

Evaluation of wound healing potential of *Chicorium intybus*. L (Asteraceae) in rats

NAVEED AHMED SYED, TARIQUE NOORUL HASAN and SYED MOHAMMED MUSHEER AALAM

For author affiliations, see end of text.

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ABSTRACT

The plant *Chicorium intybus* is used in traditional medicinal system as skin nourisher and to treat several skin ailments such as warts. The objective of this study was to evaluate ethanolic extract of *Chicorium intybus* for their wound healing activity in rats. The extract was tested on three groups of male Albino rats, consisting of six animals each, were placed individually in cages and all animals were experimentally wounded in the posterior neck area of 2cm x 2cm aseptically. Normal saline was applied topically to wounds of Group 1 animals as placebo control. Wounds of Group 2 and 3 rats were treated with ethanolic extract of *Chicorium intybus* at 50mg/mL and 100mg/mL concentrations, appreciably accelerated wound healing in rats compared with controls. Complete wound epithelization was observed, fresh hair began to grow in the entire wounded area within 18days in animals treated with the 100mg/mL ethanolic extract of *Chicorium intybus* compared with only 80.3 mm² area contraction without hair growth in control rats. The study permits the conclusion that ethanolic extract of *Chicorium intybus* has wound healing potential.

Keywords: Wound healing, *Chicorium intybus*, Rat

Traditional herbal medicine practitioners have described the therapeutic efficacies of many indigenous plants, for various diseases [1]. Natural products are source of synthetic and traditional herbal medicine. They are still the primary health care system in some parts of the world [2]. The presence of various life-sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential wound healing properties.

Burns, trauma and wounds are still a major problem in developing countries, often having severe complications. An important aspect of the use of traditional medicinal remedies and plants in the treatment of burns and wounds is the potential to improve healing. Several plants and herbs have been used experimentally to treat skin disorders, including wound injuries, in traditional medicine [3]. There has been renewed interest in screening higher plants for novel biologically active compounds, particularly those that effectively intervene in human ailments [4].

Chicorium intybus (common name Chicory, Kasni, Endive) of Asteraceae family is very popular since antiquity as food and medicine in human life. It is cultivated throughout India but especially in the fields of western and southern India. Green leaves of Kasni

are being eaten as salad. Traditionally, it has been used for hepatic conditions and liver rejuvenation [5] and has shown protective effects in mice with high levels of liver damaging enzymes [6]. Besides, this has been a primary component of variety of herbal formulations, especially in cough relief. The roots of *Chicorium intybus*, are roasted, ground and mixed with coffee for the benefit of drinkers of that beverage [7]. The nonvolatile oils of *Chicorium intybus* are essential fatty acids for mammals, which could be the pathway intermediate and precursors for certain lipid hormones [8, 9]. Its roots contain nucleotide sugar-Uridin-5'-diphosphoglucose, series of glucofructosans between sucrose and inulin beside glucose and fructose. It also contains vitamins like, vitamin A, C, Riboflavin, Thiamine and Niacin [10]. In the previous study, ethanolic extract of *Chicorium intybus* exhibited strong antimicrobial activity [11, 12]. Further, its medicinal potential has yet to be studied in detail and therefore, this present study was initiated with the aim of investigating the medicinal and therapeutic properties of *Chicorium intybus* by evaluating its effects on wound healing in injured rats using two different concentrations of ethanolic extract of *Chicorium intybus*.

Table 1. Effect of topical application of ethanolic extract of *Chicorium intybus*

Animals group	No of animals	Type of treatment	Wound healing time (days) (mm ²) Mean ± S.E.M				
			4	8	12	16	
Group 1	6	Normal saline	195.16±0.70	160.33±1.49	134.33±0.88	106.50±0.42	80.33±1.42
Group 2	6	50mg/mL*	186.16±0.94 [†]	134.16±0.94 [†]	80.16±1.40 [†]	39.66±0.66 [†]	13.66±0.75 [†]
Group 3	6	100mg/mL*	180.16±1.16 [†]	93.50±0.76 [†]	26.50±0.76 [†]	13.33±0.71 [†]	0.0 [†]

* Ethanolic extract of *Chicorium intybus*[†] P<0.001 significantly different from control (Group 1)

MATERIALS AND METHODS

Plant material, the plants were selected based on ethno pharmacology. Young plants were purchased during February 2007 from the local market of Tiruchirappalli, Tamilnadu, India, and were authenticated by Dr. Sahajahan A., Department of Botany, Jamal Mohamed College, Tiruchirappalli. A voucher of specimen has been deposited at the Department of Botany Herbarium.

Preparation of the plant extract, the *Chicorium intybus* leaves were cut into small pieces washed with distilled water and dried in oven 50°C for 5-7 days until fully dried. The leaves were ground to a fine using a grinder (Kenstar KTR01) and stored at 4°C [3]. The fine powder (250g) was soaked with 500ml of ethanol for 20hrs at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No: 1). The resulting extract was concentrated and dried to a weight of 9.2gm by placing in a water bath at 40°C [13]. This was then made into a 50mg/mL and 100mg/mL suspension in normal saline during the study [14].

Experimental animals, adult male albino rats were divided randomly into 3 groups of 6 rats each. Each rat that weighed between 180-200 gm was housed separately (one rat per cage) [3]. Animals were maintained in an environmentally controlled room with 12 h light/dark cycle and allowed free access to feed with standard pellets (Hindustan Lever Ltd., Bangalore) and water *ad libitum* [15].

