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Research Article

WOUND HEALING ACTIVITY OF FRUITS OF *ANAMIRTA COCCULUS LINN* IN RATS

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ABSTRACT

Objective: The fruits of *Anamirta Cocculus Linn* are used traditionally in India for the treatment of bronchitis, chronic skin diseases, foul ulcers, dermatophytosis, phthisis, fungal infections, vertigo and vitiated conditions of vata & kapha. The purpose of the present study was to evaluate the wound healing activity of the ethanolic extract of its fruit in excision wound model.

Method: Albino Wistar rats of either sex were divided into three groups, viz, wounded control, wounded rats administered standard drug Ciprofloxacin (2 %), and wounded rats administered A.C. 200 mg/kg, respectively. In excision wound model, wound contraction was studied.

Results: Increased wound contraction was observed in extract-treated group when compared to control. Wound healing activity was statistically significant ($p < 0.05$) & ($p < 0.001$) in animals treated with 200 mg/kg of the extract.

Conclusion: The ethanolic extract of *A. Cocculus* possesses significant wound healing activity.

Keywords: *Anamirta Cocculus*, Wound healing, Dermatophytosis, Wound model.

INTRODUCTION

In recent years, among the world population, there is an increasing trend towards the usage of herbal medicines which may be probably due to the side effects and enormous cost involved in modern medicines. At present people are turning towards the herbal wound healers so as to prevent allergy and other complications that are often encountered due to the application of synthetic wound healers.[1] A wound may be described acute or chronic[2], by the method of closure, by its presenting symptoms or by the appearance of the predominant tissue types in the wound bed. It is a breakdown in the protective function of the skin; the loss of continuity of epithelium, with or without loss of underlying connective tissue (i.e. muscle, bone, nerves)[3] following injury to the skin or underlying tissues/ organs caused by surgery, a blow, a cut, chemicals, heat/ cold, friction/ shear force, pressure or as a result of disease, such as leg ulcers or carcinomas[4]. Wound healing can be defined as a complex dynamic process that results in the results in the restoration of anatomic continuity and function. [5]. It is a complex process characterized by homeostasis, reepithelization, granulation tissue formation and remodeling of the extracellular tissue [6]. Wounds heal by primary intention or secondary intention depending upon whether the wound may be closed with sutures or left to repair, whereby damaged tissue is restored by the formation of connective tissue and re-growth of epithelium [7]. Several drugs obtained from plant sources are known to increase the healing of different types of wounds. Though some of these drugs have been screened scientifically for evaluation of their wound healing activity in different pharmacological models and patients, the potential of many of the traditionally used herbal agents remains unexplored. In few cases active chemical constituents were identified. [8]

Anamirta Cocculus is commonly known as fish berry. It is a wild woody climber found in South east Asian and Indian Subcontinent & belongs to the family Menispermaceae. [9] This plant is distributed throughout India in dense forest. Fruit is a drupe, nearly spherical, 1cm in diameter when dry, smooth and hard. The fruits of *Anamirta Cocculus* used in curing different types of diseases like bronchitis, chronic skin diseases, foul ulcers, dermatophytosis, phthisis, inflammation, fungal infections, vertigo and vitiated condition of vata and kapha. [10] It has also been suggested to possess antioxidant, antimicrobial action.

Hence, this study was undertaken to evaluate the wound healing activity of the ethanolic extract of Anamirta Cocculus in experimentally induced wound in rats.

MATERIALS AND METHODS

Collection of Plant Materials

Fruits of the *Anamirta Cocculus* plant were collected from the deciduous forest of Wayanad District of Kerala in India, in the month of April, 2011. The Botanical identification and plant material authentication was done by Dr. H .B. Singh Scientist F & Head, Raw Materials Herbarium & Museum, N.I.S.C.A.I.R., Dr .K .S Krishnan marg, New Delhi and specimen was submitted and preserved for future reference (Voucher specimen No.1397/194).

Extraction and preparation of sample

The fruits were gently washed in tap water and completely dried under room temperature (30 ± 2 ºC) for 4 weeks protected from direct heat or sunlight. The fruits of the plant were dried under shade, separated and made to dry powder.

It was then passed through the 40mesh sieve. Then150g of weighed powered fruit of *Anamirta Cocculus* was packed in Soxhlet apparatus and extracted with ethanol (90%) for 36 hrs and completion of extraction was confirmed by pouring a drop of extract from the thimble on a filter paper, which does not show the presence of any oil spot on that. After complete extraction the solvent was evaporated and concentrated to dry residue.

