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Research Article

EVALUATION OF *IN VIVO* AND *IN VITRO* WOUND HEALING ACTIVITY OF AQUEOUS EXTRACT OF *ACALYPHA INDICA*

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ABSTRACT

The present study was required to evaluate the wound healing activity of aqueous extract of *Acalypha indica*. The wound healing activity was evaluated by *in vivo* and *in vitro* models. Excision and Incision wound models used for *in vivo* segment and CAM Assay used for *in vitro* segment. Wistar albino rats of either sex, weighing 120–150 g were used for *in vivo* studies. Animals were divided into 3 groups of 6 each. The group 1 was left untreated and considered as a control, group 2 was received standard drug Povidone-iodine ointment, group 3 received 1 % aqueous extract of *A. indica* mixed with ointment base. These ointments topically applied once daily till complete epithelialization. The % of wound contraction was calculated by weight variation of the trace paper. Results showed that the wound contracting capacity of *A. indica* was significantly greater than ($P < 0.05$) the control, and also comparable to standard Povidone-iodine ointment. The incision wound model was evaluated by measuring the tensile strength of regenerated tissue. The wound breaking strength was estimated at 10th day by tensile tester. Testing results showed that *A. indica* produced a considerable increase in the tensile strength when compared with the control group; similar effects were comparable to standard drug treated group. The angiogenic activity of *A. indica* treated CAM showed the number of new blood capillaries than control CAM, also considerable with a standard drug treated CAM. The results suggest that aqueous extract of *A. indica* posses a significant wound healing potential in normal wound.

Keywords: Wound healing, Excision wound model, Incision wound model, CAM assay, Povidone-iodine ointment

INTRODUCTION

The skin is the largest organ of the body, accounting for about 15 % of the total adult body weight. It carried out many essential functions, including safeguards against external physical, chemical, and biologic attackers, as well as prevention of excess water loss from the body and a role in thermoregulation[1]. Wounds are inevitable events in everyday life. Injuries may occur due to physical, chemical, thermal, microbial or an immunological insult to the tissue. The wound is determined as a disruption of cellular and anatomical continuity of the tissue. A combination of cellular and biochemical events leading wound healing and re-building of the injured tissue strength and integrity. Clinically, one often faces under healing, over healing and non-healing. Therefore, it is intended to treat the wound to reduce the time required for healing or considered to reduce the undesirable effects[2]. It is well-known that the rate of wound healing can be enhanced by providing the best possible environment, i.e. complete asepsis, removal of devitalized tissue, apposition of wound edge and regular dressing. In case of fracture, healing can be improved by proper reduction and immobilization. In addition, these basic measures, using certain herbs which pose antiseptic, astringent, anti- inflammatory, antimicrobial and bio stimulatory property can also enhance the rate of healing. Medicinal plants are increasing the rate of tissue healing by providing essential substances, taken at various steps of regeneration and proliferation. These medicinal plants are cheaper and safer than allopathic drugs are available[3]. This is similar to the traditional ways of using herbs to heal wounds; as a poultice or juice extracted from the fresh plants. Aqueous extracts of some herbs are used to clean and disinfect wounds. Flamed leaves of some plants are used to dress injured skin, promote wound healing and can be used to ward off infection[4]. Presently, when the research on Ayurvedic preparation has achieved new heights, the role of these herbs and bio stimulators in tissue healing has been well proved scientifically and they form the basis of different herbal preparation that have flooded the market. The plant *Acalypha indica* (*A. indica*) commonly known as Indian acalypha and it belongs to the family of Euphorbiaceae. It is an erect annual herb and general plant in Indian gardens, backyards of houses and waste places throughout the plains of India[5]. The plant traditionally used as an expectorant against asthma, pneumonia, emetic, emmenagogue (Promotes menstruation) and anthelmintic. The plant *A. indica* contains Acalyphine which is used in the treatment of sore gums[6]. The root, stem and leaf of *A. indica* possesses clinical activity. The plant is reported to have a post-coital anti fertility effect, anti venom properties, wound healing effects, antioxidant activities, anti-inflammatory and diuretic effects[7]. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Useful clinical effects of plant materials typically result from combinations of secondary products present in the plant[8]. The present study was undertaken to examine the wound healing activity of aqueous extract of *A. indica* has been carried out using *in vivo* and *in vitro* models.

MATERIAL AND METHODS

Collection and Drying of plant materials

Entire plant with roots of *A. indica* was collected from the herbal garden, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India. The collected plant was authenticated by Mr. V. Chelladurai (Retired) Research Officer-Botany; Central council for Research in Ayurveda and Siddha, India and voucher specimens (No. 1957) were kept in the Pharmacognosy Lab, Department of Pharmacy, Annamalai University for future reference. The Collected plant should be washed thoroughly three times with purified water and once with distilled water. The plant materials were air shade dried and then powdered using electric blender to make a coarse powder. The powdered samples were placed in sealed containers for extraction purposes. Class A glass wares (Borosil Ltd., Mumbai, India) were used for this study and the

chemical used in this work obtained from Hi-Media laboratories, Mumbai, India.