Experimentally induced wounds, an area of 2cm by 2cm was excised from the nape of the neck, in previously shaved, disinfected with 70% alcohol and injected with sodium pentobarbitone solution at 35mg/kg of body weight [4], to the depth of the muscle, avoiding incision of muscles layer itself. A fresh surgical blade was used for the perpendicular cut in each animal and tension of skin was kept constant during the procedure [3]. The excision wound margins were traced at 4 days intervals on transparent sheet and plotted on a graph paper with a millimeter scale. The measurement of wound size was continued up to 18 days [16]. The wounds were then packed with sterile gauze soaked in the dressing agent. A further layer of dry sterile gauze was placed on top of this and then secured with adhesive zinc oxide plaster. Change of wound dressing was done at 4days of interval until complete wound re-epithelization had taken place [17].

Topical application of extract, a thin layer of normal saline was applied topically twice daily to Group 1 animals as a control. Group 2 and 3 were

treated with ethanolic extract of *Chicorium intybus* at 50mg kg⁻¹ day⁻¹ and 100mg kg⁻¹ day⁻¹.

Statistical analyses, results were expressed as Mean ± S.E.M The statistical difference between the groups in the term of the mean rate of wound healing was calculated using Student *t*-test.

RESULTS

The results of present study revealed that the application of an ethanolic extract of *Chicorium intybus* on the experimentally excised wound surface at two different concentrations accelerated the wound healing process by decreasing the wound area. The wound epithelizations at different days are shown in Table 1. Application of the ethanolic extract of *Chicorium intybus* at 100mg/mL (group 3) resulted in significant complete wound healing (*p*<0.001) with in 18days as opposed to only 80.33±1.42 mm² surface healing with normal saline (group 1) treated controls. On the day 4, the ethanolic extract of *Chicorium intybus* at 100mg/mL treated animals exhibited a considerable increase of 180.16±1.16 mm² (*p*<0.001) surface of wound contraction as compared with 186.16±0.94 mm² (*p*<0.001) wound healing activity with the ethanolic extract of *Chicorium intybus* at 50mg/mL (group 2) and the normal saline treated controls. A significant increase in wound contraction was observed in the subsequent days because of treatment with ethanolic extract of 50mg/mL and 100mg/mL as compared with the control. On day 18, complete (0.0 mm²) and 13.66±0.75 mm² contraction was observed with 100mg/mL and 50mg/mL, respectively, against only 80.33±1.42 mm² in controls. Fresh hair grew and almost completely covered the wounded area on 18th day in rats treated with ethanolic extract of *Chicorium intybus* at 100mg/mL concentration. No mortality was noticed amongst the animals in all the three groups (experimental and control).

DISCUSSION

Wound healing is a complex and dynamic process of restoring cellular structures and tissues layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing process, commencing in fibroblastic stage whereby the area of the wound undergoes shrinkage. It has 3 phases; inflammatory, proliferative and maturational and is dependent upon the type and extent of damage, the general state of the hosts health and the ability of the tissue to repair and remodeling of the damage tissues [13]. There are still no very effective

substances for promoting the wound healing process despite many advances in the pharmaceutical sciences. Consequently, there is a growing interest in traditional medicine in recent times [4]. This is also the case in the treatment of wounds. In developing countries, remedies prepared from herbal plants have been widely used for the treatment of soft tissue wounds and burns by medical personnel trained in western medicine as well as by traditional practitioners [3]. In several such studies, crude extracts of *Morinda pubescens* [4], *Catharanthus roseus* [13], *Ageratum conyzoides* [17] and *Flabellaria paniculata* [14] have exhibited strong wound healing properties in rats. Increase in tensile strength, collagen concentration, and stabilization of the fibers observed following the treatment were attributed to the wound healing properties of the above mentioned plants.

The result of the current study revealed that application of the ethanolic extract of *Chicorium intybus* at the concentration of 100mg/mL might have accelerated the wound healing process by stimulating different biological events such as fibroplasias, collagen synthesis, and wound contraction: hence, complete wound healing was achieved within 18days in the treated rats. In the previous studies have revealed that the *Chicorium intybus* has anti inflammatory, hepatoprotective, in combination with olive oil effective in all cases of poisoning. Its decoction is effective against insomnia [5, 8]. It has also been reported that candidate plants extracts are anti helminthic [18] as well as anti fungal. The activities may be due to presence of different kinds of oils, sugars and certain secondary metabolites; particularly the antifungal activity is due to the presence of sesquiterpene lactones, 8-deoxylactucin and 11 β , 13-dihydroxylactucin [19]. Further, in the previous studies, ethanolic extract of *Chicorium intybus* exhibited strong antimicrobial activity against a wide range of human pathogens [11, 12]. This antimicrobial activity could also be a key factor in rapid wound healing process against infectious microorganisms. Thus, the wound healing property of ethanolic extract of *Chicorium intybus* may be attributed to the phyto constituents present in the plant, and the quicker process of wound healing could be a function of the additive effects of the phyto constituents. At this stage, it is very difficult to zero in to the component(s) of the extracts is responsible for this wound healing property. However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

In conclusion, the ethanolic extract of *Chicorium intybus* at the concentration of 100mg/mL promote wound healing activity in rats, and it holds promise for treating various types of wounds in human beings. However, further investigations are required to elucidate their exact mechanism(s) of wound healing by *Chicorium intybus* and its feasibility for humans.

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CURRENT AUTHOR ADDRESSES

Naveed Ahmed Syed, Department of Biochemistry, Islamiah College,
Vaniyambadi- 635751, Tamilnadu, India. Email:
naveedbio@yahoo.com (Corresponding author)

Tarique Noorul Hasan, Department of Food Science and Nutrition,
College of Food and Agriculture Science, King Saud University,
K. S. A., India. Email: tarique_noorul_hasan@yahoo.com

Syed Mohammed Musheer Aalam, Department of Biotechnology,
Jamal Mohamed College (Autonomous), Tiruchirappalli- 620020,
Tamilnadu, India. Email: musheer666@gmail.com