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Research Article

EFFECT OF ETHANOLIC EXTRACT OF *CINNAMOMUM TAMALA* LEAVES ON WOUND HEALING IN STZ INDUCED DIABETES IN RATS

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ABSTRACT

Objective: Diabetes is a chronic hyperglycemic metabolic disorder; leads to generates many complications including delay in wound healing after any injury. These non healing wound ends upto organ or limb salvage. The available modern allopathic medications are not able to full control over these complications. There are several evidences that complication associated with diabetes can easily be treated by using herbal or folklore medicines. The leaves of *Cinnamomum tamala* used by traditional peoples in the treatment of hyperlipidemia, diabetes, and associated wound healing problems. The aim of our study was to find the active extract from various extracts of *C. tamala* leaves responcible for wound healing activity in diabetic rats.

Methods: 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 ethanolic extract of *C. tamala* leaves was applied topically in excision wound model while in incision and dead space wound model the ethanolic extract of *C. tamala* leaves (100 mg/kg) was give orally for 16 days.

Results: In the excision wound model the wound area and day of epithelization both were significantly decreased in ethanolic extract of *C. tamala* leaves treated rats. In incision wound model the significantly higher tensile strength was observed in rats treaded orally with ethanolic extract treated. 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 ethanolic extract of *C. tamala* leaves

Conclusion: The results suggested that the ethanolic extract of leaves of *Cinnamomum tamala* can be used in treatment of dalayed wound healing in diabetic rats.

Keywords: *Cinnamomum tamala*, Collagen, Diabetes, Granulation, Hydroxyproline, Tensile strength, Wound healing

INTRODUCTION

Diabetes has become a most serious health problem for modern world. The number of patients with diabetes and its complications increasing day by day and reached upto 220 million in this year [1]. Diabetes is a group of disorders characterized by hyperglycemia, polyurea, polyphagia & polydypsia resulting due to abnormalities in glucose metabolism[2]. Diabetes is associated with glycation of essential proteins and enzymes of our body, due to presence of high blood sugar level. The advance glycation leads to formation of glycated proteins, which are abnormal proteins and reduces the normal functioning of body. This diabetes can generate many defects in tissue repair and regeneration including connective tissue abnormality. In diabetic patient decrease in collagen content of skin can generates impaired losse connection in cells and tissue at wound or injured area [3]. Diabetes mellitus is known to be associated with a variety of alterations in connective tissue metabolism, as a result of which diabetics face the problem of poor wound healing. Loss of collagen observed in diabetes may be due to decreased levels of synthesis or enhanced catabolism of newly synthesized collagen, or both [4]. Diabetic wounds are slow to heal that can persist for many days required adequate and appropriate care for complete healing. Such wounds are difficult and tough to manage. The wound healing process is the series of repaiment of connective tissue including migration, inflammation, proliferation and maturation of cells at injured area [5]. As per WHO the effective treatment of diabetes and its complications can be possible by using herbal or traditional medicines [6]. In literature survey we had found that the *Cinnamomum tamala* leaves has beneficial effect in management of diabetes. The extracts of *Cinnamomum tamala* leaves showed presence of tannins and phenolic compounds which are having potant antioxidant activity. The oxidative stress is responsible for induction of diabetic complications. Hence in present study ethanolic extract of *Cinnamomum tamala* leaves was used to investigate wound healing activity in STZ induced diabetes in rats.

MATERIALS AND METHODS

Plant Material: The leaves of *Cinnamomum tamala* were purchased from herbal drug supplier of Mandsaur (M.P.) and authenticated in Department of Pharmacognosy at B. R. Nahata College of Pharmacy-SIRO, Mandsaur (M.P.) India.

Preperation of extract: Dried leaves of *Cinnamomum tamala* were extarced with ethanol by successive solvent extraction technique by using soxhlet apparatus for 72 hrs. The extract was 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 temperartue, water and feed. All expermental protocols were approved by Institutional animal ethical committee (IAEC) of B. R. Nahata College of Pharmacy-SIRO, Mandsaur (M.P.) India under proposal number.

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 [7, 8].

Preparation of ointment of extracts: The ethanolic extract (10 % w/w) of the dried leaves of *Cinnamomum tamala* well triturated in pistal mortar with steric acid ointment base and stored in glass container for further use in excision cutaneous wound healing model in diabetic rats.

