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Wound Healing Activity of *Taraxacum officinale* Alcoholic Extract in Albino Rats.

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Abstract: *Taraxacumofficinale (Asteraceae) a perennial or biennial herb in traditional medicine is used in treating fractures, kidney and gallstones. The objective of this study was to evaluate the wound healing activity of alcoholic extract of Taraxacumofficinale in albino rats.*
Incision, Excision and Burn wound models were used to evaluate the wound healing activity of alcoholic extract of Taraxacumofficinalein swiss variety of albino rats. For each model animals were divided into four groups of six animals each. Group I served as control, group 2nd received reference drug, group III and IV received herbal formulation of low and high dose respectively. The effects of vehicles on the rate of wound healing were assessed by the rate of wound closure, period of epithelialisation, tensile strength and histopathology of granulation tissue.
The alcoholic extract of Taraxacumofficinale promoted wound healing activity significantly in all the three models. High rate of wound contraction ($p < 0.001$) decrease in period epithelialisation& high skin-breaking strength was observed in animals treated with TO formulation 1% w/v. Histopahalogical studies of the granulation tissue from TO formulation treated animals showed lesser number of inflammatory cells, and increased collagen formation than the control.
Hence the data of the present study indicated that alcoholic extract of Taraxacumofficinalehas wound healing activity in different wound models.

Keywords: *Taraxacumofficinale, Wound healing, Incision wound, Excision wound, Burn wound.*

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I. Introduction

The earliest medicines used by mankind were obtained from plants. Evidences of this are the thousand years' old traditions and records of popular healing.*Taraxacumofficinale* (Asteraceae), commonly known as dandelion or "Handh" in (Kashmir) is a small perennial or biennial herb with a long tap root. It has a wide distribution commonly found in grasslands, lawns, roadsides, pavements and vegetable fields.

In Kashmir valley the plant, *Taraxacumofficinale* is considered to be very good for ladies after child birth. The dried leaves are used as a poultice around fractured limbs. The root is diuretic and tonic and used as a remedy for chronic disorders of kidney and gallstones. (Kaul 1997) However there are no experimental reports on wound healing activity of dandelion in literature. In this paper the wound healing efficacy of the alcoholic extract of dandelion is reported for the first time.

II. Materials And Methods

1.1. Plant materials:

The fresh plant materials (Whole Plant) of *Taraxacumofficinale* (TO) was collected from Langate block of kupwara district of Kashmir valley in the month of April and identified by Professor Ab. Rashid Malik, HOD Botany, Govt Degree College Sopore. The voucher specimen has been procured in the herbarium records of pest control and Ayurvedic Drug Research laboratory S.S.L. Jain P.G. College, Vidisha (MP).

1.2. Preparation of alcoholic extract:

The plant materials were shade dried powdered and about 500 gm. packed in soxhlet apparatus. Extraction was done by continuous hot percolation method using methanol as solvent for 72 hrs. The extract was evaporated in vacuum evaporator. The crude extract was subjected to preliminary physiochemical screening.

1.3. Chromatographic separation:

The semisolid crude extract obtained after vacuum evaporation was separated by TLC and column chromatography, (Harborne 1984) using solvent system n butanol: ammonia (1:1) which give four spots on TLC and also four fractions were yielded on column chromatography. Out of these fractions, fraction III was used for experimental bioassay.

1.4. Preparation of formulation :

The extract was formulated as 0.5% W/v and 1% W/v in gum acacia dissolved in normal saline. TO 0.5% w/v was used as a low dose and TO 1% w/v as high dose for topical application.

1.5. Experimental animals :

The present study was carried over on healthy wistar albino rats of either sex weighing between 150-250 gm. The experimental work was carried out under the supervision of IAEC as per the guidelines of CPCSEA.

1.5.1. Study Design :

For each experimental model the animals were divided into four groups of six animals each.

1. Group I served as control - received vehicle topically.
2. Group II served as standard - received nitrafurazone 0.2% w/w.
3. Group III received 0.5% w/v herbal formulation.
4. Group IV received 1% w/v herbal formulation.

