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Influence of *Cinnamomum camphora ethanolic extract* on Biophysical and Biochemical Parameters of Cutaneous Wounds in Rats

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ABSTRACT

The ethanolic extract of *Cinnamomum camphora* leaves (100mg/kg/day for 16 days) was evaluated for its wound healing activity in rats using excision, incision and dead space wound models. Extract treated animal's exhibit 95% reduction in wound area when compared to controls which was 84%. The extract treated wounds are found to epithelize faster as compared to controls. The wet and dry granulation tissue weight including Hydroxyproline, elastin and collagen tissue content increased significantly (P< 0.001) when compared with control.

Keywords: Excision wound, Dead space wound, incision, Hydroxyproline, elastin, collegan

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INTRODUCTION

Healing of wounds, a fundamental response to tissue injury occurs by a process of connective tissue repair. A fibrous scar is the end product of this process, the pre-dominant constituent of which is collagen. Collagen and other components of the ground substance are synthesized by the highly vascular granulation tissue that is formed within the wound space. Collagen provides strength and integrity to the dermis[1]. Delayed wound healing is a condition which is known to be associated with a variety of connective tissue abnormalities. The collagen content of the skin is decreased as a result of reduced biosynthesis and/or accelerated degradation of newly synthesized collagen. These abnormalities contribute to the impaired wound healing observed in patients[2].

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.[3] Aqueous and methanolic extract of the leaves has long been used in traditional medicine to kill microorganisms and provide antioxidant activities.[4] Literature survey showed that there is no significant scientific work done on wound healing property of *Cinnamomum camphora* in normal animals. Hence an attempt was made to investigate influence of *Cinnamomum camphora* on Biophysical and Biochemical Parameters of Cutaneous Wounds in albino Rats.

EXPERIMENTAL

Plant material:

Cinnamomum camphora leaves were collected locally from herbal drug supplier of Mandsaur district, Madhyapradesh, India.

Animals

Wistar rats weighing 150–180 g were used. The animals were maintained with pelleted food (Purina), while tap water was available *ad libitum*. The procedures involving animals and their care conformed to the international guidelines, Principles of Laboratory Animals Care.

Preparation of the aqueous extract

Dried fine powder of *Cinnamomum camphora* leaves was Soxhlet-extracted with ethanol as solvent. The ethanolic extract filtered on Whatman paper and lyophilized gave a green residue stored at RT until use.

Acute Toxicity Studies

The acute toxicity study was carried out in adult female albino rats by "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No.420. Fixed dose method as in Annex 2d: Test procedure with a starting dose of 2000 mg/Kg body weight was adopted. The animals were fasted overnight and next day extract of the plant *Cinnamomum camphora* leaves (suspended in 0.5 % w/v sodium CMC) was administered orally at dose level 2000 mg/kg. Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally for mortality after 24 hour till 14 days. The observations were tabulated according to 'Irwin's Table'.[5,6]

Surgical procedures and treatment

Two types of wounds on the Zero day namely excision and dead space wounds were created in experimental rats.

Excision wounds[7]

Excision wounds were used for the study of rate of contraction of wound and epithelization, All wounds were of full-thickness type extending up to the adipose tissue. Animals were anaesthetized with 120 mg/kg (i.p.) of ketamine hydrochloride and the right side of each rat was shaved. Excision wounds sized 300 mm^2 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. Animals were divided into three groups of 6 animals in each. The normal controls (Group 1) were applied with Vaseline, experimental controls (Group 2) were applied with the ethanolic extract of *Cinnamomum camphora leaves*, and the positive controls received an application of soframycin ointment (Group 3). The treatment was done topically in all the cases. The extract was applied at a dose of 100mg/kg/dayfor 10 days. Wound areas were measured on days 1, 5 and 11 for all groups, using a transparency sheet and a permanent marker. Recording of wound areas were measured on graph paper. The day of scar falling, after wounding without any residual raw wound was considered as the day of epitheliazation.

Incision Wound[8]

Animals were anaesthetized with slight vapour 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 and oral treatment was started. The sutures were removed on the 8th post wound day and the treatment was continued. The skin-breaking strength was measured on the 11th day by tensiometer.

Dead space wounds[9]

Dead space wounds wound was used for the study of biochemical parameters and of the rate of tissue formation. Dead space wounds were created by implanting sterile cotton pellets (10 mg each), one on either side in groin and axilla on ventral surface of each rat by technique of D' Arcy *et al* as described by turner. Animals were divided into two groups of 6 animals in each. The normal controls (Group 1) were provided plain water only, experimental controls (Group 2) were given *Cinnamomum camphora leaves ethanolic* extract orally in a dose of 100mg/kg/day for 10 days. On the 10^{th} post wounding day the granulation tissue formed on the implanted cotton pallets was carefully removed under anesthesia. After noting the wet weight of granulation tissue the tissue was dried at 60ºC for 12 hrs and the dry granulation tissue weight was recorded to the dried tissue 5 ml 6N HCl was added and kept at 110ºC for 24 hrs. The neutralized acid hydrolisate of the dry tissue was used for the determination of hydroxyproline[10]. The collagen and elastin were calculated by level of hydroxyproline using reference formula[11].

Statistical analysis: The means of wound area measurement between groups at different time intervals were compared using one-way ANOVA, followed by Tukey's tests. One-way ANOVA was used to examine the mean differences in wound healing between the groups in excision and dead space wound models. Data was analyzed using the Graph Pad Software (5.0- demo version) and *P* value of < 0.05 were considered to be significant.

