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Research Article

EVALUATION OF WOUND HEALING ACTIVITY OF HERBAL GEL CONTAINING THE FRUIT EXTRACT OF *COCCINIA INDICA* WIGHT AND ARN. (CUCURBITACEAE)

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ABSTRACT

The present work was to investigate wound healing activity of *Coccinia indica* (Wight and Arn) fruits belonging to family Cucurbitaceae. Herbal gel containing ethanolic fruit extract and aqueous fruit extract of *C. indica* was formulated and evaluated on excision wound model and incision wound model. Excision wound measuring about 500 mm^2 was created on the albino rats placed in group (n=6) and the gel applied topically on the wounded area which was measured at interval of 3 days until epithelization and complete wound closure. Blank gel and Framycetin sulphate cream (FSC) 1% w/w served as the control and standard treatment respectively. Topical application of ethanolic extract gel on excision wound in rats caused a significant ($P<0.01$) higher rate of wound healing (99.49%) and reduced epithelization period. In incision wound model, ethanolic extract gel significantly ($P<0.01$) increased the breaking strength as compared to control (486.10±5.86) than aqueous extract (415.78±6.43). The result suggest that treatment with ethanolic extract gel of *C. indica* fruits may have beneficial influence on the various phases of wound healing like wound contraction and resulting in faster healing than aqueous extract.

In conclusion, the observation and results obtained in this study indicated that the ethanolic extract gel of *C. indica* fruits significantly stimulated wound contraction. These findings could justify, role of this plant material in the management of wound healing.

Keywords: *Coccinia indica,* Herbal formulation, Wound healing activity.

INTRODUCTION

A wound may be defined as a break in the epithelial integrity of the skin or may also be defined as loss or breaking of cellular and anatomical or functional continuity of living tissue[1-2]. Wound healing processes are well organized biochemical and cellular events leading to the growth and regeneration of wounded tissue in a special manner[3]. Healing of a wound is an important biological process involving tissue repairs and regeneration. It involves the activity of an intricate network of blood cells, cytokines and growth factors which ultimately leads to the restoration to the normal condition of the injured skin or tissue[4]. The aim of wound care is to promote wound healing in the shortest time possible, with minimal pain, discomfort and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration[5]. Wound healing processes are known to be influenced by among other factors by infections, nutritional status, drugs and hormones, types and sites of wound and wasting diseases like diabetes[6]. In folklore medicines, medicinal plants have been used widely in facilitating wound healing with high degree of successes. This has inspired many researches which are aimed at validating the claims and discovering mechanisms which are possibly explains the potential of these herbs on wound repair processes.

Coccinia indica Wight and Arn (Fam. Cucurbitaceae) a climbing or prostrate, much branched, perennial herb widely distributed in both wild and cultivated states on the plains of India. Whole plant, fruits, leaves and roots of *C. indica* are being reported to the literature as having medicinal value. In Ayurveda it is used as galactagogue, antipyretic; cures leprosy '*vata*' the burning sensation of the body, consumptions, jaundice, diseases of blood and in inflammation[7-8]. Pectin extracted from the fresh fruit also shows hypoglycemic effect[9]. The root is cooling, aphrodisiac, stops vomiting urinary losses, burning of hands and feet, given for uterine discharges[10]. The ethanolic extracts of leaves, stems and fruits showed significant anti-inflammatory activity[11-12]. The methanolic extract of leaves and fruits of *C. indica* possesses significant antimicrobial activity with different potency of selected micro-organisms[13-14]. The anti-oxidant effect of an ethanolic extract of *Coccinia indica* leaves was studied in streptozotocin induced diabetic rats[15]. The hepatoprotective effect of an ethanolic extract of *C. indica* fruits were evaluated against carbon tetrachloride induced hepatic damage in rats[16]. In the present study, we investigated ethanol and aqueous Extract of *Coccinia indica* (Wight and Arn) fruits (Cucurbitaceae) formulated as a gel for wound healing activity on excision and incision wound model.

