# EFFECT OF CINNAMOMUM TAMALA LEAVES EXTRACTS ON COLLAGEN CROSS-LINKING AND TENSILE STRENGTH IN WOUND HEALING IN DIABETIC RATS

**Rupesh Soni[1], Amit Vairagi[2] and D. N. Srivastava [2]**

[1]. Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur-342003 (Raj)
[2]. Department of Pharmacology & Toxicology, B. R. Nahata College of Pharmacy, Mandsaur-458001 (Madhya Pradesh)
E mail id: amitvairagi86@gmail.com

## ABSTRACT

OBJECTIVE: Diabetes can result in development of several complications, including delayed wound healing. Diabetic wounds are slow, non-healing wound that can persist for weeks despite adequate and appropriate care. Cinnamomum tamala leaves are used to treat various diseases like wound healing and diabetes . In Present study different fractions of *Cinnamomum tamala* leaves extract used to investigate, their effect on collagen cross-linking and tensile strength in wound healing in streptozotocin induced diabetes in rats.

METHODS: Wistar albino rats (150-200gm) were made diabetic by single intraperitoneal injection of streptozotocin (45mg/kg). Incision and dead space wound were implemented back side of animals. *Cinnamomum tamala* leaves extract was given orally at a dose of 100 mg/kg for 14 days in incision and dead space wound healing models. After 14 days tensile strength, wet & dry weight of granulation tissue and hydroxylproline level was measured.

RESULTS: In incision wound model extract treated rats showed significant increase in wound breaking or tensile strength. The increase in tensile strength shows deposition of collagen and collagen cross- linking as well as tensile strength of skin. In Dead space wound healing models extract treated rats showed significant increase in wet & dry granulation tissue weight and hydroxyproline level. This shows more synthesis of collagen at damaged area or wound because hydroxyproline is the main constituent for collagen synthesis.

CONCLUSION: From above study it is concluded that extract of *Cinnamomum tamala* leaves significantly increases collagen cross-linking and tensile strength in wound healing in diabetic rats.

## INTRODUCTION

Diabetes is a syndrome of disordered metabolism , due to the combination of hereditary and environmental causes. Diabetes mellitus refers to the group of diseases that lead to high blood glucose level due to defects in either insulin secretion or insulin action. The acute sign of diabetes: Polyurea, resulting compensatory thirst and increase fluid intake, blurred vision, weight loss, lethargy, and changes in energy metabolism.

Diabetes develops due to a diminished production of insulin (in type 1) or resistance to its effect (in type 2 and gestational) and both lead to hyperglycaemia i.e. increase blood glucose level.

**Diabetes complications:-**

When glucose remains high in the blood for long time various protein became glycosylated.it alters tissue structure and protein function. There various complications associated with the diabetes like:- Angiopathy vascular damage (like macroangiopathy, microangiopathy, infection, hyperosmola, ketoacidosis etc.).

Cardiovascular abnormalities manifestations, Skin condition associated with diabetes

Atypical, asymptomatic and contribute to sudden death

**Wound healing in Diabetes**

Patients with diabetes often have wounds that are difficult to heal. The initial barrier to healing is an increased blood glucose level , which causes the cell walls to become rigid , impairing blood flow through the critical small vessels at the wound surface , impeding red blood cell permeability and flow . Impairment release of oxygen by hemoglobin results in oxygen and nutrient deficits in the wound. A less optimal immune function also contributes to poor wound healing in patients with diabetes. When blood glucose levels are persistently elevated , chemotaxis and phagocytosis are compromised. Chemotaxis is the process by which white cells are attracted to the site of infection , while phagocytosis is the ingestion of bacteria by white cells. Both processes are important in controlling wound infections.

Diabetic infections take a longer time to heal because of delayed macrophage introduction and diminished leukocyte migration, which causes a prolonged inflammatory phase in the wound healing cascade. Protein - calorie malnutrition and the resultant body composition changes are an additional consideration in wound healing . Patients with diabetes often have a progressive loss of lean body mass, which is replaced with a metabolically inactive fat mass. The absence or deficiency of insulin in diabetes mellitus causes impaired metabolism of carbohydrates , fat and proteins , which are necessary for cellular activities and tissue synthesis in wound healing. Insulin is required for glucose to enter cells as to provide a source of energy for uptake of amino acids to synthesize proteins and for inhibition of adipose tissue lipolysis. Glucose is also needed to supply energy for fibroblastic and polymorphonuclear (PMN) activities during wound healing. Altered glucose metabolism , as seen in diabetes mellitus, leads to defective metabolism of these nutrients and reduces fibroblastic / PMN activity , causing impaired wound healing.

Plant *Cinnamomum tamala* leaves are used in case of all type of diabetes. Major chemical constituents found like Eugenol,Linalool, Cinnamaldehyde, Cinnamic acid. It has been shown antibacterial, hypoglycaemic, antioxidant, antifungal, antidermatophytic, antiviral, antimicrobial, hypolipidemic action in previous studies.

## MATERIALS AND METHODS

**Plant material:-***Cinnamomum tamala* leaves.

**Chemicals:-**Streptozotocin, glucose, gluco strips, mupirocin ointment (2%w/w) , diethyl ether, ethanol,sterilized cotton, hydroxyl proline, ehlrich reagent.

