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Evaluation of *Centella Asiatica* Leaf Extract for Wound Healing in Sterptozotocin Induced Diabetic Rats

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ABSTRACT

The plant *Centella asiatica* is used in traditional medicine to treat a variety of disorders. The objective of this study presented in this report was to evaluate the wound healing potential of ethanolic extract of the plant in streptozotocin-induced diabetic rats. The study was carried out by using Wistar albino rats by creating excision and dead space wounds. The measured wound area of the *Centella asiatica* treated group was reduced significantly when compared to diabetic control animals. Significant increase in the weight of the granulation tissue and the hydroxyproline content were observed. The histological study of the healing tissue obtained from the experimental diabetic animals showed the fast lay-down of collagen when compared to the normal and diabetic control group. The fasting blood glucose values of the diabetic experimental group animals were significantly reduced when compared to the diabetic control animals. We noticed the correlation between the wound contraction rate and the blood glucose values. The results of the present study clearly demonstrate that the ethanolic extract of *Centella asiatica* possesses a definite prohealing action in normal healing as well as in the diabetes induced wound healing.

Keywords: *Centella asiatica,* Wound healing, Sterptozotocin, Wound models, Diabetes

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INTRODUCTION

Wound healing is a complex (but orderly) phenomenon involving a number of processes, including induction of an acute inflammatory process by wounding, regeneration of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of extracellular matrix proteins, remodeling of connective tissue and acquisition of wound strength [1]. When tissues are disrupted following injury, collagen is needed to repair the defect and restore anatomic structure and function. An aim of the study is to explore the potential offered by natural products for wound healing in diabetes.

Centella asiatica L. is commonly known as Indian pennywort. The plant is widely distributed in Asia, Africa, North and South America [2]. This plant is very useful for wound healing, ulcer protection, antimicrobial action. Both topical and oral administration of the plant extracts have been shown to accelerate wound healing [3]. Ethanolic extract of plant possesses a definite prohealing action in normal healing as well as in the steroid depressed wound healing [4]. Pharmacological studies with the extracts of this plant are found to have sedative, antidepressant, analgesic and anticonvulsive effects [5]. *Centella asiatica* L is reputed for its medicinal use in chronic and obstinate eczema, psoriasis, syphilis and leprosy [6].

Diabetic wounds are slow, non-healing wounds that can persist for weeks despite adequate and appropriate care. Such wounds are difficult and frustrating to manage [7]. Diabetic wound healing is an enigmatic and debilitating complication and poses a serious challenge in clinical practice. The exact pathogenesis of the poor wound healing with the wound is not clearly understood, but evidence from studies involving both human and animal models reveal several abnormalities in the various phases of the wound healing process [8].

The present study has been undertaken to investigate the effects of ethanol extract of *Centella asiatica* L on the different parameters of wound healing alone and in the streptozotocin induced diabetic wound healing in rats.

Centella asiatica Linn

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MATERIALS AND METHODS

Plant Material

Leaves of Centella asiatica were collected during September from the local areas of Udupi district, Karnataka, India, and were authenticated by Professor Gopalkrishna Bhat, Department of Botany, Poorna Prajna College, Udupi. A voucher specimen (No.pp525) has been deposited at the Department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal, India.

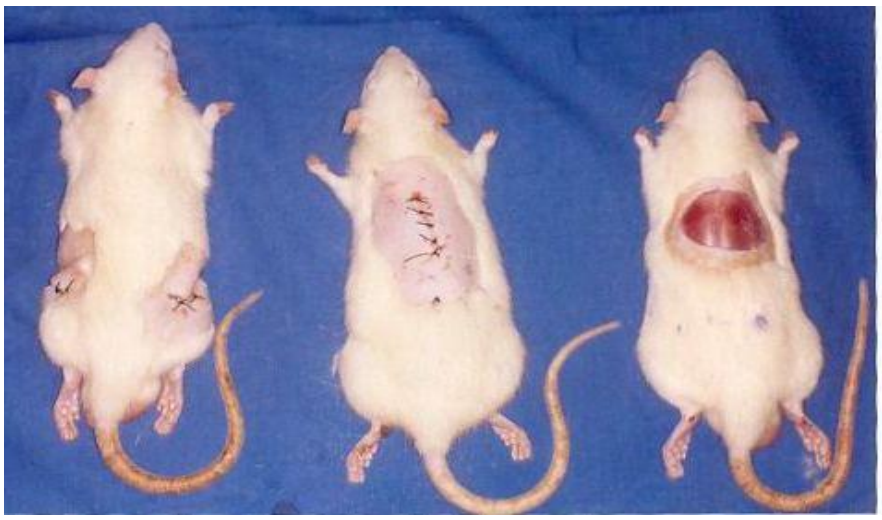
Preparation of Ethanol Extract

Leaves of Centella asiatica were dried in shade and powdered. The powder (75g) was extracted with 700 ml of 95% ethanol in a soxhlet apparatus at 60°-75°C and concentrated. The yield was 10 –15%.

