoparia dulcis* may be attributed the phytoconstituents present in it, which may be due to their individual or additive effect that fastens the process of wound healing. Between these three extract petroleum ether extract showed better wound healing activity. It is due to the presence of triterpinoids and flavonoids in petroleum ether extract.

Table No.1: Wound area in different post wound days of rats treated with different extract of *Scoparia dulcis*

S.No	Excision wound model wound area mm²±SEM post wound days					
1	Treatment	0	4	8	12	16
2	Simple ointment base	498.83±4.54	384.66±1.38	275.16±1.51	176.00±1.41	74.0±1.71
3	Standard	492.50±2.10	257.16±1.57	169.33±1.92	53.00±1.65	0
4	PEESD ointment 10%	490.83±1.16	383.0±1.06	208.00±0.96	54.50±0.76	6.16±0.91
5	CESD ointment 10%	498.0±1.15	408.5±0.76	278.00±1.00	66.50±0.95	9.0±0.51
6	EESD Ointment 10%	491.83 ± 0.45	401.5 ± 0.84	315.83±1.37	74.00±0.51	9.33±0.75

Results expressed in Mean ± SEM from six observations

Table No.2: Excision wound model Percentage of wound contraction activity of *Scoparia dulcis*

S.No	Excision wound model Percentage of wound contraction Post wounding days					
1	Ointment base	0	4	8	12	16
		0	22.88±0.277	44.83±0.303	64.71±0.283	85.16±0.343
2	Standard	0	47.78±0.320	65.61±0.440	89.23±0.33	100
3	PEESD ointment 10%	0	22.52±0.215	57.92±0.195	88.97±.154	98.74±0.142
4	CESD ointment 10%	0	17.97±0.153	44.17±0.200	86.64±0.192	98.19±0.115
5	EESD Ointment 10%	0	18.36±0.172	35.75±0.277	84.95±0.104	98.10±0.154

Results expressed as Mean ± SEM from six observations

Table No.3: Wound healing activity of *Scoparia dulcis* in incision wound model

S.No	Groups	Treatment	Tensile strength(g) Mean weight in gram ±S.E.M
1	Group I	Ointment base	758.33 ± 67.59
2	Group II	Standard	1575 ± 148.18
3	Group III	PEESD ointment 10%	1508.33 ± 134.42
4	Group IV	CESD ointment 10%	1358.33 ± 71.20
5	Group V	EESD Ointment 10%	1359.36 ± 72.23

