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Formulation And Evaluation Of Anthraquinone Fraction Of *Rubia cordifolia* Linn.

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Abstract

Medical knowledge has followed the growth and fading of great civilizations which enriched its flow with traditional experiences. *Rubia cordifolia* was responsible for treating all types of skin disorders thousands of years. Therefore the current research is focused on innovative and novel topical formulation of anthraquinone fraction containing herbal ingredient. The extract were incorporated into a gel base to prepare gels and there evaluation parameters were studied. The antibacterial and wound healing activity of extract and gel were investigated in mice. The gels prepared by anthraquinone fraction of ethanol extract of *R. cordifolia* root were found to be active against all the bacteria's used in this study. The extract and gel were topically applied on the excision wound surface of mice and effect produced in terms of contracting ability, closure, decrease in surface area; tissue regeneration and histopathology of wound were significant. Thus the study demonstrated the antibacterial, wound healing action of the extract, fraction and its gels and effective in the functional recovery of the healing of wounds and in skin diseases. Bioprospecting and the further study on active components of this plant may provide a better understanding in the process of wound healing with goal of elucidating their active potential compounds.

Keywords*: Rubia cordifolia,* Wound healing, Antimicrobial activity, Excision wound.

INTRODUCTION

Research on wound healing agents is one of the developing areas in modern biomedical sciences. Many traditional practitioners across the world particularly in countries like India and China with age old traditional practices have valuable information of many lesser-known hitherto unknown wild plants used by the traditional healers for treating wounds and burns. Several drugs of plant, mineral and animal origin are described in the traditional texts of Indian systems of medicine like Ayurveda for their healing properties under the term 'Vranaropaka'. Besides the classical systems of Indian Medicine, the folk and the tribal medicine also employ a number of plants and animal products for treatment of cuts, wounds and burns. Some of these plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models and human subjects, but the potential of most of the plants remain unexplored.[1]

In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases.[2] Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, diarrhorea, scabies, venereal disease, ulcers, snake bite, etc.[3] More than 80% of the world's population still depends upon traditional medicines for various skin diseases.[4] Herbal medicines in wound management involve disinfection, debridement and providing a moist environment to encourage the establishment of the suitable environment for natural healing process.[5]

Rubia cordifolia also known as, Manjishtha, Indian madder, distributed throughout India.[6] It is found throughout the hilly districts of India from northwest Himalayas eastwards, ascending to 8000 ft and southwards to Ceylon. The roots of this plant are of high medicinal value and are recognized as official.[7] This perennial herbaceous prickly creeper or climber is upto 10m long, found throughout the country ascending to 3750 m and grow well in light (sandy), medium (loamy) and heavy (clay) soils.[8] *Rubia cordifolia* is an important medicinal plant which is used for treatment of various ailments in Ayurvedic system of medicine. The biological investigations have shown that many of the medicinal properties claimed for the plant in the historical texts do, indeed, have sound scientific basis.[9] It has a variety of uses such as blood purifier, immunomodulator [10], antiinflammatory [11] and antioxidant.[12] It is helpful in treating skin diseases, in blood purification, increasing appetite and in stimulation and contraction of uterus. Thus, the present study aims to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin and to investigate the medicinal use of *Rubia cordifolia* as a wound healing promoter that had been cited in folkloric literature.

MATERIALS AND METHODS

Plant Material and extract preparation

The roots of the plant *R. cordifolia* were procured from the local market of Pune. About 200 g of powdered roots (dry) were extracted with ethanol (95%) using soxhlet apparatus for 4-6 hours. Alcohol removal carried out under pressure afforded a semi solid mass with a yield of 9%.

Preparation of Anthraquinone Rich fraction

The prepared Ethanol extract was subjected to hydrolysis with 5 to 10% hydrochloric acid under reflux for two hour and then it was filtered and extracted with non polar solvent such as diethyl ether. The organic layer was separated and

remaining aqueous layer again extracted with ether. The successive organic layer were collected and evaporated under rotary evaporator to get the Anthraquinone fraction.

Gel formulation

A 0.2%, 0.5% and 1% gel of Anthraquinone fraction were formulated using Carbopol 940 (Vishal chemicals, Mumbai) in the concentration of 1%.

Animals

The protocol of the study was approved by the Local Ethical Committee for animal experimentation. The mice were obtained from the Serum institute, Pune, India and kept in animal house in standard conditions. They were provided food and water ad libitum during the whole period of the experiment.

An acute toxicity study was conducted according to the staircase method. Toxicity studies conducted as per internationally accepted protocol drawn under OECD guidelines 420 in Swiss albino mice at a dose level of extracts up to 5000 mg/kg. The animals were physically active and were consuming food and water in a regular way. Male albino Swiss mice weighing 25-30 g were used in wound healing model experiments.