Animals

Healthy Wistar albino rats of either sex and of approximately the same age, weighing between 150-250 g were used for the study. They were individually housed, maintained in clean polypropylene cages and fed with commercially pelleted rat chow (M/s Hindustan Lever Ltd. Mumbai) and water ad libitum. The experimental protocol was subjected to scrutiny of Institutional Animal Ethical Committee for experimental clearance (No.IAEC/KMC/UA/2000).

Wound Models



Dead space wound Incision wound Excision wound

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Acute Toxicity Studies

Healthy albino rats of either sex (n=6) were orally fed with increasing doses (1,2,4,8 and 16g/kg body weight) of ethanol extract for 14 days. The doses of up to 4g/kg body weight did not produce any sign of toxicity and mortality.

Experimental Procedure

The animals were divided into 3 groups of 6 animals each.
Group I: Control group with wound alone;
Group II: Test group with wound and streptozotocin;
Group III: Test group with wound, streptozotocin and treated with extract;

The wounding procedures were carried out using ketamine (1ml/kg body weight) anaesthetized rats, at the dose level of 800mg/kg body weight. The extract was given daily to the rats orally in the case of dead space wound for ten days. In the case of excision wound, extract was given every day to the rats until the day of epithelization.

Induction of Diabetes

Animals of group 2 and 3 were weighed and their fasting blood glucose levels were determined before inducing diabetes. The animals were then injected with single dose of streptozotocin (STZ, 50mg/kg) in cold 0.1M citrate buffer, pH 4.5 (freshly prepared) in the tail vein to induce diabetes. Control animals were injected with 0.1M citrate buffer. Fasting blood glucose was measured three days later to ensure the induction of diabetes (initial). Animals with a blood glucose level of ≥200mg/dl were considered diabetic. Blood glucose levels and body weights were monitored on a weekly basis thereafter. The blood samples were also collected on day 11 to determine blood glucose (final). Fasting blood glucose measurements were done using blood drawn from the tail vein. A glucometer was calibrated using the hexokinase method with standards and quality controls.

Excision Wound Model

A circular piece of full thickness (approximately 500mm^2) was cut off from a predetermined area on the back of rat [9]. Wounds were traced on 1mm^2 graph paper on the day of wounding and subsequently on the alternate days, until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction. Number of days required for falling of eschar without any residual raw wound gave the period of epithelization.

Dead Space Wound Model

These wounds were created by implanting two polypropylene tubes (0.5cm X 2.5cm each), one on either side in the lumbar region on the dorsal surface of each rat. On the 10^{th} post-

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wounding day, the granulation tissue formed on the implanted tubes was carefully dissected out. The wet weight of granulation tissue was noted. The breaking strength of granulation tissue was measured by the method of Lee [10]. Later these granulation tissues were collected, dried at 60°C for 24hr and weighed and the weight was noted. The dried granulation tissue was then utilized to estimate protein and hydroxyproline content [11] and protein [12]. A section of wet granulation tissue was subjected to histopathological examination so as to determine the lay-down of collagen using Haematoxylin and Eosin stains.

Statistical Analysis

The results were analyzed using one way analysis of variance (ANOVA) with post hoc Scheffe's test. *P* values <0.05 were considered statistically significant.

RESULTS

Table 1 shows the effect of the *Centella asiatica* leaf extract on various biochemical parameters. The oral administration of the extract significantly increased the rate of wound contraction and decrease in epithelisation period (*P*< 0.001) (Table1 & 3) when compared to diabetic control. The wet and dry tissue weight, breaking strength of the granulation tissue was significantly increased in the diabetic with extract treated group in compared to diabetic control (*P*< 0.001, (*P*< 0.05) (Table1)

Table 1 – Effect of ethanol extract of *Centella asiatica* in streptozotocin induced diabetes in rats in excision and dead space wound model.
[Values are mean ± SD of 6 replications]