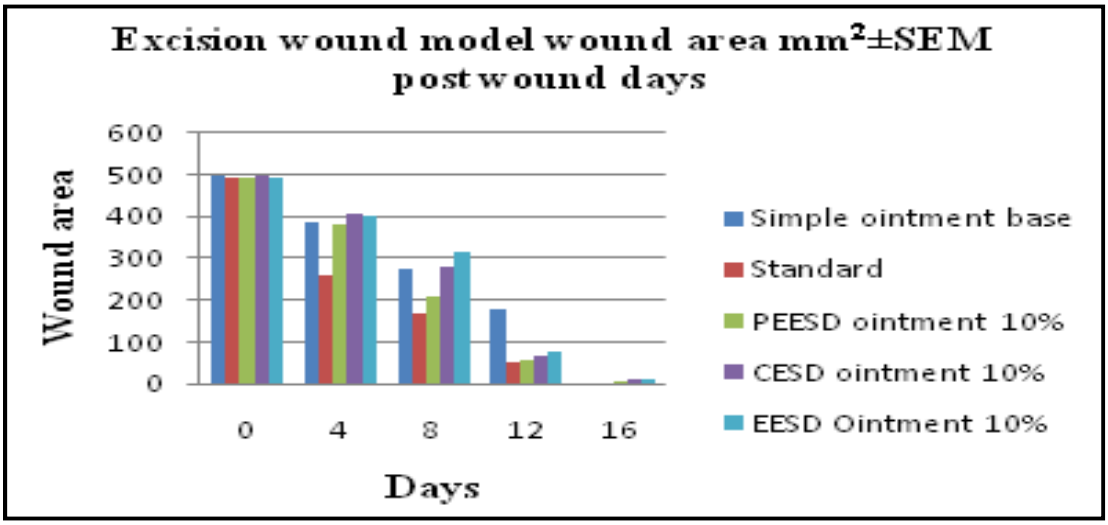

Figure No.1: Excision wound model wound area in post wound days

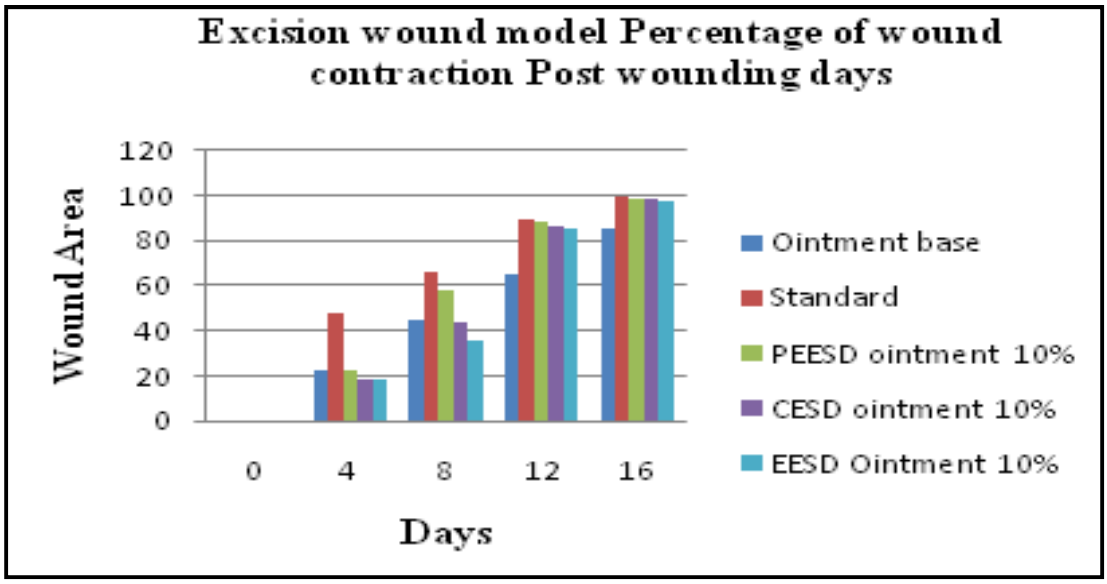

Figure No.2: Excision wound model Percentage of wound contraction in post wound days

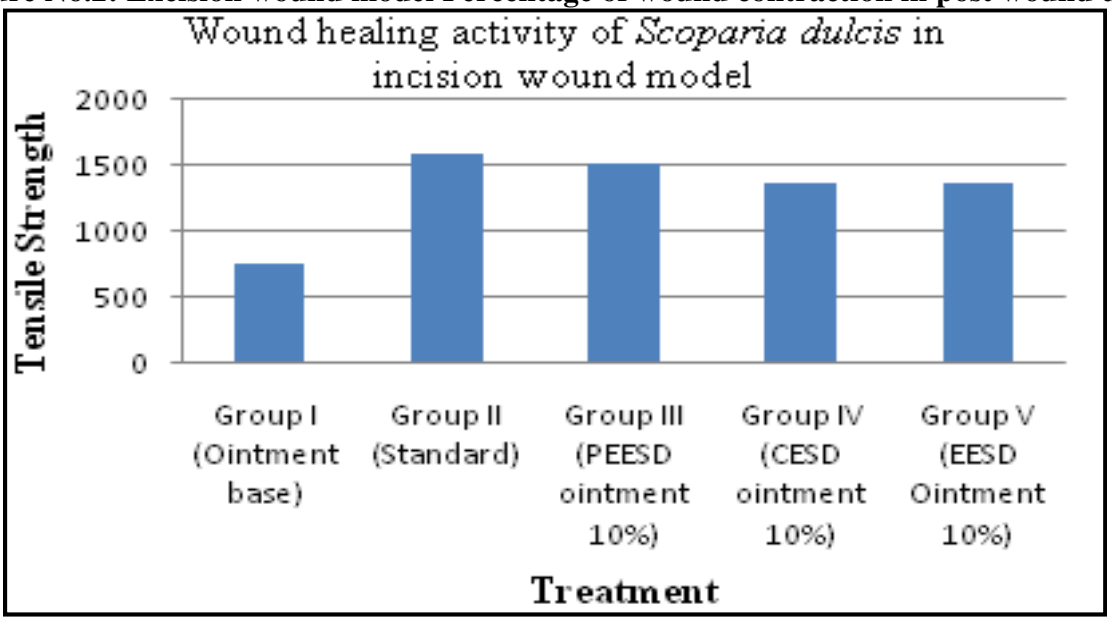

Figure No.3: Incision wound model Percentage of wound contraction in post wound days

CONCLUSION

It is concluded from this study that the *Scoparia dulcis* possess significant wound healing potential, which substantiates the traditional use of the *Scoparia dulcis* in the treatment of different types of wounds. However further research may be undertaken to identify and isolate the pytoconstituent (s) responsible for the wound healing property and to study the exact mechanism of action.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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