Excision wound healing model in diabetic 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[9] and a permanent marker. Recording of wound areas were measured on graph paper and % wound closure was calculated by formulla[10]. The day of scar falling, after wounding without any residual raw wound was considered as the day of epitheliazation [11, 12].

Treatment Groups: For 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 ethanolic extract of leaves of *Cinnamomum tamala* (100 mg/kg).

Incision wound healing activity in diabetic rats: 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 (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[13, 14].

Treatment Groups: incision 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 plane vehicle of 0.5 % w/v sodium CMC orally.
- Group III (DT): Diabetes Treated; Diabetic rats treated with 100 mg/kg of ethanolic extract of dried leaves of *Cinnamomum tamala* suspended in 0.5 % w/v sodium CMC suspension orally.

Dead space wound healing activity in diabetic rats: Animals were anaesthetized with slight vapour inhalation of di-ethyl 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 back 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 [15]. This dried tissue was further used to estimate hydroxyproline [16], collagen [17] and elastin [18] level in skin of normal and diabetic rats.

Treatment Groups: For 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 plane vehicle of 0.5 % w/v sodium CMC orally.
- Group III (DT): Diabetes Treated; Diabetic rats treated with 100 mg/kg 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 epithelization was recorded in excision wound model[9, 10, 11, 12]. In incision wound model the tensile strength was measured [13, 14]. In dead space wound model the weight of wet & dry granulation tissue [15], amount of hydroxyproline [16], collagen [17] and elastin [18] were measured.

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

Results: There were significant increase in wound healing parameters during treatment with ethanolic extract of dried leaves of *Cinnamomum tamala* was observed as compared to control groups of normal and diabetic rats.

Effect on wound parameters of excision and incision wound model:

As shown in Table No. 1, the ethanolic extract of *Cinnamomum tamala* leaves showed maximum effect 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 ethanolic extract 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 with decrease in blood glucose level. In incision wound model the tensile strength of ethanolic extract treated rats was found increased along with decrease in blood glucose level with comparison to diabetic control rats.

Table.1: Effect of ethanolic extract of *C. tamala* leaves treatment in excision and incision wound model.

S. No.	Parameters Groups	Wound Area (mm^2)	% Wound Closure	Day of Epithelization	Tensile Strength (gm/mm^2)	Blood Glucose Level (mg/dl)
1	Normal Control (NC)	47.67±2.525	84.11± 0.876	24.17± 0.909	238.5± 3.146	75.83± 1.956
2	Diabetic Control (DC)	110.0± 3.602 ***	63.49± 1.286***	42.33± 0.614 ***	159.3±2.165 ***	386.5± 7.140 ***
3	Diabetic Treated(DT)	13.83± 1.558 ***	95.45± 0.499***	16.67± 0.333 ***	309.7±1.713***	14.07± 0.166***

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 DT were compared with DC. P value considered as P<0.05 Significant (*), P<0.01 Very Significant (**), P<0.001 Highly Significant (***).

Effect on wound parameters of dead space wound model

As shown in Table No. 2, the highest effect of ethanolic extract of *Cinnamomum tamala* leaves as seen 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 ethanolic extarct treated rats with comparison to diabetic control rats.

Table 2: Effect of ethanolic extract of *C. tamala* leaves treatment in dead space wound model

S. No.	Parameters Groups	Wet Grenulation Tissue Weight (mg)	Dry Grenulation Tissue Weight (mg)	Hydroxyproline (µg/ml)	% Collagen	% Elastin
1	Normal Control (NC)	219.0± 3.120	56.50± 1.118	5.418 ± 0.119	40.42± 0.892	235.2± 5.190
2	Diabetic Control (DC)	116.5±4.145***	34.83±1.167***	3.587±0.104***	26.76± 0.780***	155.7±4.540***
3	Diabetic Treated (DT)	329.2± 2.455***	102.0± 2.543***	8.628± 0.112***	64.37± 0.837***	374.5±4.872***

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 DT were compared with DC. P value considered as P<0.05 Significant (*), P<0.01 Very Significant (**), P<0.001 Highly Significant (***)

DISCUSSION

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 upto 220 million in this year [1]. Diabetes is a group of disorders characterized by hyperglycemia resulting due to abnormalities in glucose metabolism [2]. This diabetes can generate many tissue abnormalities including connective tissue abnormality like loss tissue integrity, weak tensile strength, and decreased elasticity. In diabetic patient decrease in collagen content of skin can generates impaired and non healing abnormalities in wound or injured area [3].