Treatment	Excision wound	Dead space wound		
	Epithelization period (days)	Wet tissue weight (mg/100g rat)	Dry tissue weight (mg/100g rat)	Breaking strength (g)
Wounded control	21.5±1.5	240.5±20.5	30±2.5	285±15.5
Diabetic control	33.6±4.76[a]	192±12.5[b]	24±3.8	190±10.5[a]
Diabetic + *Centella asiatica*	23.5±1.5[p]	242±18.5[p]	31±4.5[r]	272±12.5[p]

P values: [a]:<0.001,[b]:<0.01,[c]:<0.05 vs control with diabetic control; and [p]:<0.001,[r]:<0.05 vs diabetic control with diabetic with *centella asiatica*

Table 2 – Effect of ethanol extract of *Centella asiatica* in streptozotocin induced diabetes in rats in dead space wound model.
[Values are mean ± SD of 6 replications]

Treatment	Blood glucose (mg%) Initial	Blood glucose (mg%) Final	Protein (mg g^{-1})	Hydroxyproline (mg/g tissue)
Wounded control	71.33±0.84	71.16 ± 0.70	48.30 ± 5.0	14.72±4.02
Diabetic control	300.83 ± 41.11[a]	322.50 ± 38.68 [a]	44.60 ± 4.60[b]	10.35±3.65[b]
Diabetic + *Centella asiatica*	296.00 ± 47.07	230.50 ± 30.42[p]	46.40 ± 4.90[q]	13±5.5[q]

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P values: [a]:<0.001,[b]:<0.01, vs control with diabetic control; [p]:<0.001,[q]<0.01, vs diabetic control with diabetic with *centella asiatica*

Table 3 – Effect of ethanol extract of *Centella asiatica* in streptozotocin induced diabetes in rats in excision wound model. [Values are mean ± SD of 6 replications]

Treatment	Percent wound contraction in days									
	4	8	12	16	20	22	24	28	32	34
Wounded Control	12.46±2.46	48.43±2.4	71.4±1.84	84±4.2	96.5±4.3	99.1±1.5				
Diabetic control	15.8±3.2[a]	1±5.8[a]	18±4.1[a]	39.9±5.5[a]	61±6.5[b]		70.2±4.4	81.3±3.2	96.8±4	99.5±1.5
Diabetic + *Centella asiatica*	6.5±1.1[p]	18.5±3.5[p]	37.5±2.7[p]	74.6±5.5[p]	86±9.5[q]		98.5±3.5	99.5±3.5		

P values: [a]:<0.001,[b]:<0.01, vs control with diabetic control; [p]:<0.001,[q]<0.01, vs diabetic control with diabetic with *centella asiatica*

The protein and hydroxyproline content of the granulation tissue of the experimental animals were found to be moderately high (*P*<0.01) when compared to the diabetic animals which did not receive the *Centella asiatica* leaf extract. Wound healing was significantly correlated with the blood glucose values. Significant reduce in the blood glucose level in the final sample which was taken after 11th day in diabetic with extract treated group. We observed that extract treated diabetic wounds were clean, with healthy granulation tissue being produced.

