Journal of Comprehensive Pharmacy

Research Article

Available Online at: www.jcponline.in

ISSN NO: 2349-5669

Wound healing activity of topical application of *Raphanus sativus* var. gel in experimental animal models

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ARTICLE INFO

Article history:

Received 10 August 2014

Accepted 1 September 2014

Available online 27 October 2014

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ABSTRACT

Aim The extracts of *Raphanus sativus* var. (Family: Brassicaceae) roots were evaluated for their wound healing activities in rats using excision wound model respectively.

Methods The effects of test samples on the rate of wound healing were assessed by the rate of wound closure, period of epithelialisation and wound breaking strength. Framycetin ointment I.P. was used as reference standard for the activity comparison.

Results The results of the study revealed that the animals treated with methanol and aqueous extracts of *Raphanus sativus* var. showed faster rate of wound healing compared to other extracts under study.

Conclusion The present work justifies the use of *Raphanus sativus* var. roots for wound healing activity as claimed in the folklore literature.

Keywords *Raphanus sativus* var, Brassicaceae, Wound healing, Framycetin, Epithelialisation.

INTRODUCTION

The wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues. Healing of wound is a biological process that is initiated by trauma and often terminated by scar formation. The process of wound healing occurs in different phases such as coagulation, epithelization, granulation, collagenation and tissue remodeling. In India, there has been interest in the potential of medicinal plant for development of drugs with wound healing properties as taught in a popular form of Indian medicine known as Ayurveda [1].

Wound healing is the process of repair that follows injury to the skin and proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin [2]. Several medicinal plants have been used since time immemorial for treatment of cuts, wounds and burns and showed promising effects. Some very common

plants like *Aloe vera*, *Azadirachta indica*, *Carica papaya*, *Celosia argentea*, *Centella asiatica*, *Cinnamomum zeylanicum*, *Curcuma longa*, *Nelumbo nucifera*, *Ocimum sanctum*, *Phyllanthus emblica*, *Plumbago zeylanica*, *Pterocarpus santalinus*, *Terminalia arjuna* and *Terminalia chebula* have been extensively reported in Ayurveda, Siddha and Unani systems of medicines for their wound healing potential [3].

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 [4].

Hence the main objective of the present study to evaluate the wound healing property of *Raphanus sativus* var. *longipinnatus* belonging to the Family Brassicaceae. The plant has been reported to possess anti-inflammatory activity and also contains major

phytoconstituents, which are responsible for number of Activities.

Therefore in the present study, the aqueous extract of roots of *Raphanus sativus* var. is taken to evaluate the wound healing activity as there is no scientific data available to prove the traditionally mentioned uses of healing wound.

MATERIALS AND METHODS

Plant collection and Authentication

Root of *Raphanus sativus*, a common vegetable used as a salad in winters, were collected from market of Nellore. The Root peels were scrapped and allowed to dry in shade for two weeks. To prevent the loss of active phytoconstituents, samples were kept under constant observation to avoid any fungal growth and authenticated by K. Audiseshamma, Asst. professor in Botany, D.K.W Govt Degree College.

Chemicals and equipments used

Ointment base, Soframycin (Framycetin ointment), Distilled water. Equipments: Borosilicate Soxhlet Extractor, Weighing balance.

Animals used

Male Wistar rats (150-200g) were used for study. All animals had free access to pelleted food and water ad libitum. Temperature was maintained at 23±10C. The experimental protocol was approved by Institutional animal ethical committee of Narayana Medical College, Nellore, A.P as per guidelines issued by Committee of Purpose of Control and Supervision of Experiment on Animals (CPCSEA) India. (regulation no:1423/po/11/CPCSEA/114/2011).

Aqueous Extraction of Roots of *Raphanus sativus*

The root peels of raddish were shade dried. The dried root peels were grinded in an electric grinder to obtain fine powder. The powder sample was stored in sterilized air tight container at room temperature (25-30°C). Further the dried root peel powder of raphanus sativus was transferred into the thimble of a soxhlet apparatus and extracted using distilled water for 18hours. Appearance of colorless solvent in the siphon tube was the indication of exhaustive extraction and based on that further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to thick paste on the water bath, maintained at 50°C to get aqueous extract. Finally extract was air dried thoroughly to remove all traces of the solvent and calculate the percentage yield. The dried extract was then stored in an air tight container till used.

Preliminary photochemical testing of extract

The extraction was subjected to chemical tests to detect the chemical constituents present in them. 0.5 grams of extract was dissolved in 5ml of distilled water and filtered. The filtrate was used to determine the presence of various phytoconstituents as described in the following section.

