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

ABSTRACT
Diabetes can result in development of several complications, including delayed wound healing. Diabetic wounds are slow, non-healing wounds that can persist for weeks despite adequate and appropriate care. Cinnamonum 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.

MATERIALS AND METHODS: Wistar rats (150-200g) were made diabetic by single intraperitoneal injection of streptozotocin (50mg/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 tissue proteoglycan level was measured.

RESULTS: In incision wound model treated rats showed significant increase in wound breaking or tensile strength. The increase in tensile strength showed deposition of collagen and collagen cross linking as well as tensile strength of skin. In Dead space wound healing models treated rats showed significant increase in wet & dry granulation tissue weight and wet & dry tensile strength of skin. Tissues treated with Cinnamomum tamala leaves extracts showed better hydroxyproline in the main component for collagen synthesis.

CONCLUSION: From the results 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: Polyuria, resulting compensatory thirst and increased 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 hyperglycemia i.e. increase blood glucose level.

RESULTS

DISCUSSION & CONCLUSION
Tissues were dried in a bath of ethylene glycol at -60ºC to constant weight and were hydrolyzed in 6N HCl, at 130ºC for 4 hr in sealed tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to chromatography - T orchestra for 20 min the reaction was terminated by addition of 0.4 M phosphate acid and color was developed with the help of Ehrlich reagent and measured at 546 nm using spectrophotometer.

Statistical analysis
The statistical was done by Graph pad prism software version 5 and result was expressed in Mean±SEM and data was compared by one way ANOVA L.Effect of thiamine t-test and p<0.05 is considered as significant, p 0.01 very significant and p<0.001 is considered as highly significant.

MATERIALS AND METHODS

Plant materials: Cinnamomum tamala leaves.

Chemicals: Streptozotocin, glascose strips, murine omentin (25k/Ω), diethyl ether, ethanol, sodium chloride, hydrogen peroxide, ethyl alcohol, propranolol.

Extraction: Leaves are coarsely powdered, weighed and filled in closed 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
Wistar albino rats (150-200g) were made diabetic by a single injection of streptozotocin (50mg/kg i.p.) prepared in normal saline after overnight fasting. Blood was drawn from the tail vein 72 hr for the injection and the glucose level were estimated using glucoseometer (Accu CHECK). Wounds were made on the rats showing elevated blood glucose (>250mg/dl).

Wound healing in diabetic rats
Animals were divided into six groups, (six animals each). All six groups of animals were anesthetized with anesthetic ether, and a paravertebral long incision of 1 cm length was made through the skin and cutaneous muscle at a distance of 1.5 cm from the spinous process. The wound was drawn closer and saturated 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. Natures were removed on the 9th post wound and the application of drug was continued. The skin breaking strength was measured on the 14th day by tension.

Grouping of animals

Group 1: Normal control

Group 2: Diabetic control

Group 3: Diabetic with Ethanolic extract of CT

Group 4: Diabetic with Pet. Ether extract of CT

Group 5: Diabetic with Acetic extract of CT

Group 6: Diabetic with Chloroform extract of CT

Dead space wound healing in diabetic rats

Dead space wounds were inflicted by implanting sterile cotton pellets (10mg each) on one side left 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 dined tissue 3 ml tissue was added and kept at 100°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

Tissues were dried in a bath of ethylene glycol at -60ºC to constant weight and were hydrolyzed in 6N HCl, at 130ºC for 4 hr in sealed tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to chromatography - T orchestra for 20 min the reaction was terminated by addition of 0.4 M phosphate acid and color was developed with the help of Ehrlich reagent and measured at 546 nm using spectrophotometer.

The statistical was done by Graph pad prism software version 5 and result was expressed in Mean±SEM and data was compared by one way ANOVA L.Effect of thiamine t-test and p<0.05 is considered as significant, p 0.01 very significant and p<0.001 is considered as highly significant.

REFERENCES
2. Turner B. A., "Streptozotocin in human disease - onset and control diabetes in rats via increased glucose release and prevention of age-related complications".