MATERIALS AND METHODS

Plant material

Fruits of *Coccinia indica* (Wight and Arn) Family Cucurbitaceae were procured from the local market. The plant material was identified and authenticated at Department of Botany, R. T. M. Nagpur University, and Nagpur. A voucher specimen (9196) was deposited in the institute.

Drugs and chemicals

Framycetin sulphate cream (FSC) 1%w/w, diethyl ether, ethanol, sterilized cotton were used.

Animal

Wistar rats (150-220 g) were used for evaluation of wound healing activity. The animals were housed in a 12h light: dark cycle in a temperature controlled room, with free access to water and food. The protocol of the experiments was approved by the Institutional Animal Ethical Committee (IAEC) of Manoharbhai Patel Institute of Pharmacy, Gondia (M.S.) and according with the guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Ethical guidelines were strictly followed during all the experiments.

Extraction of plant material

The fruits were washed thoroughly, dried under the shade and pulverized. The coarse powder (500g) extracted successively with petroleum ether and ethanol using soxhlet apparatus. Finally the aqueous extract was prepared by masceration. The extracts were dried using rotary vaccum evaporator and stored in desicators until further use.

Formulation of Gel

The gel was composed of carbapol 5%; propylene glycol 1%, triethanolamine 0.8 ml, extract 10%, methyl paraben and propyl paraben added as a preservative and distilled water in a quantity sufficient to prepare 100gm gel. Two formulations were prepared,

one containing ethanolic extract (Test I gel) and aqueous extract (Test II gel) of *C. indica* fruits.

Pharmacological screening of extracts

Grouping of Animals

The albino rats were divided randomly into four groups, each group containing 6 rats.

Group 1- received application of blank gel, served as a control.

Group 2- received application of standard drug i.e., Framycetin sulphate cream (FSC) (1%w/w).

Group 3- received application of Test I gel.

Group 4- received application of Test II gel.

Wound healing activity

Excision and Incision wound models were used to evaluate wound healing activity.

Excision wound model

Excision wound were used for the study of rate of contraction of wound and epithelization[17]. The rats were anaesthetized with slight vapors inhalation of diethyl-ether. A circular wound of about 500 sq. mm. was made on depilated ethanol sterilized dorsal thoracic region of the rats. The areas of the wound were measured immediately by placing transparent polythene graph paper over the wound and then tracing the area of wound on it. This was taken as the initial wound area reading. All the samples control (blank gel), standard (Framycetin sulphate cream 1%w/w), Test-I gel and Test-II gel were applied once daily for18 days, starting from the day of wounding. The observations of percent wound closure were made on the 3rd, 6th, 9th, 12th, 15th and 18th post wounding days. The wound area of each animal was measured at interval of 24-48 hours using tracing paper method. The percentage of wound contraction was calculated from the days of measurement of wound area. The period of epithelization was calculated as the number of days required for the fall of dead tissue remnants of the wound without any residual raw wound.

Incision wound model

In incision wound model, under light ether anesthesia the animal was secured to operation table in its natural position. One paravertebral straight incision of 6 cm was made on either side of the vertebral column with the help of scalpel blade. The wounds were closed with interrupted sutures in 1 cm apart. The gel was topically applied once in a day. The sutures were removed on the 8th post wound day. The skin breaking strength of the wounds were measured on the 10th day by continuous constant water flow technique[18].

Statistical Analysis

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnet's test at P<0.01 significance level.

RESULTS

Herbal gel or ointments containing different medicinal plants have been reported to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort and scarring to the patient[19]. The medicinal plants that are so used owe their efficacy to a direct action on the wound repair processes, or to the anti-inflammatory and anti-microbial properties. Some of the plants employed in wound care have also been shown to possess a combination of these and other related properties. Significant promotion of wound healing activity was observed in both ethanol and aqueous fruit extracts of *Coccinia indica* (Wight and Arn) in excision and incision wound model.