**Extraction:-** Leaves are coarsely powdered , weigh and filled in soxhlet apparatus for extraction. Ethanol, Pet. Ether, Chloroform and Acetone solvent were used for extraction . The % yield was calculated for each extract after drying.

**Induction of diabetes:[8]**

Wistar albino rats (150-200gm) were made diabetic by a single injection of streptozotocin (45mg/kg i.p.) prepared in normal saline after overnight fasting. Blood was drawn from the tail vein 72 hr the injection and the glucose level were estimated using glucometer (Accu-Chek). Wounds were made on the rats showing elevated blood glucose (≥250mg/dl). Blood glucose levels were estimated at the time of creation of excision wounds.

**Incision wound healing in diabetic rats:[1]**

Animals were divided into six groups, (six animals each). All animals of six groups were anesthetized with anaesthetic ether, and a paravertebral long incision of 4 cm length was be made through the skin and cutaneous muscle at a distance about 1.5 cm from the middle on right side of the depilated back. After the incision was made, the two ends of the wound was drawn closer and sutured at 0.5 cm intervals using sterile surgical thread (No. 000) and a curved needle (No.11). *Cinnamomum tamala* leaves extract was given orally at a dose of 100 mg/kg for 14 days. Sutures were removed on the 9th post wound day and the application of drug was continued. The skin breaking strength was measured on the 14th day by tensiometer.

**Grouping of animals:**

Group1: Normal control
Group2: Diabetic wound control
Group3: Diabetic wound with Ethanolic extract of CT
Group4: Diabetic wound with Pet. Ether extract of CT
Group5: Diabetic wound with Acetone extract of CT
Group6: Diabetic wound with Chloroform extract of TC

**Dead space wound healing in diabetic rats:[2]**

Dead space wounds were inflicted by implanting sterile cotton pellets (10mg each) one on left side in the groin and axilla on the ventral surface of each rat. *Cinnamomum tamala* leaves extract was given orally at a dose of 100 mg/kg for 14 days. On the 14th post wounding day, the granulation tissue formed on the implanted cotton pellets were carefully removed under anesthesia. After noting the weight was recorded. To the dried tissue 5 ml tissue was added and kept at 110.c for 24 hr. the neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyproline.

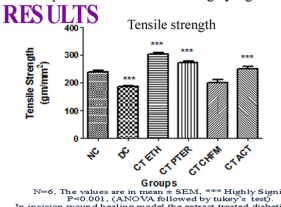
**The assay procedure for hydroxyproline content:[3]**

Tissues were dried in a hot air oven at 60-70°C to constant weight and were hydrolyzed in 6N HCL at 130°C for 4 hr in sealed tubes. The hydrolysate was neutralized to ph 7.0 and was subjected to chloramines –T oxidation for 20 min the reaction was terminated by addition of 0.4 ml perchloric acid and color was developed with the help of Ehrlich reagent t 60°C and measured at 554 nm using autoanalyzer.

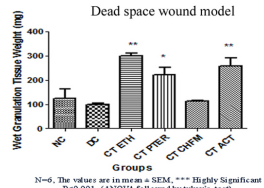
**Statical analysis**

The statistical was done by Graph pad prism software demo version 5 and result was expressed in Mean±SEM and data was compared by one- way ANOVA followed by tukey's test and $p<0.05$ considered as significant, p, 0.01 very significant and $p<0.001$ is considered as highly significant.

## RESULTS




N=6, The values are in mean ± SEM, *** Highly Significant P<0.001, (ANOVA followed by tukey's test).
In incision wound healing model the extract treated diabetic animals showed higher tensile strength as compared to control animals.

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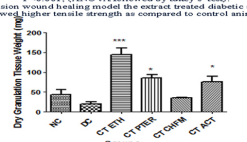
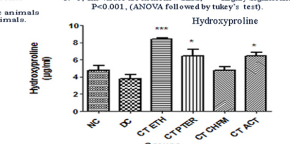



N=6, The values are in mean ± SEM, *** Highly Significant P<0.001, (ANOVA followed by tukey's test).

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## DISCUSSION & CONCLUSION

Collagen is the predominant extra cellular 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. Deposition of newly synthesized collagens at the wound site increases the collagen concentration per unit area and hence the tissue tensile strength[4]. Treatment of wounds with *Cinnamomum tamala* extract increased the maximum levels of collagen in the granulation tissue, as compared to the untreated control animals.

The increased weight of the granulation tissues also suggests that *Cinnamomum tamala* 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 incision wounds treated with the *Cinnamomum tamala* 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. Recent studies have shown that phytochemical constituents like flavonoids[5] and triterpenoids[6] are known to promote the wound healing process mainly due to their astringent and antimicrobial properties, which appear to be responsible for wound contraction and increased rate of epithelialization.

The present study demonstrates that the *Cinnamomum tamala* leaves extract accelerates wound healing in experimental rats. The results suggest that *Cinnamomum tamala* extract treatment may have a beneficial influence on the various phases of wound healing like fibroplasia , collagen synthesis and contraction resul -ting in faster healing.

Postoperative wounds are commonly known to be complicated by infection. Earlier studies have shown that antimicrobial activity of various plants supports the wound healing. Further the plant has been evaluated for antimicrobial activity by previous researchers [7] ; hence present research supports traditional claims of the plant in wound healing.

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