Preparation of plant extract

About 10 g of the powdered samples of *A. indica* was placed with double sterilized distilled water in a 100 ml Erlenmeyer flask and then boiling the mixture for 5 min. The extract was cooled and filtered through Whatman no 1 filter paper, collected filtrate was then centrifuged 5000 rpm 10 min. The collected supernatant was evaporated to dryness under reduced pressure using a Rota vapor and the resulting paste like form extracts were kept in vacuum desiccators[9].

Models for wound healing activity

***In vivo* Models**

Wistar albino rats of either sex, weighing 120–150 g were used for *in vivo* studies, the experiment was conducted in conformity with the internationally accepted principles for laboratory animal use and the experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC), Central animal house, Annamalai University, Annamalai Nagar, Chidambaram, India (160/1999/CPCSEA). The animals were housed in polypropylene cages in standard environmental conditions (20–25°C), feed with standard rodent diet *ad libitum* and tap water. The animals were acclimatized to laboratory conditions for a week prior to the initiation of the experiment. 12 hours before the start of the experiment, rats were deprived of food, but given free access to water[10].

Excision wound model

The animals were anesthetized with anesthetic ether by open mask method and placed on an operation table in its natural position. An excision wound was inflicted on the dorsal thoracic region 1–1.5 cm away from the vertebral column on either side and 5 cm away from the ear. The skin impressed area was excised to the full thickness to obtain a wound area about 500 mm^2 and 2 mm diameter and 2 mm depth. Hemostasis was achieved by blotting a wound with a cotton swab soaked in normal saline. Animals were divided into three groups of 6 each. The group 1 was left untreated and considered as a control, group 2 was received standard drug Povidone-iodine ointment, group 3 received 1 % aqueous extract of *A. indica* mixed with ointment base. These ointments topically applied once daily starting from the day operation till complete epithelialization. Wound areas were measured on days 3,7,11 and 19 th[11]. The wound area was drawn with permanent marker on a transparent sheet and marked area of the transparent sheet was isolated and measuring the weight for calculation. Percentage wound contraction was calculated as:

% of the wound closer = (Initial wound size – specific day wound size) × 100 / Initial wound size

Incision wound model

Para vertebral straight incision of 6 cm length was made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete hemostasis, the wounds were closed by means of interrupted sutures placed at approximately 1 cm apart. Animals were treated daily with drugs, as mentioned above under excision wound model from 0 days to 9th post wounding day. The wound breaking strength was estimated at 10 the day by tensile tester (Instron 6021)[11]. Tensile strength was calculated using the following formula:

Tensile strength = Total breaking load / Cross-sectional area

***In vitro* Model**

Chick Chorioallantoic Membrane (CAM) model

In this study, the angiogenic activity of aqueous extract of *A. indica* was analyzed by CAM Assay. The test samples (herbal extract) were stored air tight glass vials at 4°C light controlled environment. 100 µg/µl solution of test sample was prepared in Phosphate buffered saline (PBS) and sterilized by passing through a syringe filter (0.22 µm). Vascular endothelial growth factor (hVEGF) 50 µg/µl was prepared (standard) in sterile PBS. Nine-day-old fertilized Hen eggs were procured from hatchery and were cleaned and decontaminated using alcohol. A small window of 1.0 cm^2 is made on the shell of the egg and opened, Gelatin sponges were cut in approximately 2 mm^3 pieces and loaded with 2 µl of test solution and 2 µl standard solution and placed at the junction of two large vessels on CAM, after that the window is resealed with tape. The sealed eggs are incubated at 37°C in a well-humidified chamber for 72 h. Then, the eggs are opened, new blood vessel formation is observed in CAM containing sponge with herbal extract which are compared with CAM containing sponge with hVEGF (standard) and CAM containing sponge without herbal extract (Control). Fixed CAMs were imaged under constant illumination and magnification under a stereo microscope fitted with a digital camera (CANON). Images were analyzed on MS power point keeping the image size constant. A ring was drawn around the graft and the size was kept constant[11,12].