1.6. Wounding procedure:

The studies were carried out using ether anaesthetized rats in three different wound models.

1.6.1. Incision Wound:

A longitudinal Para vertebral incision of 6cm long was made through the skin and cutaneous tissue on the back as described by Ehrlich and Hunt (1969).

The parted skin was closed with interrupted sutures 1cm apart. The wounds were left undressed and topical application of the drug was given once a day. The sutures were removed on 7th the post wound day and the application was continued. The skin breaking strength was measured on 10^{th} day evening after the last application, as described by Lee (1968).

1.6.2. Excision Wound:

The rats were inflicted with excision wounds as described by Morton & Malone (1972). The dorsal fur of the animals was shaved and the area of wound to be created was outlined on the back of animal with methylene blue. A full thickness of the excision wound was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open. They were treated topically with different formulations in each group. The parameters studied were wound closure, epithelialisation time and collagen content. The measurement of the wound areas of the excision wound model were taken on every alternate day. The recorded wound area was measured with graph paper. The period of epithelialisation was calculated as the number of days required for falling off the dead tissue remnants without any residual raw wound. In the excision wound model the granulation tissue formed was excised on 10^{th} day stained with haematoxylin and eosin and observed for histological changes under microscope.

1.6.3. Burn Wound Model:

Partially thickened burn wounds were created by pouring hot molten wax at 80°c on the shaven back of the rat through a cylinder of 300mm^2 circular opening. (Bairy*et. al.* 1997) The wax allowed remaining on the skin till it solidified, the cylinder was then removed with the wax adhering to it, which left a partial thickness circular burn wound. Immediately after injury and on subsequent days the ointments were applied topically.

III. Statistical Analysis

Results are expressed as mean ± SEM. The comparisons between experimental groups were made using one way ANOVA following by Dunnet's test $p< 0.05$ was considered to be significant.

IV. Results

IV.1. Preliminary phytochemicalscreening:

The phytochemical analysis of the TO alcoholic extract revealed the presence of alkaloids, saponins, flavonoids and Terpenoids.

IV.2. Effects on excision and incision wound:

Both high as well as low concentrations of herbal extract produced a significant decrease in period of epithelialisation when compared to control ($P < 0.01$) Treatment with standard drug nitrafurazone (0.02% W/v) also produced significant reduction in period of epithelialisation. ($p< 0.001$) It was found that high dose of herbal extract 1% was comparatively more effective than low dose (0.5%) in reducing period ofepithelialisation. (Table1.)

The breaking strength of 10 day old invasion wound was increased by all treatment. The high dose of herbal extract was found to be more effective that low dose in increasing the breaking strength. (Table 1)

IV.3. Effect on burn wounds:

Like the excision wound model, application of TO 0.5%, TO 1% as well as Nitrafurazone 0.02% topically shortened the period of epithelialisation significantly ($p< 0.01$) and also produced a significant decrease in wound area as compared with control. The high dose of TO 1% was found to be more effective when compared with low dose of TO 0.5% and 0.02% nitrafurazone standard drug. (Table 2)

TABLE 1. Showing the effect of *Taraxacum officinale* (TO) on the period of epithelialization and wound contraction 50% in burn wound models.

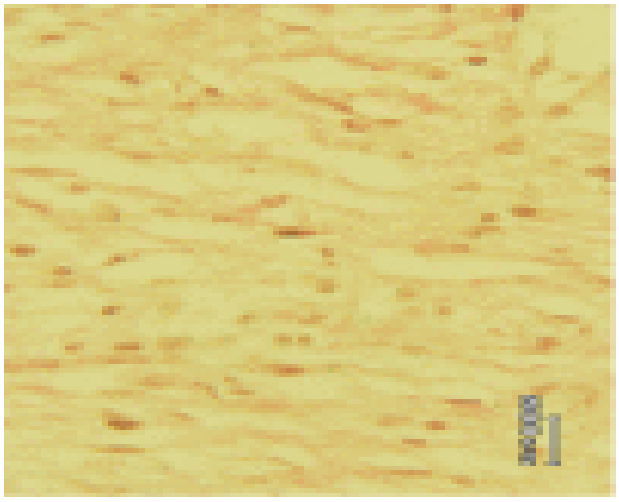
(All values are meant SEM, n = 6 P** < 0.01 vs. control