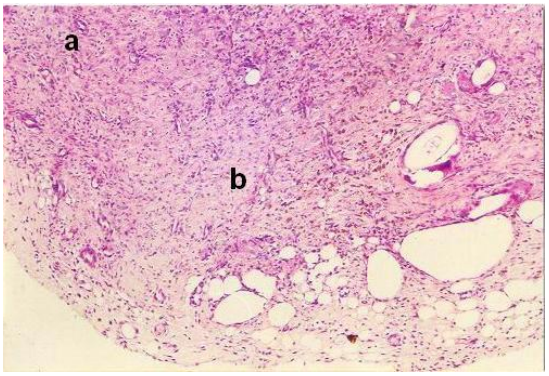

Figure 1

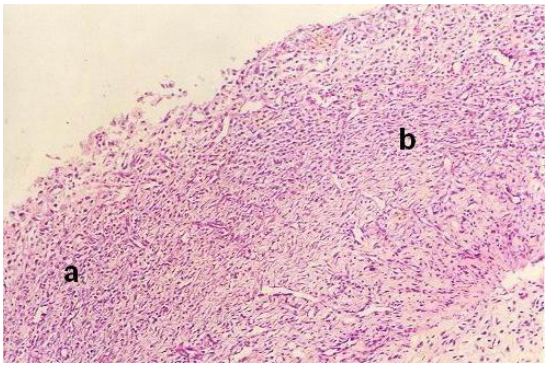

Figure 2

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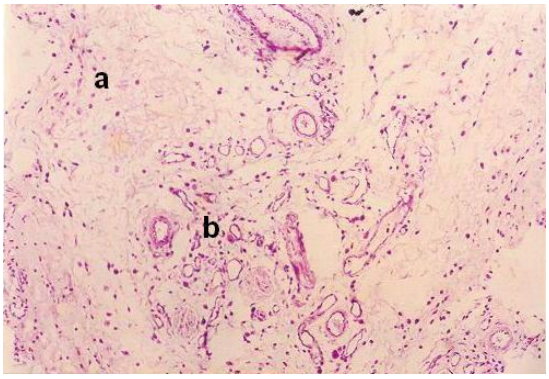

Figure 3

Fig.1-3 – Histopathology of 10 days old granulation tissue.

1 – Control (a) maximum number of fibroblasts, (b) Collagen bundles are indistinguishable.
2 – Diabetic control- (a) Poorly developed matrix shows maximum number of cells, (b) Poor collagen formation.
3 – Diabetic with extract treated (a) Moderate cell population with some matrix formation, (b) Developing neovascularisation.
[H and E, taken with Olympus PM 20 photomicroscope 20 X magnification]

Histological observations of the 10 days old granulation tissue in diabetic and extract treated group (Figure 3) revealed a well developed matrix. Collagen was well organized and formed bundles between the cells. There was better neovascularization in the extract treated group as compared to the normal control and diabetic control. Due to the better formation of ground substance the cells appeared to be spread apart in extract treated group when compared to control groups (Figure 1 and Figure 2). In this study, the extract treated groups showed better epithelialization, collagenation and neovascularization as compared to control wounds. Neovascularization and inflammatory response in extract treated groups indicate the entire process of inflammation results in stimulation of fibroblasts in synthesizing collagen.

DISCUSSION

Granulation, collagen maturation and scar formation are some of the many phases of wound healing, which run concurrently, but independent of each other. The use of a single model is inadequate and no reference standard exists that can collectively represent the various phases of wound healing. Hence, two different models have been used in the present study to assess the effect of *Centella asiatica* on the various phases of wound healing.

The results of the present study clearly demonstrate that the ethanolic extract of *Centella asiatica* possesses a definite prohealing action in diabetic induced rats. An increase in granulation tissue breaking strength and hydroxyproline content of treated wounds may be due to increase in collagen concentration and stabilization of fibers [13]. Increase in wet and dry granulation tissue weight indicated high protein concentration and collagen bundle formation. The increased granulation mass is predominantly contributed by fibroblasts. The plant extract also antagonized the poor healing in diabetes, to some extent on collagen synthesis, maturation and organization to form bundles. Thus it has the potential for antagonizing the slow healing process in diabetes. The researchers showed that the combination of transforming growth factor–R1 and fibroblast growth

factor had marked positive effects on biochemical parameters of wound healing and reversed the tensile strength deficit of diabetic wounds [14]. The constituents identified as the most important for the plants pharmacological activities are the triterpinoid acids, asiatic acid, madecassic acid, triterpinoid saponins and asiaticosides. Asiatic acid was the only component responsible for the collagen synthesis stimulation [15]. In recent years oxidative stress has been implicated in a variety of degenerative processes and diseases. These include acute and chronic inflammatory conditions such as wound healing [16]. Oxygen free radicals play a important role in the failure of ischemic wound healing and antioxidants improve the healing in ischemic skin wounds [17]. The active component of *Centella asiatica* has been shown to significantly increase the level of antioxidants like vitamin E, vitamin C, SOD, catalase and glutathione peroxidase [18].

Centella asiatica may thus achieve the following effects to improve tissue healing: i) an increased blood supply which increases the oxygen supply to the wound by blocking vasoconstructive compounds; and ii) greater migration of epidermal cells and extensive reorientation of collagen fibers caused by a stronger cross-linking [19]. This could be the reason for the faster healing in the extract treated rats. Since *Centella asiatica* is ubiquitous and abundantly grown, it could be a fairly economic therapeutic agent for wound management as a prohealer as well as to control abnormal healing.

CONCLUSION

The present study suggests that the oral administration of *Centella asiatica* leaf extract plays a significant role in the healing of wounds through the process of collagen formation in a streptozotocin induced diabetic rats. The data further suggests that *Centella asiatica* may be useful in the management of diabetic wounds due to its positive wound healing activity and hypoglycemic effects

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