RESULTS

Excision wound model

The excision wound model was measured by wound contraction capacity at various time intervals until the complete wound healing process. Contraction of the wound area was observed from the 19th day of the 3rd day. It was observed in the injured area, completely closed on the 19th. Proof of this is documented in the photos. Figure 1 showed the photograph taken before starting the treatment. Figure 2 depicts the percentage of the wound contraction of different treated groups in 19th day. The percentage of wound contraction was calculated by weight variation of the trace paper, which is carved from the wound area marked trace paper. Table 1 represents the effect of aqueous extract of *A. indica* effect on percentage of wound contraction in the excision wound model. The control rats showed a time dependent decreases the wound size from 0.01725 to 0.0050 mg from the day 3rd to 19th. The group treated with standard drug (Povidone – iodine ointment) showed 0.01463 to 0.0036 mg from the day 3rd to 19th day. 1 % w/w aqueous extract incorporated with simple ointment treated group showed decreased the wound size from 0.0126 ± 0.0006 to 0.0028 ± 0.0003 mg from the day 3rd to 19th day.

Incision wound model

The incision wound healing model was evaluated by measuring the tensile strength of regenerated tissue. 1 % aqueous extract of *A. indica* mixed with ointment base. These ointments topically applied once daily till complete epithelialization. The 10-day post-surgical skins breaking strength measurement results are depicted in Table 2 skin braking strength of the animals treated with aqueous extract of *A. indica* showed 82.387 ± 0.686, standard drug Povidone-iodine ointment showed 86.113 ± 0.897, control group (animals treated with simple ointment) showed 63.523 ± 1.201 and control group (the skin samples were excised from the normal animals on experimental day) showed 106.18 ± 1.022.

CAM assay

The angiogenic activity of aqueous extract of *A. indica* was analyzed by formation of new blood capillaries. The formation of new capillaries was observed at the end of the 3rd day of incubation. The capillaries were identified and marked with red triangles

showed in Figure 3. The number of the blood capillaries formed in CAM containing aqueous extracts was compared with untreated CAM and CAM containing standard solution. Among all three samples, aqueous extract of *A. indica* showed (Avg.10) several numbers of formations of blood capillaries, this effect was comparable to standard drug (Avg.13.7) and higher than control (Avg.4.3) treated group.

Table 1: Effect of wound healing activity of aqueous extract of *A. indica* on the excision wound model

Post wounding days	Weight of trace paper (mg)		
	Control (Simple Ointment)	Standard (Povidone-iodine)	Extract (1 % w/w of Aq. Extract of *A. indica*)
3rd day	0.0172 ± 0.0012	0.0146 ± 0.0096	0.0126±0.0006
7th day	0.0142 ± 0.0008	0.0123 ± 0.0008	0.0103±0.0015
11th day	0.0130 ± 0.0005	0.0116 ± 0.0004	0.0097±0.0006
19th day	0.0050 ± 0.0008	0.0036 ± 0.0004	0.0028±0.0003*

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dunnett's $P < 0.05$ calculated by comparing treated groups with the ONLY WOUND group

Table 2: Effect of wound healing activity of aqueous extract of *A. indica* on the incision wound model

S. No.	Group	10th past wounding day
		Tensile strength
1	Control	106.18 ± 1.022
2	Control (Treated)	63.523 ± 1.201
3	Std	86.113 ± 0.897
4	Ext	82.387 ± 0.686**

Values are expressed as the mean ± S.D; n = 6, Statistical significance (p) calculated by one way ANOVA followed by Dunnett's $P < 0.05$ calculated by comparing treated groups with the ONLY WOUND group. The $P < 0.001$, consider extremely significant

Table 3: Effect of Angiogenic activity of aqueous extract of *A. indica* on CAM model

S. No.	Group	Number of blood capillaries present in CAM
1	Control	4.33 ± 0.66
2	Standard	13.66 ± 0.33
3	*A. indica* Extract	10.00 ± 0.57

Values are expressed as the mean ± S.D; n = 3, Statistical significance (p) calculated by one way ANOVA followed by Dunnett's $P < 0.05$ calculated by comparing treated groups with the ONLY WOUND group. The $P < 0.001$, consider extremely significant

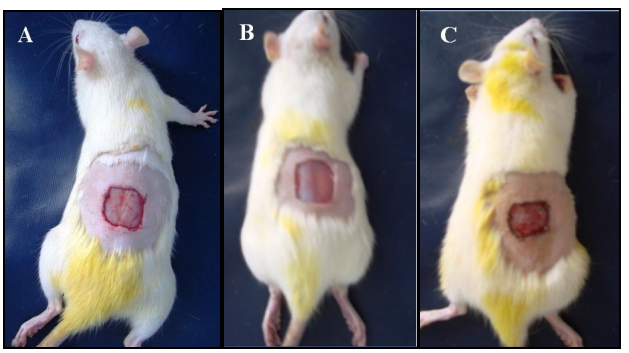

Figure 1: Excision wound on - 0 day A- Group I - Normal control, B - Group II - Povidone iodine ointment, C- Group III- Aqueous extract of *A. indica*