Excision wound model

The dorsal skin of the mice was shaved. The mice were divided into five groups of six animals each. The animals were depilated on the paravertebral area prior to wound creation and predetermined area of 7mm× 7mm skin in its full thickness was excised under ether anesthesia.[13] Group I – V were treated with plane base, gels of different concentrations of anthraquinone fraction (0.2%, 0.5%, 1%) and anthraquinone fraction itself respectively, once a daily for 15 days. Wounds were left undressed to the open environment and the animals were kept individually in separate cages.

Measurement of wound area

The progressive changes in wound area were measured in mm at every 3 days interval. Progressive decrease in the wound size was monitored periodically.

Histological Examination

At day 16 the experiment was terminated and the wound area was removed from the surviving animals for histological examination. The tissue was processed in the routine way for histological evaluation. Five micrometer thick sections were stained with haematoxylin and eosin, the routine stain used in the histopathology, and recommended as a general survey stain. The tissue samples were evaluated for the following histological criteria; the extent of reepitheliasation, the maturation and organization of the epidermal squamous cells, the thickness of the granular cell layer, the degree of the tissue formation.

The different animal groups were assessed blindly by the pathologist and results were compared with the control groups.

Phytochemical screening methods

Ethanol extract was evaluated for presence of various phytoconstituents by performing different qualitative chemical tests reported. It showed the presence of anthraquinone glycosides, saponins, tannins and phytosterols.[8, 14]

Statistical analysis

The relative wound area results were compared using one- way analysis of variance (ANOVA) followed by Dunnett's tests. *P* values less than 0.05 were considered as indicative of significance.

RESULT

Wound area

A better healing pattern with complete wound closure was observed in mice treated within 15 days while it took about 25-30 days in control mice with different concentration of gel of anthraquinone fraction (Table 1). There was a significant reduction in wound area from day three onwards in treated mice and also on later days the closure rate was much faster than when compared with control mice. The table 1 shows the effect of gel and anthraquinone fraction of *R.cordifolia* on excision wound model in mice.

Table 1.Effect of gel and anthraquinone fraction of *R. cordifolia* on excision wound model in mice

Sr. no	Groups	Wound area in mm				
		3 days	**6 days**	**9 days**	**12 days**	**15 days**
1.	Control	7.41 ± 0.008	747 ± 0.005	7.44 ± 0.02	7.4 ± 0.009	7.35 ± 0.01
2.	0.2% Gel	6.99 ± 0.01*	6.90 ± 0.01*	6.75 ± 0.016*	6.67 ± 0.01*	6.59±0.012*
3.	0.5% Gel	6.0 ± 0.02**	5.7 ± 0.01**	5.4 ± 0.008**	5.4 ± 0.005**	5.3 ± 0.01**
4.	1% Gel	5.7 ± 0.01**	5.5 ± 0.02**	5.0 ± 0.01**	4.7 ± 0.03**	4.9 ± 0.01**
5.	1% EE	5.6 ± 0.01**	5.3 ± 0.01**	5.1 ± 0.01**	4.9 ± 0.02**	4.7 ± 0.02**

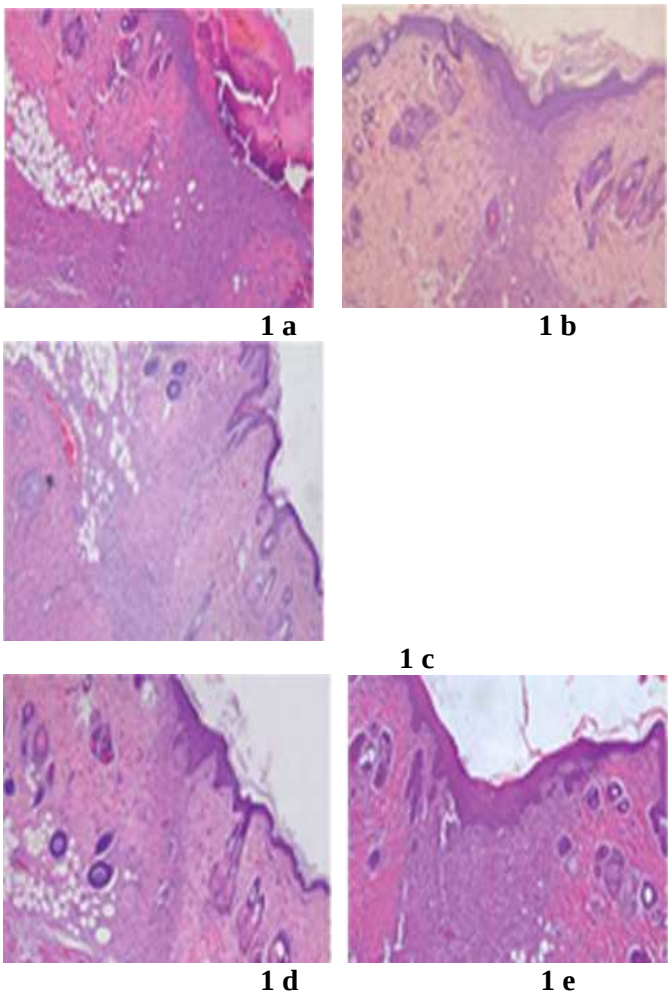
EE, Ethanol Extract.
(Values expressed as Mean ± SEM), * p< 0.05, ** p< 0.01, *** p< 0.001.