Abnormalities related with diabetic wounds include delayed inflammation, altered neovascularization, decreased synthesis of collagen, and defective macrophage function. Diabetic wounds are also prone to infections due to impaired granulocytic function and cellular chemo taxis [19]. The streptozotocin has been used as diabetogen to produce high level of blood glucose and production of complications of diabetes [7]. This complication mechanism involved oxidative stress in body produces the delayed wound healing [20]. The phytochemicals like flavonoids, terpanoids, phenolics and tannis [21] are potent antioxidants and can alter the oxidative stress in diabetic patient [22].

In present study photochemical screening showed the presence of high amount of phenolic and tannin compounds in ethanolic extract of *Cinnamomum tamala* leaves. The Phenolics and tannins are the potent antioxidants reported in literature[23] . Sharma et al [24], and Kar et al, [25] reported that Ethanolic extract of *Cinnamomum tamala* leaves exhibits antihyperglycemic activity The high blood glucose level is responsible for delayed wound healing. The controlling of blood glucose level is a important factor to promote wound healing in diabetes[26]. The ethanolic extract of *C. tamala* leaves treated rats showed significant decrease in blood glucose level during wound healing process.

Deep skin wounds in diabetic and non-diabetic cases heal by contraction and granulation tissue formation and re-epithelialization. In excision wound model the ethanolic extract of *C. tamala* leaves treated group exhibits faster wound contraction and re-epithelialization [27]. The % wound closure was also more in extract treated rats. 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 wound healing process, the pre-dominant constituent of this 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 repaired dermis [28].

In incisional skin-wound models made on the back of db/db mice, delayed repair was characterized by reduced angiogenesis, delayed formation of granulation tissue, decreased collagen content, and low breaking strength [29]. In incision wound model the increased amount of tensile strength was observed in ethanolic extact of *C. tamala* leaves.

In the dead space wound model the rats of ethanolic extarct treatment group showed increased inflammation, granulation andskin strengthening in the form of increase in wet & dry weight of granulation tissue with elevated level of hydroxyproline, collagen and elastin content. The hydroxylproline is the constitutory amino acid of collagen and elastion and these are responsible for granulation, strengthening, and remodeling during tissue repair process after injury. Collagen is a predominant extracellular matrix protein in granulation tissue of regenerating wounds. Collagen synthesis is increased during wound healing after tissue injury. Collagen provides strength to tissue and plays a major role in homeostasis as well as in epithelialization[30].

CONCLUSION

The ethanolic extract of *Cinnamomum tamala* leaves was evaluated for wound healing activity in diabetic rats. The all four phases (hemostasis, inflammation, gtanulation and remodeling) of wound healing studied by excision, incision and deadspace wound models. The high blood glucose level is the root cause of delayed wound healing in patients of diabetes. The treatment of ethanolic extract of *C. tamala* leaves 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 antidiabetic, antioxidant and antimicrobial activities of phytoconstituents like phenolics and tannins which present in ethanolic extract of *Cinnamomum tamala* leaves. Further studies are needed to identify active compound responsible for faster wound healing activity with detailed mechanism of action.

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REFERENCES

1. Zimmet PZ. Diabetes epidemiology as a tool to trigger diabetes research and care Diabetologia. 1999;42: 99-518.
2. Teixeira CC, Rava CA, DaSilva PM, Melchior R, Argenta R, Anselmi F, Almeida CR, Fuchs FD. Absence of antihyperglycemic effect of jambolan in experimental and clinical models. J Ethnopharmacol. 2000;71(1-2):343-347.
3. Goodson WH, Hunt TK, Wound healing and the diabetic patient. Surg Gynecol Obstet. 1979;149:600-608.
4. Lien YH, Tseng MM, Stern R., Glucose and glucose analogs modulate collagen metabolism. Exp Mol Pathol. 1992;57:215–219.
5. Raghow R, The role of the extracellular matrix in postinflammatory wound healing and fibrosis. Fed Am Soc Exp Biol J. 1994;8:823-831.
6. The WHO Expert Committee on Diabetes Mellitus. Technical Report Series 646. Geneva: World Health Organization, 1980.
7. Seifter E, Rettura G, Pedawer J, Stratford F, Kambosos D, Levenson SM. Impaired wound healing in streptozotocin diabetes. Prevention by supplemental vitamin A. Ann Surg. 1981;194(1):42-50.