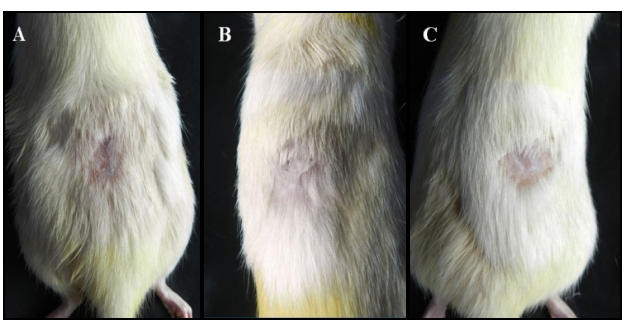

Figure 2: Excision wound on – 19th day A- Group I - Normal control, B - Group II - Povidone iodine ointment, C- Group III- Aqueous extract of *A. indica*

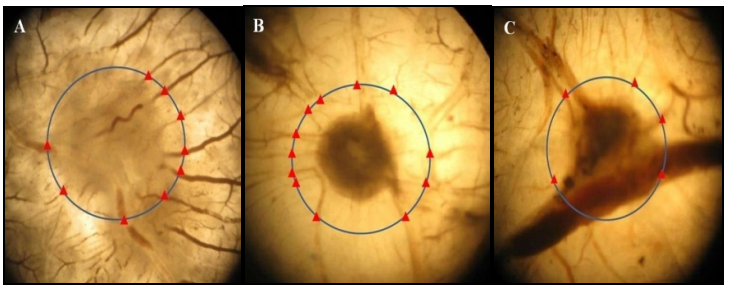

Figure 3: (A) CAM treated with *A. indica*; (B) CAM treated with Standard, (C) CAM untreated. The red color triangles in the figure represent the formation of new blood capillaries

DISCUSSION

The aim of the present work is to evaluate the wound healing effectiveness of aqueous extract *A. indica* by *in vivo* and *in vitro* models. Wound healing is an extremely complex phenomenon involving a number of well orchestrated events including continuous, overlapping and precisely programmed phases. The events of each phase must occur in a defined and regulated manner. Interruptions, aberrancies, or prolongation in the process can lead to delayed wound healing[13]. One of the events that obstruction wound healing is colonization of the wound bed by microorganisms and is important for the removal of contaminating micro-organisms. In the lack of efficient decontamination, however, inflammation may be prolonged, since microbial clearance is incomplete. Both bacteria and endotoxins can lead to the prolonged elevation of pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF-α and elongate the inflammatory phase. If this continues, the wound may enter into chronic state and fail to heal[14]. Currently, a number of classes of drugs have been widely used in wound management. Among that antibiotics play a key role to reduce the contamination. The basis of such expectation is that while antibiotics protect tissue growth process, which is a most essential part of the wound healing profile. The wound healing activities of medicinal plants have since discovered in folklore. The considerable successes recorded have led to an investigation into medicinal plants with a view to validating these acclaimed properties[15]. The aqueous extract of *A. indica* has already been reported in our previous study of its antibacterial activity. The study has been extended to the belief that the plant has wound healing activity. In the present study, excision and incision wound models carried out for the *in vivo* segment. Plant extracts mixed with an ointment base because that is the most convenient form of topical application. The contraction capacity of the excision wound model measured in different time intervals. Initial findings from the results of the three groups, (control, standard and aqueous extract of *A. indica*) were reduced on the 19th days. A comparison of these results indicates that aqueous extract of *A. indica* better than untreated control group and the standard drug treatment for wound healing activity showed that the group is relatively equal effect. Incision wound model evaluated by measuring the tensile strength. The results from an incision wound healing study disclosed that aqueous extract of *A. indica* produced a significant increase in the tensile strength when compared with the control group (Treated group) and almost similar effects were comparable to standard drug treated group. The angiogenic activity of aqueous extract of *A. indica* was analyzed by Chick chorioallantoic membrane assay for *in vitro* segment. Angiogenic activity was measured by the number of newly formed capillaries. Similar indication apparent from the results that aqueous extract of *A. indica* treated CAM showed the number of new blood capillaries more than control treated CAM. Comparison of this result with standard drug treated CAM indicates that nearly equal effect. From the all above experiments brings out with results that of aqueous extract of *A. indicia* may suitable for acute wound management.

CONCLUSION

The aqueous extract showed significant wound healing activity on *in vivo* and *in vitro* wound models. These results offer pharmacological evidence on the traditional use of *A. indica* for healing wounds. Further studies are needed to better assess the potential value of *A. indica* extracts as wound healing agents.

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