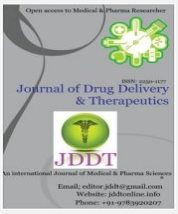
Available online on 25.12.2017 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research



Open Access Research Article

Wound Repair and Regenerating Effect of Eugenol Isolated from Ethyl Acetate Soluble Fraction of Ethanolic Extract of *Cinnamomum tamala* Leaves in STZ Diabetic Rats

Rupesh Soni[1], D.N. Srivastava[2]

[1]DDM College of Pharmacy, Gondpur Banehra (Upper)-177213, Tehsil - Amb, Distt. Una (H.P.), India

[2]B. R. Nahata College of Pharmacy, Mandsaur-458001 (M.P.), India

E-mail address: rupeshsoni77@gmail.com

ABSTRACT

Diabetes is a chronic hyperglycaemic disorder; leads to developed several complications including delayed wound healing after any injury. These non-healing wound ends up to organ or limb salvage. The available modern medications are not capable to fully control over these complications. There are several evidences that these complications can easily treated by using herbal of folklore medicines. The leaves of *Cinnamomum tamala* used by traditional peoples in the treatment of diabetes and associated wound healing. In our previous study we had found that the ethanolic extract of leaves of *Cinnamomum tamala* is most active in treatment of wound healing in diabetic rats. The aim of our study was to find the active isolated eugenol from ethyl acetate soluble fraction of ethanolic extract of *C. tamala* leaves responsible for wound healing activity in diabetic rats. The Wistar albino rats were made diabetic by single i.p. injection of Streptozotocin (60 mg/kg). The excision, incision and dead space wound were created on back side of rats. The eugenol isolated from ethyl acetate soluble fraction of ethanolic extract of leaves of *Cinnamomum tamala* was applied topically in excision wound model while in incision and dead space wound model the eugenol isolated from ethyl acetate soluble fraction (100 mg/kg) was give orally for 16 days. In the excision wound model the wound area and day of epithelisation both were significantly decreased eugenol isolated from ethyl acetate soluble fraction treated rats. In incision wound model the significantly higher tensile strength was observed in rats treaded orally with eugenol from 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 eugenol treated rats by ethyl acetate soluble fraction. The results suggested that the eugenol isolated from ethyl acetate soluble fraction of ethanolic extract of leaves of *Cinnamomum tamala* can be beneficial in treatment of wound healing in diabetic rats.

Cite this article as: Soni R, Srivastava DN, Wound Repair and Regenerating Effect of Eugenol Isolated from Ethyl Acetate Soluble Fraction of Ethanolic Extract of Cinnamomum tamala Leaves in STZ Diabetic Rats, Journal of Drug Delivery and Therapeutics. 2017; 7(7):235-237

INTRODUCTION:

Modern world is facing a critical health problem that is diabetes. The number of patients with diabetes and its complications increasing day by day and reached up to 220 million in this year [1]. Diabetes is a group of disorders characterized by hyperglycaemia resulting due to abnormalities in glucose metabolism[2]. Diabetes is associated with glycation of essential proteins and hormones, due to presence of high blood sugar level. In diabetic patient decrease in collagen content of skin can generates impaired and non healing abnormalities in wound or injured area [3]. Diabetic wounds are slow, non-healing wound that can persist for weeks despite adequate and appropriate care. Such wounds are difficult and tough to manage. The wound healing process is the sequence of repaiment of connective tissue including migration, inflammation, proliferation and differenciation of cells [4]. As per WHO the effective treatment of diabetes and its complications can be possible by using herbal or traditional medicines [5]. In our previous study we had found that the ethanolic extract of *Cinnamomum tamala* leaves has beneficial effect in healing of wounds in diabetic rats. The Ethyl acetate fraction of *Cinnamomum tamala* leaves showed presence of tannins and phenolic compounds which are having potent antioxidant activity. The oxidative stress is responsible for induction of diabetic complication. Hence in present study eugenol isolated from ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves was used to investigate wound repair and regeneration activity in diabetic rats.

MATERIALS AND METHODS:

The eugenol is isolated as active constituent from ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum tamala.* Wistar albino rats of either sex weighed between 120-150 gm were used for the wound healing activity. Induction of Diabetes: Rats were made diabetic by a single injection of Streptozotocin (60 mg/kg, i.p.) prepared in citrate buffer (0.1 M, pH 4.5) after overnight fasting. Blood was drawn from the tail vein 24 h after the injection and the glucose level was estimated by glucose oxidase method by using Accu-Chek Glucometer before and 72 hrs after STZ injection. Animals showed blood glucose level more than 250 mg/dl were selected for further cutaneous wound healing activity in diabetic animals. The eugenol isolated from ethyl acetate fraction of ethanolic extract (10 % w/w) of the dried leaves of *Cinnamomum tamala* well triturated in pastel mortar with steric acid ointment base and used further in excision cutaneous wound healing model in diabetic rats.

Excision wounds sized 300 mm^2 and 2 mm^2 depth were made by cutting out piece of skin from the shaven area. Wound areas were measured on days 0, 4, 8 and 16 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.

Excision wound model:

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

Group II (DC): Diabetic Control; Diabetic rats topically treated with Plane steric acid ointment.

Group III (DT): Diabetes Treated; Diabetic rats topically treated with ointment of eugenol of ethyl acetate soluble fraction of ethanolic extract of leaves of *Cinnamomum tamala* (100 mg/kg).

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.

Incision wound model:

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

Group II (DC): Diabetic Control; Diabetic rats treated with vehicle of 0.5 % w/v sodium CMC orally.