In excision wound model, the mean percentage closure of wound area was calculated on the 3, 6, 9, 12, 15 and 18 post wounding days. (Table I) Application of Test I gel showed significant (P<0.01) wound healing activity. It produced highest rate of wound healing (96.49%), reducing the epithelization period to (17.02 ± 1.15) compared to the control with epithelization period of (24.79 ± 3.53). Test II gel treated animals showed wound healing (90.51) which is lesser than Test I gel and standard dug Framycetin sulphate cream (1%w/w). Standard showed wound healing (98.14) and epithelization period of (16.58±1.87). The figure 1 shows % wound healing activity.

The incision wound study was also carried out to measure the breaking strength on the day 10 regenerated tissues. (Table II) Test I and Test II gel treated animal showed increase in tissue breaking strength (486.10±5.86) and (415.78±6.43) respectively when compared to control (275.21±4.75). The mean breaking strength significant in animals treated with standard drug Framycetin sulphate cream (1% w/w) (517.68±.27).

Table 2: Effect of topical application of Test I gel and Test II gel of *Coccinia indica* fruits on healing of incision wound model

Groups	Breaking strength (g)
Control	275.21±4.75
Standard	517.68±6.27*
Test I gel	415.78±6.43*
Test II gel	486.10±5.86*

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test, and P<0.01* when compared to control group.

Table 1: Effect of topical application of Test I gel and Test II gel of *Coccinia indica* fruits on healing of excision wound model

Group	0-day	3rd day	6th day	9th day	12th day	15th day	18th day	Period of epithelization
Control	511.29±5.83 (0.00)	489.65±6.37 (4.23)	405.29±5.65 (20.73)	359.09±10.58 (29.76)	278.99 ±18.02 (45.43)	191.51 ±14.41 (62.54)	88.22 ±6.24 (82.74)	24.79±3.53
Standard	510.26±4.16 (0.00)	407.47±4.5* (20.14)	308.5 ±6.74* (39.49)	202.59±4.31* (60.29)	56.10 ±2.34* (89.00)	30.13 ±2.37* (94.09)	09.47 ±1.33* (98.14)	16.58±1.87*
Test I gel	507.65±5.66 (0.00)	465.31±2.93* (8.34)	350.40±4.86* (30.97)	291.26±5.10* (42.65)	155.48 ±3.61* (69.37)	80.29 ±3.38* (84.18)	48.13 ±2.46* (90.51)	19.03±1.13*
Test II gel	509.71±3.30 (0.00)	444.34±2.98* (12.82)	317.46±4.10* (37.71)	228.61±2.59* (55.14)	66.45 ±2.13* (86.96)	37.89 ±2.33* (92.56)	17.84 ±1.38* (96.49)	17.02±1.15*

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test, P<0.01* when compared to control group.