Chemicals

Ciprofloxacin, Tween 80, Anesthetic ether and all other chemicals were of analytical grade.

Acute Toxicity Studies

Acute oral toxicity study of ethanolic extract of *Anamirta Cocculus (Linn.)* was carried out according to OECD guidelines 423.

Wound healing activity

Albino Wistar rats of either sex weighing 150-200 g were selected. Animals were depilated at the 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 ether anesthesia, wounding was performed aseptically. Excision wound model in Albino Wistar rats were selected for assessing the wound healing activity. The study protocol was approved by Institutional Animal Ethics Committee (Proposal No: 02/2012).

Animals in the first group which served as a control received the vehicle only and the ethanolic extract was suspended in Tween

80(1%w/v) and was administered orally at a dose of 200 mg/kg body weight daily for a period of twenty days or till the excised wound healed which ever was earlier to the animals in the second group. Animals in the group three received Ciprofloxacin 10mg/kg.[11] The rats were anaesthetized prior to excision procedure.

Excision wound

For the excision wound study, each group containing six animals was selected. A circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats under light ether anesthesia in aseptic condition and observed throughout the study. The animals were housed individually. The observation of percentage wound closure were made on 4th, 8th and 12th post wounding days and thereafter on alternate days until healing was complete.[12]

Statistical analysis

The results were expressed as mean ± SEM of 6 animals in each group. The data were Statistically evaluated by one-way ANOVA followed by Dunnet's test or comparison of test & standard group with control. Values of $p < 0.05$ & $p < 0.001$ were considered statistically significant.

RESULTS

Acute Toxicity Study

There was neither change in behavioral pattern or any sign of toxicity during the observations up to72 h for mortality. The extract was safe up to a maximum dose of 2000mg/kg b. w. The biological evaluation was carried out at a dose of 200mg/kg b. w.

Table 1: Effect of ethanolic extract of A.C. fruits on Excision Wound Model when administered orally

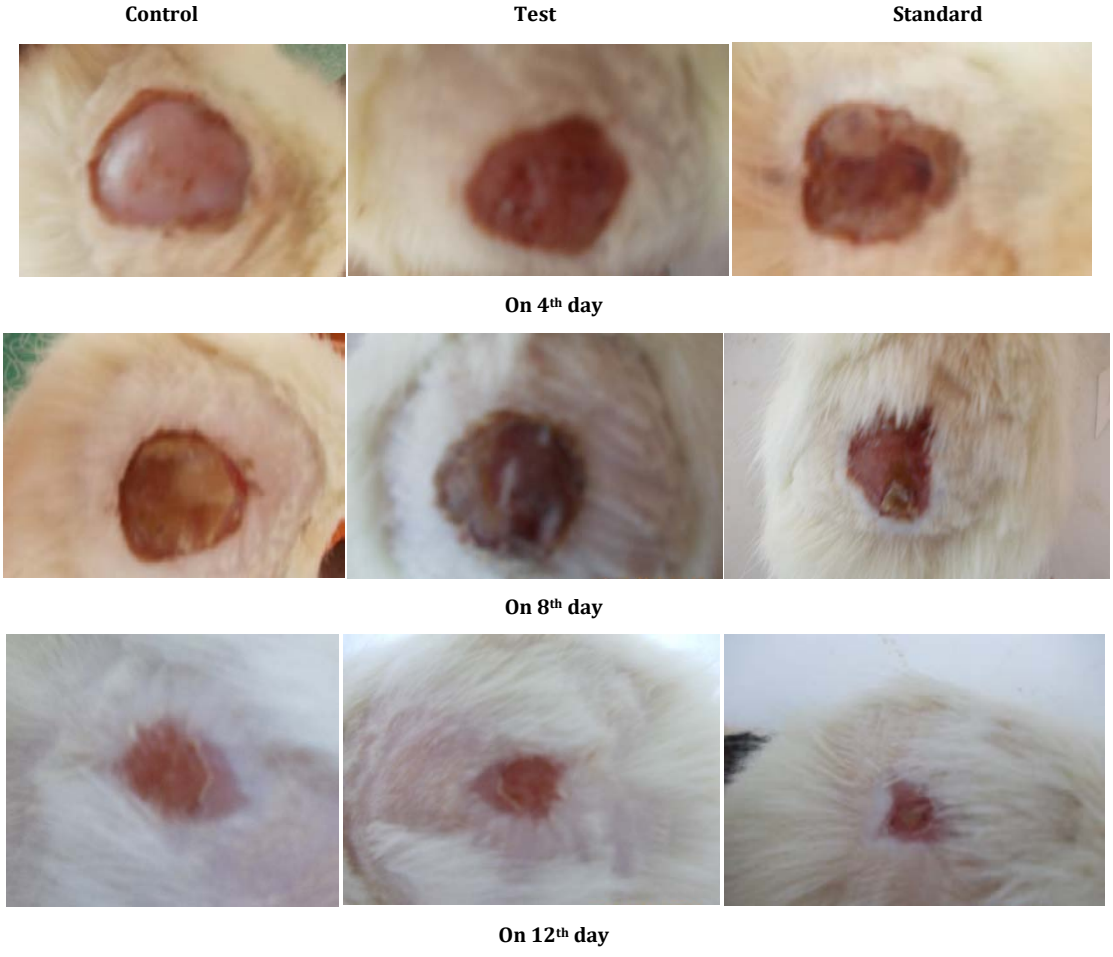
Groups	Dose (mg/kg)	Percentage contraction (sq m m. ±S.E.M.)		
		4th day	8th day	12th day
Control	1 ml of vehicle	12.70±0.1344	17.91±0.2159	25.84±0.2382
Test A.C.	200	21.34± 0.03152	63.65±0.03713*	70.75±0.1594***
Ciprofloxacin	10	30.24±0.01335*	76.72 ±0.01844***	97.38±0.1627***