Histological Evaluation

Histological evaluation was carried out for the treated and untreated samples. There was a marked infiltration of the inflammatory cells, increased blood vessel formation and enhanced proliferation of cells as a result of treatment with *R. cordifolia* gel and extract. There was full thickness reepitheliasation, in which epidermis was thin and well organized, comparable to the normal adjacent skin which was not involved in the wound generation and healing process. The granular layer was well formed and one cell in thickness. The All the animals when treated with different concentrations of gel of ethanol extract showed comparable results when compared with control. There was a full thickness epidermal regeneration which covered completely the wound area.

The epidermis was thick and disorganized, especially when compared with the adjacent normal skin. In all, complete epitheliasation, vasculirisation and hair follicles formation were observed in treated mice (Fig. 1 a-e).

Early dermal and epidermal regeneration in treated mice also confirmed that the extract had a positive effect towards cellular proliferation, granular tissue formation and epitheliasation.



1 a **1 b**

1 c

1 d **1 e**

1 a Control (Plane gel base)
1 b Treated with 0.2% Gel
1 c Treated with 0.5% Gel
1 d Treated with 1% Gel
1 e Treated with 1%Extract

Figure1. (a –e) Effect of gel and anthraquinone fraction of *R. cordifolia* on excision wound model in mice (Histopathological slides)

DISCUSSION

Wound healing is a very complex, multifactor sequence of events involving several cellular and biochemical processes. The aim in these processes is to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin.[15] Healing process, a natural body reaction to injury, initiates immediately after wounding and occurs in four stages. The first phase is coagulation which controls excessive blood loss from the damaged vessels. The next stage of the healing process is inflammation and debridement of wound followed by re-epitheliasation which includes proliferation, migration and differentiation of squamous epithelial cells of the epidermis. In the final stage of the healing process collagen deposition and remodeling occurs within the dermis.[16, 17]

The results in this study are in support that wound healing and repair is accelerated by applying gel of *R. cordifolia,* which was highlighted by the full thickness coverage of the wound area by an organized epidermis. The enhanced capacity of wound healing with the plant could be explained on the basis of anti-inflammatory effects of the plant that are well documented in the literature.[18]

Study on animal models showed enhanced rate of wound contraction and drastic reduction in healing time than control, which might be due to enhanced epitheliasation. The animals treated with 1% gel and extract showed significant results when compared with different groups and control. The treated wound after six days itself exhibit marked dryness of wound margins with tissue regeneration.[19]

However, histological evaluation showed that, increased cellular infiltration from haematoxylin and eosin staining in treated cases may be due to chemo tactic effect enhanced by the crude extract which might have attracted inflammatory cells towards the wound site. Increased cellular proliferation may be due to the mitogenic activity of the plant extract, which might have significantly contributed to healing process.

Early dermal and epidermal regeneration in treated mice also confirmed that the extract had a positive effect towards cellular proliferation, granular tissue formation and epitheliasation.[4]

The embellin isolated from the ethanol extract of plant *Emblica officinalis* containing condensed tannins when formulated as a gel possess significant wound healing property as that of gel prepared by *R. cordifolia* ethanol extract. [20]

A number of secondary metabolites/active compounds isolated from plants have been demonstrated in animal models (in vivo) as active principles responsible for facilitating healing of wounds. Some of the most important ones include tannins from *Terminalia arjuna*,[21] oleanolic acid from *Anredra diffusa*,[22] polysaccharides from *Opuntia ficus-indica*,[23] gentiopicroside, sweroside and swertiamarine from *Gentiana lutea*,[24] shikonin derivatives (deoxyshikonin, acetyl shikonin, 3-hydroxy-isovaleryl shikonin and 5,8-*O*dimethyl acetyl shikonin) from *Onosma argentatum*,[25] asiaticoside, asiatic acid, and madecassic acid from *Centalla asiatica*,[26,27,28] quercetin, isorhamnetin and kaempferol from *Hippophae rhamnoides*,[29] curcumin from *Curcuma longa*.[30]

CONCLUSION

The gel and the extract both showed the wound healing activity in mice. Histological evaluation shows there was a marked infiltration of the inflammatory cells, increased blood vessel formation and enhanced proliferation of cells as a result of treatment with *Rubia cordifolia* gel and extract. This study thus demonstrates the

wound healing activity of ethanolic extract and its gel formulation of the roots of the plant *R. cordifolia* and found to be effective in the functional recovery of the healing of wounds and also in histopathological alterations.

As infections being a major cause of morbidity and mortality in wound patients, these herbal extracts may prevent infection that leads to high risk of sepsis, and thereby prevents the prolongation of inflammatory phase. Further study on the fractionation of active components and the mutual effect of these plant extract machinery on infecting microbial species may provide a better understanding of the infection management in the process of wound healing.

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