Group III (DT): Diabetes Treated; Diabetic rats treated with 100 mg/kg of eugenol of ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum tamala* suspended in 0.5 % w/v sodium CMCsuspension orally.

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 post-wounding 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. This dried tissue was further used to estimate hydroxyproline, collagenand elastinlevel in skin of normal and diabetic rats.

Dead space wound model:

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

Group II (DC): Diabetic Control; Diabetic rats treated with vehicle of 0.5 % w/v sodium CMC orally.

Group III (CtPii-EAC): Diabetic rats treated with 100 mg/kg of eugenol of ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum tamala* 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 epithelisation was recorded in excision wound model. 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 oneway analysis of variance followed by dunnett's test. $P<0.05$ considered as significant.

RESULTS AND DISCUSSION:

There was significant increase in wound healing parameters during treatment with eugenol isolated from ethyl acetate soluble fraction of ethanolic extract of dried leaves of *Cinnamomum tamala* as compared to control groups of normal and diabetic rats. Effect on wound parameters of excision and incision wound model as shown in Table 1, the effect of eugenol of ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves on wound area; % wound closure and day of epithelialization in excision wound model and tensile strength & blood glucose level in incision wound model in diabetic rats. The eugenol of 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. epithelisation was decreased with decrease in blood glucose level. In incision wound model the tensile strength of euegnol of ethyl acetate fraction treated rats was found increased along with decrease in blood glucose level with comparison to diabetic control rats.

Effect on wound parameters of excision and incision wound model: As shown in Table 2, the effect of eugenol of ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* 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 eugenol of ethyl acetate fraction treated rats with comparison to diabetic control rats.

In present study photochemical screening showed the presence of high amount of phenolics and tannin compounds in ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves. The Phenolics and tannins are the potent antioxidants reported in literature. Sharma et al, and Kar et al reported that ethanolic extract of *Cinnamomum tamala* leaves exhibits antihyperglycemic activity The high blood glucose level is responsible for delayed wound healing and eugenol isolated from ethyl acetate fraction treated rats showed significant decrease in blood glucose level during wound healing process.

Table 1: Effect of eugenol isolated from ethyl acetate fraction of ethanolic extract of Cinnamomum *tamala* treatment in excision and incision wound model.

S. No.	Groups	Wound Area (mm^2)	% Wound Closure	Day of Epithelisation	Tensile Strength (gm/mm^2)	Blood Glucose Level (mg/dl)
1.	Normal Control (NC)	54.83±0.94	82.23±0.38	24.67±1.05	233.0±3.92	74.00±3.04
2.	Diabetic Control (DC)	126.2±1.86*	59.32±0.62*	44.00±1.41*	157.8±2.11*	402.3±8.87*
3.	Diabetic Treated (DT)	5.55±0.47*	98.21±0.14*	10.83±0.40*	335.0±1.76*	70.33±2.95*

Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and diabetic treated groups were compared with DC. P value considered as P<0.001 Significant (*)

Table 2: Effect of eugenol isolated from ethyl acetate fraction of ethanolic extract of *Cinnaomum tamala* treatment in dead space wound model.

S. No.	Groups	Wet Granulation Tissue Wt. (mg)	Dry Granulation Tissue Wt. (mg)	Hydroxyproline (µg/ml)	% Collagen	% Elastin
1.	Normal Control (NC)	223.5±3.45	54.33±1.22	6.00±0.25	44.81±1.92	260.7±1.12
2.	Diabetic Control (DC)	223.5±3.45	35.50±1.23*	3.63±0.12*	27.10±0.89*	157.7±5.21*
3.	Diabetic Treated (DT)	223.5±3.45	124.5±2.39*	9.35±0.18*	69.76±1.39*	405.9±8.09*

Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and diabetic treated groups were compared with DC. P value considered as P<0.001 Significant (*)

CONCLUSION:

The isolated eugenol from ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves was evaluated for wound healing activity in diabetic rats. The all four phases (hemostasis, inflammation, granulation and remodelling) of wound healing studied by excision, incision and dead space wound models. The high blood glucose level is the root cause of delayed wound healing in patients of diabetes. The treatment of eugenol isolated from ethyl acetate soluble fraction promotes wound healing by decrease in blood glucose level, faster contraction of wound and increased granulation of tissue with increased tensile strength. This action may be due to anti diabetic, antioxidant and antimicrobial activities of phytoconstituents like phenolics and tannins which present in ethyl acetate soluble fraction of ethanolic extract of *Cinnamomum tamala* leaves. Further studies are needed to identify active faster wound healing activity with detailed mechanism of action.

REFERENCES:

1. Goodson WH,Hunt TK, Wound healing and the diabetic patient,Surg Gynecol Obstet 1979,149,600.
2. Kumar R, Kohli K, Kajal H. A study of drug prescribing pattern and cost analysis among diabetic patients in a tertiary care teaching institute in north India. Journal of Drug Delivery and Therapeutics, 2013; 3(2):56-61. doi:10.22270/jddt.v3i2.431
3. Nayak BS, Pereira L, Muhraj D, Wound healing activity of Carica papaya L. in experimentally induced diabetic rats. Indian J Exp Biol, 2007, 45,739-743.
4. Prasad V, Jain V, Girish D, Dorle AK, Wound-healing property of Momordica charantia L. fruit powder,J Herb Pharmacother,2006, 6,105-15.
5. Reddy KG & Enwemeka SC, A simplified method for the analysis of hydroxyproline in biological tissues,Clin Biochem, 1996, 29,225-229.