Preparation of topical formulation

A topical formulation of the herbal extract was prepared using simple ointment I.P. The herbal extracts were incorporated into the topical formulations in two concentrations as shown in Table No. 1.

Table No. 1: Topical Formulations of Aqueous Extract of roots of *Raphanus sativus*

S. No.	Ingredients	Formulation I	Formulation II
1.	Wool fat	5g	5g
2.	Cetostearyl Alcohol	5g	5g
3.	Hard Paraffin	5g	5g
4.	Herbal Extract	5g	10g
5.	White Soft Paraffin	q. s to 100g	q. s to 100g

Evaluation of wound healing activity

The rats of either sex weighing 150-200 g were selected. Animals were depilated at dorsal thoracic region before wounding. They were housed individually with free access to food and water, the basal food intake and body weight to the nearest gram was noted. Under light chloroform wounding was performed aseptically. Excision wound models in rats were selected for assessing the wound healing activity. The prepared formulations were applied topically once in a day to respective groups.

Excision wound model

In the excision wound model rats were anaesthetized with chloroform prior to creation of the wounds. The dorsal surface was shaved with an electric clipper. A full thickness of the excision wound of 500 mm² was created by using toothed forceps, a surgical blade and pointed scissors. The entire wound left open. Wounds were traced on 1 mm² graph paper on the day of wounding and subsequently a gap period of two days till 16 days and then on alternate days until healing was complete. The groups were treated in the same manner as mentioned in the treatment protocol. The ointment was topically applied once in a day. The progressive changes in wound area were monitored on 2,4,6,8,10,12,14, and 16 days of post wounding.

Table No.2: Phytochemical constituents present in *Raphanus sativus* root extract

S.NO	TEST	AQUEOUS EXTRACT
1	Carbohydrates	
	Benedicts test	+
	Fehling's test	+
2	Proteins	
	Biuret test	+
	Millon's test	-
3	Amino acids	
	Ninhydrin test	-
	Tyrosine test	-
4	Alkaloids	
	Mayer's test	+
	Dragendroff's test	+
5	Glycosides	
	Borntragers test	-
6	Flavonoids	
	Lead acetate test	+
7	Phytosterols	
	Salkowski test	+
8	Fats and oils	
	Solubility test	-
	Stain test	-
9	Phenolics and tannins	
	Lead acetate test	+
	Acetic acid test	+
10	Volatile oils	
	Solubility test	+

(+) Indicates positive result, (-) Indicates Negative result

Statistical analysis

All the data was expressed as Mean $\pm$ S. E. M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad 5.0). Statistical significance was set accordingly.

RESULTS

Preliminary phytochemical investigation

The aqueous root extract was subjected for preliminary phytochemical screening for carbohydrates, proteins, amino acids, alkaloids, glycosides, flavonoids, phytosterols, fats & oil, phenolics & tannins, volatile oils. The phytochemical screening revealed the presence of phyto constituents given in the Table No.2.

Wound healing activity

Wound represents the major health problem both in terms of morbidity and mortality. The processes involved in wound healing are epithelization, contraction and connective tissue deposition. The healing process depends to a large extent on the regulated biosynthesis and deposition of new collagens and their sub-sequent maturation in the tissue re pair process inflammatory cells promote the migration and proliferation of endothelial cells leading to leovascularization of connective tissue cells.

Which synthesize extracellular matrixes including collagen and keratinocytes result into re-epithelization of wounded tissue. Inflammation, collagen maturation and scar formation are some of the many phases of wound healing which run concurrently but independent of each other.

The % rate of wound contraction in control group, rats was 3.16% to 80.34% from Day 2 to Day 16 while complete epithelization and healing were observed on Day 21. In Standard group the % rate of wound contraction in rats; treated topically with Soframycin (1% w/w) was from 3.34% to 83.68% from Day 2 to Day 16 while complete epithelization and healing were observed on Day 18. In Test group I the % rate of wound contraction in rats, treated topically with Formulation I (5% w/w) was from 3.6% to 79.8% from Day 2 to Day 16 while complete epithelization and healing were observed on Day 20 While Formulation II (10% w/w) showed increase in wound contraction from 2.5% to 84.0% from Day 2 to Day 16 while complete epithelization and healing were observed on Day 17 respectively which is shown in Table No. 3 and Graph No. 1.

