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Wound repair and regenerating effect of ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum camphora* leaves in wistar albino rats

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ABSTRACT

The leaves of *Cinnamomum camphora* are used in folk medicine for treatment of several skin disorders, anti-inflammatory disorders, various antimicrobial diseases and antioxidant activities. The aim of our study was to find the active fraction from ethanolic extract of C. *camphora* leaves responsible for wound healing activity in rats. The wistar albino rats were made wounded by the excision, incision and dead space wound on back side of rats. The ethylacetate soluble fraction of ethanolic extract of leaves of *Cinnamomum camphora* was applied topically in excision wound model while in incision and dead space wound model the Ethyl acetate soluble fraction (100 mg/kg) was give orally for 16 days. In the excision wound model the wound area and day of epithelization both were significantly decreased Ethyl acetate soluble fraction treated rats. In incision wound model the significantly higher tensile strength was observed in rats treaded orally with ethyl acetate soluble fraction. There were significant increase in weight of wet & dry granulation tissue with increased amount of hydroxyproline, collagen and elastin was observed in treated rats by ethyl acetate soluble fraction. The results suggested that the ethyl acetate soluble fraction of ethanolic extract of leaves of *Cinnamomum camphora* can be beneficial in treatment of wound healing in wistar albino rats.

Keywords: *Cinnamomum camphora,* ethanolic extract, anti-inflammatory

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INTRODUCTION

Wound is a rupture in the epithelial integrity of the skin which resulted from violence or trauma and may be followed by disruption of the structure and function of underlying normal tissue.[1] It is a clinical entity and is as old as mankind, often considered as major problem in clinical practice.[2] Each year, millions of people experience burns, suffer from chronic wounds, or have acute wounds that become complicated by infection, dehiscence or problematic scarring.[3] Wound healing is any process which acts, or induced to act, to restore the integrity of the damaged tissues in order to replace lost tissues.[4] It encompasses the activity of an intricate network of blood cells, cytokines, and growth factors which ultimately leads to the restoration to normal condition of the injured skin or tissue.[5]

Many factors can interfere with wound healing process, thus causing improper or impaired wound healing. The factors include poor nutrition, insufficient oxygenation, infection, prolonged inflammation, age, diabetes and other diseases, drugs, smoking, alcoholism, depression and others.[6]

Impaired wound healing can result into severe morbidity leading to long hospitalization of patients. The aim of treating wounds is to shorten the time taken for healing and to reduce risks of undesired complications.[7] Research on wound healing agents is one of the developing areas in modern biomedical sciences and many studies have been conducted using different wound healing models. Many traditional practitioners across the world have valuable information of many plants for treating wounds and burns. The presence of bioactive constituents in plants has urged researchers to screen medicinal plants with a view to determine potential wound healing activities and isolate chemical entities associated with wound healing.[8]

Several indigenous drugs have been described in folkloric Indian medicine for the management of cuts, bruises, burns and wounds. One of them, *Cinnamomum camphora leaves*, commonly known as Kapur tree, is an herb that grows in the fields of India. The leaves of this plant contain Tannins, Flavonoids, Saponins.[9] Aqueous and methanolic extract of the leaves has long been used in traditional medicine to kill microorganisms and provide antioxidant activities.[10]

Literature survey showed that there is no significant scientific work done on wound healing property of *Cinnamomum camphora* in normal animals.

The present study demonstrates the wound healing effect of crude extract and fractions of the leaves of *Cinnamomum camphora* using a host of wound models in wistar albino rats.

MATERIALS AND METHODS

Plant Material: The leaves of *Cinnamomum camphora* were purchased from herbal drug supplier of Mandsaur (M.P.) and authenticated in Govt. Herbal Medical College's Botanist at Mandsaur (M.P.) India.

Preparation of extract and fraction: Dried leaves of *Cinnamomum camphora* were extarced with ethanol by successive solvent extraction technique by using soxhlet apparatus for 72 hrs. The ethanolic extract was dried and suspended in water and fractionized with ethylacetate and dried under vaccume and stored in glass container for further use.

Animals: Wistar albino rats of either sex weighed between 120-150 gm were used for the wound healing activity. The animals were housed in central animal house faclity of B. R. Nahata College of Pharmacy-SIRO at controlled standard housing conditions of CPCSEA for temperature, water and feed. All experimental protocols were approved by Institutional animal ethical committee (IAEC).