Group	Burn	Wound
	Epithelialisation Period (Days)	Wound Contraction 50% days
Control (vehicle)	23.16 ± 0.30	11.98 ± 0.33
Standard Nitrafurazone 0.02%	19.00 ± 0.36**	10.16 ± 0.16**
To 0.50% in (vehicle)	18.10 ± 0.16**	8.33 ± 0.34**
To 1% (vehicle)	15.14 ± 0.60**	6.96 ± 0.31**

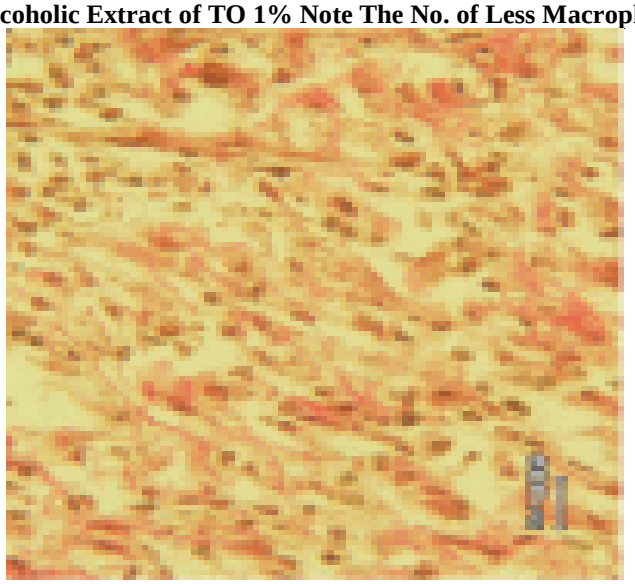
Table 2.showing effect of to extract on the period of epithelialisation and 50% wound contraction in excision wound model and breaking strength in incision wound model.

Values are mean ± SEM n = 6 p** < 0.01 vs control

Group	Excision Wound		Incision Wound
	Epithelialisation Period (Days)	Wound Contraction 50% days	Breaking Strength in gms.
Control received (gum acacia in normal saline	24.22 ± 0.24	11.96 ± 0.40	408.65 ± 13.74
Standard received nitrafurazone 0.2% w/w	18.84 ± 0.23**	08.60 ± 0.34**	492.32 ± 10.92**
TO extract 0.5% w/w	18.10 ± 0.16**	07.40 ± 0.22**	501.31 ± 11.27**
TO extract 1% w/w	13.96 ± 0.40**	6.90 ± 0.42**	526.64 ± 12.92**



Showing the Effect of Alcoholic Extract of TO 1% Note The No. of Less Macrophages & More Collagen



Control Showing More Macrophages & Less Collagen

V. Discussion

Wound healing is a process by which damaged tissue is restored as closely as possible to its normal. Wound contraction is the process of Shrinkage of the area of wound. It is mainly dependent upon the type and extent of damage the general state of health and the ability of the tissue to repair.Collagenation, wound contraction and epithelialisation are crucial phases of wound healing. The phases of inflammation, macrophasia, fibroplasia and collagenation are intimately interlinked. Thus an intervention into any one of the phases by drugs could either promote or depress, one or all phases of healing. Possibly the constituents of TO extract like alkaloids, terpenoids&flavonoids mayplay a major role in the process of wound healing. Terpenoids and flavanoids 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 epithelialisation. Several studies with plant materials have demonstrated the presence of similar phytochemical constituents, which are responsible for promoting wound healing activity, (Nayak*et. al*. 2006, Nayeem*et. al*. 2009, Shrivastava and Durgaprasad 2008, Saini*et al*2007)

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