The mean epithelization period was observed from Control Group, Standard Group, Formulation I & Formulation II was 20.83 $\pm$ 0.60, 18 $\pm$ 0.25, 20.7 $\pm$ 0.47 &

Table No.3: Effect of Formulation I & II on wound contraction

Post Wounding Days	Wound area (mm ²) (mean $\pm$ S.E.M) and Percentage of wound contraction			
	Control	Standard	Test-1	Test-2
2	484.2 $\pm$ 3.27 (3.16)	483.3 $\pm$ 2.47*** (3.34)	481.7 $\pm$ 3.07*** (3.66)	487.5 $\pm$ 1.70*** (2.5)
4	440.8 $\pm$ 2.71 (11.84)	426.7 $\pm$ 6.14*** (14.66)	385.0 $\pm$ 6.19*** (23)	410.0 $\pm$ 9.66*** (18)
6	365.0 $\pm$ 4.28 (27)	366.7 $\pm$ 8.81*** (26.66)	375.0 $\pm$ 5.00*** (25)	375.0 $\pm$ 8.46*** (25)
8	291.7 $\pm$ 12.22 (41.66)	308.3 $\pm$ 9.45*** (38.34)	326.7 $\pm$ 8.02*** (34.74)	320.0 $\pm$ 9.30*** (36)
12	256.7 $\pm$ 13.55 (48.66)	168.3 $\pm$ 4.77*** (66.34)	241.7 $\pm$ 14.00*** (48.34)	190.0 $\pm$ 5.16*** (62)
14	159.1 $\pm$ 8.10 (68.18)	103.3 $\pm$ 4.01*** (79.34)	165 $\pm$ 8.06*** (67)	113.3 $\pm$ 4.94*** (77.34)
16	98.3 $\pm$ 2.78 (80.34)	81.6 $\pm$ 3.33*** (83.68)	102.5 $\pm$ 4.42*** (79.5)	80 $\pm$ 4.65*** (84)

Values are mean $\pm$ SEM (percent) of 6 rats in each group *** P <0.01

Graph No. 1: Effect of Formulation I & II on wound contraction

17±0.25 respectively which was shown in Table No. 4 and Graph No.2

Table No. 4: Effect of Formulation I & II on Epithelization period

S.No.	Groups	Period of Epithelialisation
1	Control Group	20.83 ± 0.60
2	Standard	18 ± 0.25***
3	Formulation I	20.7 ± 0.47
4	Formulation II	17 ± 0.25***

DISCUSSION

In the present study significantly improved wound healing activity has been observed with the prepared ointment, compared to that of the reference standard and control group of animals. In the excision wound model, marketed formulation treated animals showed significant reduction in the wound area ($P < 0.001$), faster rate of epithelialisation (Table No.3), increased dry

weight of the tissue ($P < 0.001$) and increased hydroxyproline content ($P < 0.001$) when compared with the control group of animals.

According to Midwood et al [5] in his study reported that wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. He also noticed that in the maturational phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. In the present study two dose levels of *Raphanus sativus* gel was studied for their wound healing properties. The topical application of *Raphanus sativus* gel significantly accelerated the rate of wound healing, histopathological studies of healed wound revealed comparatively less inflammation, more collagen and angiogenesis as evident from the findings of the present investigation. There are numerous reports about the wound healing effects. Muhammad and Muhammad in their study noticed that the wound healing effects may be due to regulation of collagen expression and increase in tensile strength of the wounds. They also reported that enhanced healing activity has been attributed to increased collagen formation and angiogenesis [6]. Al-Henhena et al [7] in his study reported that collagen

Graph No. 2: Effect of Formulation I & II on Epithelization period

play a central role in the healing of wounds and it is a principal component of connective tissue and provides a structural framework for the regenerating tissue. A wound healing mechanism termed as angiogenesis improves the circulation to the wound site facilitating the oxygen and nutrients required for the wound healing process which involves re-epithelisation. Angiogenesis and epithelial cell proliferation are the prominent wound healing process as suggested from the research reports [8].

Conclusion

In the present study it was noticed that the epithelialisation period for both the doses of *Raphanus sativus* gel is short compared to the control groups. The findings indicate that *Raphanus sativus* had a prominent effect on the rate of wound contraction.

Acknowledgement

Authors wish to thank Principal Mr. Dr. U. Vivek Kumar and management Narayana Pharmacy College, Nellore, for providing the all facilities to carry out the research work.

Conflict of Interest

We declare that we have no conflict of interest.

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Cite this article as: Peeriga R, Munna S, Theepalapudi JS , Yakasiri NSS, Putta JG, Lavanya Mettukuru L. Wound healing activity of topical application of *Raphanus sativus* var. gel in experimental animal models. J Compr Phar 2014;1(4):142-148.