Preparation of ointment of fractions: The ethyl acetate fraction of ethanolic extracts (10 % w/w) of the dried leaves of *Cinnamomum camphora* well triturated in pistal mortar with stearic acid ointment base. and used further in excision cutaneous wound healing model in wistar albino rats.

Excision wound healing model in rats: Animals were anaesthetized with slight vapour inhalation of di-ethyl ether and the back side of each rat was shaved. Excision wounds sized 300 mm2 and 2 mm depth were made by cutting out piece of skin from the shaven area. The entire wound was left open. Animals were closely observed for any infection and those which showed any sign of infection were separated, excluded from study and replaced. Wound areas were measured on days 0, 4, 8 and 16 for all groups, using a transparency sheet[11] and a permanent marker. Recording of wound areas were measured on graph paper and % wound closure was calculated by formula.[12] The day of scar falling, after wounding without any residual raw wound was considered as the day of epitheliazation.[13,14]

Treatment Groups: For excision wound model:

1. Group I (NC): Normal Control; Normal rats topically treated with Plane stearic acid ointment.

2. Group II (CC-EOA): Fraction treated; topically treated with ointment of ethyl acetate soluble fraction of ethanolic extract of leaves of *Cinnamomum camphora* (100 mg/kg).

3. Group III (PC): Positive Control; rats topically treated with ointment of Soframycin Antibiotic cream

Incision wound healing activity in rats: Animals were anaesthetized with slight vapor inhalation of di-ethyl ether and the back side of each rat was shaved. A longitudinal paravertebral incision of six centimeters in length was made through the skin and cutaneous muscle on the back in anesthetized rats. After the incision, surgical sutures were applied at intervals of one centimeter. The wounds were left undressed (day 0). The sutures were removed on the 8th post wound day and the application of extract was continued. The skin-breaking strength was measured on the 11th day by tensiometer.[15,16]

Treatment Groups: For Incision wound model:

1. Group I (NC): Normal Control; Normal rats treated with plane vehicle of 0.5 % w/v sodium CMC orally

2. Group II (CC-EA): Fraction treated; treated with 100 mg/kg of ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum camphora* suspended in 0.5 % w/v sodium CMC suspension orally.

Dead space wound healing activity in rats: Animals were anaesthetized with slight vapour inhalation of diethyl ether and the back side of each rat was shaved. Dead space wounds were inflicted by implanting sterile cotton pellets (10 mg each), one on left side in the groin and axilla on the ventral surface of each rat. On the 11th postwounding day, the granulation tissue formed on the implanted cotton pellets was carefully removed under anesthesia. After noting the weight of the granulation tissue, the tissue was dried at 60ºC for 12 hr, and the dry granulation tissue weight was recorded.[17] This dried tissue was further used to estimate hydroxyproline[18], collagen[19] and elastin[20] level in skin of normal srats.

Treatment Groups: For dead space wound model:

1. Group I (NC): Normal Control; Normal rats treated with plane vehicle of 0.5 % w/v sodium CMC orally

2. Group II (CC-EA): Fraction treated; treated with 100 mg/kg of ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum camphora* suspended in 0.5 % w/v sodium CMC suspension orally.

Biochemical analysis: At the end of experiments the wound area, % wound closure and day of epithelization was recorded in excision wound model7. In incision wound model the tensile strength was measured. In dead space wound model the weight of wet & dry granulation tissue, amount of hydroxy- proline, collagen and elastin were measured.

Statistical analysis: The data were expressed in Mean±SEM and statistically analyzed by one way analysis of variance followed by dunet's test. $P<0.05$ considered as significant.

RESULTS:

Effect on wound parameters of excision and incision wound model:

There were significant increases in wound healing parameters during treatment with ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum camphora* as compared to control groups of normal rats. Effect on wound parameters of excision and incision wound model: As shown in Table No. 1, the effect of ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum camphora* leaves on wound area; % wound closure and day of epithelialization in excision wound model and tensile strength in incision wound model in normal rats. The ethyl acetate fraction treated rats showed significant increase in % wound closure and decrease in wound area on 16th day of treatment. The day of scar falling i.e. epithelization was decreased. In incision wound model the tensile strength of ethyl acetate fraction treated rats was found increased with comparison to normal control rats.