Values are expressed as mean± S.E.M., n=6 animals in each group, *P<0.05Vs Control, *** P<0.001 Vs Control (One -way ANOVA).

Wound healing activity

In the excision wound model, as shown in Table 1, the wound contracted progressively when treated with the extract and the reference drug. Thus the extract & standard drug promoted wound contraction significantly when compared with the control group. Thus, the extract promoted wound healing.

Photos for wound healing activity (Excision wound)



DISCUSSION

Wound healing or repair is a natural process of regenerating dermal and epidermal tissue, and may be categorized into three phases, viz, inflammation, proliferation and remodeling phase. In the inflammation phase, various growth factors such as tumor necrosis factor (TNF), interleukins (IL) are released to initiate the proliferation phase. The latter is characterized by angiogenesis, collagen deposition, granular tissue formation, epithelialization and wound Contraction [13]. In the last phase, the levels of collagen production and degradation equalize, after which disorganized fibers are rearranged thus increasing the tensile strength of the wound [14]. The capacity of wound to heal depends, in part, on its depth, as well as on the overall health and nutritional status of the individual. Following injury, inflammatory response occurs and the cells below the dermis begin to increase collagen production. Later, the epithelial tissue is regenerated. It is well known that stages in healing, namely, coagulation, inflammation, microphasia, fibroblasts formation and collagenation, are intimately interlinked [15]. The process of wound contraction and epithelization is separate and independent. The activity of fibroblast is responsible for wound contraction and involves movement of entire dermis. Epithelization involves migration and proliferation of cells. It is known that stabilization of lysosomal membranes, inhibition of cellular migration and inhibition of fibroblast contraction are responsible for their anti-healing effects [16]. Thus, intervention in any one of these phases by drugs would eventually lead to either promotion or depression of collagenation, wound contraction and epithelization [17]**.** In the present investigation, the ethanolic extract of *A. Cocculus* at the doses of 200 mg/kg showed significantly increased healing by wound contraction, when compared to the control group. Healing enhances epithelization and promotes wound contraction by increasing granulation tissue weight due to infiltration of macrophages [18].

Reactive oxygen species (ROS) play a vital role in wound healing and can trigger various beneficial oxygen free radicals. ROS also play an important role in the failure of ischemic wound healing while antioxidants improve healing in ischemic skin wounds [19]**.** Elevated lipid peroxide levels have also been demonstrated in certain inflammatory skin lesions such as wound and dermatitis [20]. Therefore, if a compound has antioxidant potential, it can be a good therapeutic agent for enhancing the wound healing process [21]**.**

CONCLUSION

The present study indicates the wound healing activity of *Anamirta Cocculus* in experimental animals using excision wound model. Several phytoconstituents like alkaloids and terpenoids are known to promote wound healing process due to their antioxidant and antimicrobial activities. This study revealed that the ethanolic extract possess good wound healing property which may be attributed to the individual or combined actions of phytoconstituents like alkaloids and triterpenoids present in it. Further investigations are needed for identification and isolation of bioactive compounds responsible for wound healing activity.

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