RESULTS:

In acute toxicity studies the extract in dose up to 2 g/kg body weight did not produce any signs of toxicity or mortality. The animals were physically active and consumed food and water in a regular way. No abnormal behavior was noticed. Significant increase in wound healing activity was observed in leaf extract treated rats. In excision wound model, animals of group 2 and 3 showed a decrease in the epithelialization period and an increased percentage of wound contraction when compared with the animals of group 1, (Table 1). On day 11 the extract treated normal animals (Group 2) and positive control (Group 3) showed high wound contraction compared with normal control groups animals (Groups 1) (*P<0.001*). The wound contraction results of extract treated animals were comparable with positive controls. The *Cinnamomum camphora leaves ethanolic* extract treated animals showed highest wound contraction.

In the dead space wound model (Table:2), the extract treated animals in groups 2 showed significantly higher levels of hydroxyproline, elastin and collagen as compared with animals in the normal controls groups (Group 1) (*P<0.001*). A significant increase was also observed in dry and wet weight of granulation tissues in the animals treated with the ethanolic extract of *Cinnamomum camphora leaves* (*P<0.001*). Overall the weights of animals were did not differ for any of the study groups.

In the animals that did not receive the leaf extract treatment, the wound appered tobe hard and crusty with undermined margins and were generally unclean with a biofilm glaze of the surface. In contrast the wounds in the animals treated with extract were clean and showed bright and red healthy granulation tissue. The wounds treated with soframycin showed the healthy granulation tissue.

Table 1: Excision Wound model parameters

S. No.	Parameters	Group-I Normal Control (%)	Group-II Ethnaolic Extract Treated (%)	Group-V Positive Control (%)
1	% Wound Closure	84.11± 0.876	95.45± 0.499 ***	98.84 ± 0.258 ***
2	Wound Area Final (mm^2)	47.67±2.525	13.83± 1.558 ***	05.32 ± 2.412 ***
3	Epithelization (Days)	24.17± 0.909	16.67± 0.333 ***	11.86± 0.542 ***

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 Normal Treated
1	Tensile Strength (gm/mm^2)	238.5± 3.146	309.7± 1.713 ***
2	Wet Granulation Tissue Wt. (mg)	219.0± 3.120	329.2± 2.455 ***
3	Dry Granulation Tissue Wt. (mg)	56.50± 1.118	102.0± 2.543 ***
4	Hydroxy-proline (µg/ml)	5.418 ± 0.119	8.628± 0.112 ***
5	% Collagen	40.42± 0.892	64.37± 0.837 ***
6	% Elastin	235.2± 5.190	374.5± 4.872 ***

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

DISCUSSION

Delayed wound healing is known to be associated with a variety of alterations in connective tissue metabolism, as a result of which patient face the problem of poor wound healing. Loss of collagen observed in wound tissue may be due to decreased levels of synthesis or enhanced catabolism of newly synthesized collagen, or both[12]. As *Cinnamomumcamphora leaves* was reported to cause antimicrobial and antioxidant effects, it was felt that it would be interesting to study its influence on the healing of wounds in normal rats. Results obtained in the present study suggest that treatment of wounded rats with *Cinnamomum camphora leaves ethanolic* extract may have a beneficial influence on wound healing.

Collagen and elastin is the predominant extracellular protein in the granulation tissue of a healing wound and there is a rapid increase in the synthesis of this protein in the wound area soon after an injury. In addition to providing strength and integrity to a tissue matrix, collagen also plays an important role in haemeostasis. Subsequent epithelialization also requires collagen. In the present study, we have examined the influence of *Cinnamomum camphora leaves ethanolic* extract on the collagen and elastin content in granulation tissues of healing full-thickness wounds in rats. Treatment of wounds with *Cinnamomum camphora leaves ethanolic* extract increased the maximum levels of collagen and elastin in the granulation tissue, as compared to the untreated normal control rats.

Glycosaminoglycans and proteoglycans are synthesized by fibroblasts in the wound area. These substances form a highly hydrated gel-like ground substance, a provisional matrix on which collagen fibres are embeded. As collagen accumulates, hexosamine levels decrease[11]. Treatment with *Cinnamomum camphora leaves ethanolic* extract increases the content of ground substance in the granulation tissues. It may be seen that the decrease in hexosamine content was associated with a concomitant increase in collagen content.

The protein and DNA content of granulation tissues indicate the levels of protein synthesis and cellular proliferation. Higher protein and DNA contents (compared to the untreated controls) of the treated wounds suggest that *Cinnamomum camphora leaves ethanolic* extract, through an as yet unknown mechanism, stimulates cellular proliferation. The collagen/DNA ratio of the granulation tissues also suggests that *Cinnamomum camphora leaves ethanolic* extract gel may increase the synthesis of collagen per cell.

The collagen molecules synthesized are laid down at the wound site and become crosslinked to form fibres. Wound strength is acquired from both, remodelling of collagen, and the formation of stable intra- and inter-molecular crosslinks. Since incisional wounds treated with the *Cinnamomum camphora leaves ethanolic* extract showed greater tensile strength, it may be inferred that it not only increases collagen synthesis per cell, but also aids in crosslinking of the protein. *Cinnamomum camphora leaves ethanolic* extract treated wounds also showed an increased rate of wound contraction, leading to quicker healing as confirmed by decreased period of epithelialization when compared to untreated control wounds.

The present study demonstrates that the *Cinnamomum camphora leaves ethanolic* extract accelerates wound healing in normal animals. The results suggest that *Cinnamomum camphora leaves ethanolic* extract treatment may have a beneficial influence on the various phases of wound healing like fibroplasia, collagen synthesis and contraction resulting in faster healing. It is quite possible that the enhanced healing of wounds in rats by *Cinnamomum camphora leaves ethanolic* extract is a result of its antimicrobial[13] and antioxidant[14,15] and/or its capacity to stimulate fibroblast function during the healing process.

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