Effect on wound parameters of dead space wound model:

As shown in Table No. 2, the effect of ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum camphora* leaves on wet & dry weight of granulation tissue, amount of hydroxyproline, collagen and elastin. In dead space wound model the weight of wet & dry granulation tissue was significantly increased with significant increase in level of hydroxyproline, % collagen and % elastin in the ethyl acetate fraction treated rats with comparison to normal control rats.

Table 1: Excision Wound model parameters

S. No.	Parameters	Group-I Normal Control (%)	Group-II CCEA (Ethylacetate soluble fraction of Ethnaolic Extract) Treated (%)	Group-V Positive Control (%)
1	% Wound Closure	84.16± 0.780	96.53± 0.307***	97.86 ± 0.145 ***
2	Wound Area Final (mm^2)	49.17± 2.120	10.83± 1.014***	04.21 ± 3.148 ***
3	Epithelization (Days)	26.33± 0.557	13.50± 0.991***	11.02± 0.654 ***

The values are in mean ± SEM, ** Very Significant P < 0.001, *Significant P < 0.05

Table 2: Incision and dead space wound parametes

S. No.	Parameters	Group-I Normal Control	Group-II CCEA (Ethylacetate soluble fraction of Ethnaolic Extract) Treated (%)
1	Tensile Strength (gm/mm^2)	232.4± 4.676	323.7± 1.911***
2	Wet Granulation Tissue Wt. (mg)	222.2± 3.049	341.5± 3.233***
3	Dry Granulation Tissue Wt. (mg)	55.33± 1.453	111.3± 1.856***
4	Hydroxy-proline (µg/ml)	5.575± 0.080	8.949± 0.065***
5	% Collagen	41.59± 0.602	66.76± 0.485***
6	% Elastin	242.0± 3.508	388.4± 2.825***

The values are in mean ± SEM , ** Very Significant P < 0.001

DISCUSSION:

The present investigation describes some unique features of the leaves extract from the plant *Cinnamomum camphora* with respect to its potential wound healing capacity in rats. Plant products are potential wound healing agents, and largely preferred because of their widespread availability, non-toxicity, absence of unwanted side effects, and effectiveness as crude preparations. Various activities were conducted in this study to evaluate the potential of *Cinnamomum camphora* a wound healing agent. One such activity is the phytochemical screening test. The phytochemical results reveal the presence of tannins, alkaloids, reducing sugars and steroids[9] in the ethylacetate soluble ethanolic leaves extract. The constituents of the leaves extract, such as tannins, terpenoids and alkaloids, may play a major role in the wound healing process observed in this study, however, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

Wound contraction, a part of the proliferative phase of wound healing, occurs through the centripetal movement of the tissues surrounding the wound, which is mediated by myofibroblasts. The increased wound contraction in the treated group may be due to the enhanced activity of

fibroblasts by ethylacetate soluble ethanolic leaves extract of *Cinnamomum camphora*.

A significant increase in collagen content due to enhanced migration of fibroblasts and epithelial cells to the wound site was observed during the wound healing process in the treated group. A close examination of granulation tissue sections revealed that tissue regeneration was much quicker in the treated group compared to that in control wounds.

Early dermal and epidermal regeneration in the treated group confirmed that the ointment containing the ethylacetate soluble ethanolic leaves extract had a positive effect toward cellular proliferation, granulation tissue formation, and epithelialization. Incomplete epithelialization with less extracellular matrix synthesis was observed in control rats. Clumps of degenerating neutrophils, necrotic changes, and the persistence of inflammatory exudates in the upper dermis with loss of epidermis were also observed up to day 16. The treated rats showed marked epithelialization, a moderate amount of extracellular matrix synthesis and new blood vessel formation.[21]

CONCLUSION

The results obtained in the present study clearly indicate that the ethylacetate soluble fraction of ethnanolic extract of leaves of *Cinnamomum camphora* are having significant wound healing activity in rats. The wound healing effect of ethylacetate soluble fraction of ethnanolic extract of leaves of *Cinnamomum camphora* may be due to the presence of more than one active principles mentioned above. Further pharmacological and biochemical investigation will clearly elucidate the mechanism of action and will be help full in projecting this plant as an therapeutic target in wound healing and other diseases.

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