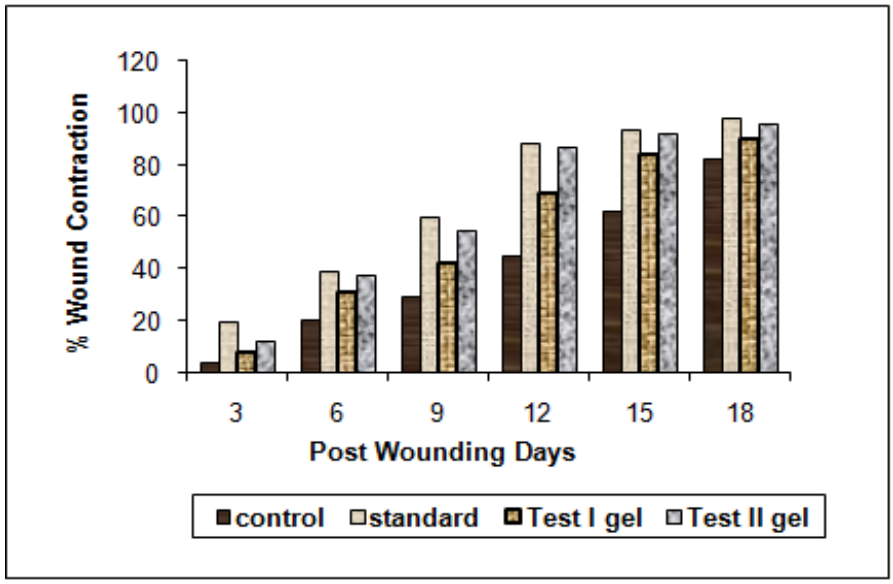

Fig. 1: Wound healing activity of *Coccinia indica* gel

DISCUSSION

Wounds are the physical injuries that results in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disrupted functional response of the several cell types to injury. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue[20]. In spite of tremendous advances in the chemical drug industry, the availability of substances capable of stimulating the process of wound repair is still limited[21]. Moreover the management of chronic wounds is another major problem due to high cost therapy and presence of side effects[22].

Wound healing is a natural process of regenerating dermal and epidermal tissues. Whenever there is wound, a set of overlapping events takes place to repair the damage. These processes have been categorized into phases which include the inflammatory, proliferative and remodeling phases[23]. In the inflammatory phase, bacteria and debris are phagocytosed and removed and cytokines and mediator are released that cause the migration and division of cells involved in the proliferative phase. Angiogenesis, collagen deposition, granulation tissue formation, epithelization and wound contraction occur in the proliferative phase[24]. During epithelization, the epithelial cells crawl across the wound bed to cover it[25]. The wound is eventually closed by a combination of all these and by the process of wound contracture. During wound contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. In the maturation and remodeling phase, Collagen is remolded and realigned along tension lines and cells that are no longer needed are removed by apoptosis[26].

The results obtained in the present study suggested that treatment of rat excision wound with Test I gel (ethanolic extract of *C. indica* fruits) has accelerated wound healing process than Test II gel (aqueous extract of *C. indica* fruits). Treated excision wound showed an increase rate of wound contraction, leading to faster healing as confirmed by the increased healed area when compared to control group. Breaking strength was measured to confirm the wound healing activity of ethanolic extract gel of *C. indica* fruits. The increase in breaking strength of treated wounds may be due to increase in collagen concentration and stabilization of fibres. The result showed that treatment with ethanolic extract gel of *C. indica* fruits may have beneficial influence on the various phases of wound healing like fibroplasias, collagen synthesis and wound contraction resulting in faster healing.

Phytochemical studies of *C. indica* revealed that it contains cucurbitacin B the major constituents present in all the parts of plant. It also contains flavonoids, glycosides, mucilage, saponins, β-carotene, β-amyrin, β-sitosterols, cephalandrines A and B, lycopene, cryptoxanhin and apo-6'-lycopental, carbohydrates and proteins. Fruits also reported taraxerone, taraxerol and 24R-24-ethylcholest-5-en-3β-ol glucoside[27- 28- 29].

Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence the drug that inhibiting lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis[30]. Tannins[31], Flavonoids[32], triterpenoids[33], and saponins[34], are also known to promote the wound healing process mainly due to astringent, anti-oxidant and anti-microbial property, which seems to be responsible for wound contraction and increase rate of epithelization.

Thus wound healing potency of *C. indica* may be attributed to the phytoconstituents present in it, which may be either due to their individual or additive effect that fastens the process of wound healing. Between the two extract studied, ethanolic fruit extract was found to possess better wound healing property. Further investigations are necessary to determine the bioactive constituents present in the extract responsible for wound healing activity. The result of this work shows that formulating *C. indica* extract into a gel is effective in wound repair and provides scientific evidence to some of ethnomedicinal properties of *Coccinia indica* (Wight and Arn).

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