8. Kumudhavalli MV, Jaykar B. Evaluation of Antidiabetic activity of Costus igneus(L) leaves on STZ induced diabetic rats. Der Pharmacia Sinica. 2012;3(1):1-4.
9. Devender R K, Shashidher B, Kumar GP, Evaluation of wound healing activity of methanolic root extract of Plumbago zeylanica L. in wistar albino rats Asian J Plant Sci Res. 2011;1(2):26-34.
10. Sushil SP, Yogesh TS, Chetan AC, Lalit PS, Naveenkumar PJ, Chhaya HG, Wound healing activity of topical application form based on herbomineral formulation. Asian J Plant Sci Res. 2012;2 (3):355-363.
11. Nayak BS, Anderson M, Periara LM, Evaluation of wound-healing potential ofCatharanthus roseus leaf extract in rats Fitoterapia, 2007;78: 540-544.
12. Malan R, Walia A, Saini V, Gupta S, Comparison of different extracts leaf of Brassica juncea Linn on wound healing activity. Euro J Exp Bio. 2011;1(2):33-40.
13. 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.
14. Ambuj N, Nilesh G, Umesh KJ, Evaluation of Wound healing activity of Tinospora cordifolia Willd. Der Pharmacia Sinica. 2012;3(1):126-130.
15. Prasad V, Jain V, Girish D, Dorle AK, Wound-healing property of Momordica charantia L. fruit powderJ Herb Pharmacother. 2006; 6(3-4):105-115.
16. Reddy KG, Enwemeka SC, A simplified method for the analysis of hydroxyproline in biological tissues. Clin Biochem, 1996;29:225-229.
17. Klein LR, Wesis PH, Induced connective tissue metabolism in vivo: reutilization of pre-existing collagen. Proc Nat Acad Sci USA, 1966;56(1):277-284.
18. Grant RA, Estimation of hydroxyproline by the AutoAnalyser. J Clin Pathol, 1964;17 :685-686.
19. Guo S, Dipietro LA, Factors affecting wound healing. J Dental Res, 2010;89(3):219.
20. King L, Impaired wound healing in patients with diabetes Nurs Stand. 2001;15(38):39.
21. Mrowicz R, Tannins: the new natural antioxidants. Eur J Lipid Sci Technol. 2007;109:549.
22. Smerq J, Sharma M, Possible Mechanism of Murraya koenigii and *Cinnamomum tamala* With Reference to Antioxidants Activity Int J Pharm Sci Drug Res. 2011;3(3):260-264.
23. Palanisamy P, Srinath KR, Yoganand KD, Choudhary PC, Evaluation of antioxidant and antidiabetic activities of Cinamomum tamala leaves in streptozotocin induced diabetic rats. Int J Pharm, 2011;2(12):157-162.
24. Sharma SR, Dwivedi SK, Swarup D, Hypoglycemic and hypolipidemic effects of *Cinnamomum tamala* Nees leaves. Indian J Exp Biol, 1996;34(4):372-374.
25. Kar A, Choudhary BK, Bandhopadhyay NG, Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats J Ethnopharmacol, 2003; 84(1):105-108.
26. Rosenthal, SP, Acceleration of primay wound healing by insulin. Archives in Surgery 1968;96:53–55.
27. Albertson S, Hummel RP, Breeden M, Greenhalgh DG, Surgery, 1993;114(2): 368.
28. Raghow R, The role of the extracellular matrix in postinflammatory wound healing and fibrosis. Fed Am Soc Exp Biol J. 1994;8:823-831.
29. Galeano M, Torre V, Deodato B, Campo GM, Sturiale A, Squadrito F, Cavallari V, Cucinotta D, Buemi M, Altavilla D, Surgery. 2001;129:467.
30. P. Chithra, G.B. Sajithlal, Gowri Chandrakasan. Influence of aloe vera on the healing of dermal wounds in diabetic rats. Journal of Ethnopharmacology 1998;59:195–201.