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Effect of *Ocimum sanctum* Linn. Leaf Extract On Wound Healing in Streptozotocin Induced Diabetic Rats.

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ABSTRACT

The medicinal plants are widely used by the traditional medicinal practitioners for curing various diseases in their day to day practice. The plant *O.sanctum* 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 *O.sanctum* 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 fasting blood glucose values of the diabetic experimental group animals were significantly reduced when compared to the controls 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 *O. sanctum* has a definite prohealing action in normal healing as well as in the diabetes induced wound healing.

Keywords: Wound healing, Sterptozotocin, Diabetes, *O. sanctum*

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INTRODUCTION

Ocimum sanctum L. has been used for thousands of years in Ayurveda for its diverse healing properties. *O.sanctum Linn.* (Sanskrit: Tulasi; family: Labiaceae), is found throughout the semitropical and tropical parts of India. Different parts of the plant are traditionally used in Ayurveda and Siddha systems for the treatment of diverse ailments like infections, skin diseases, hepatic disorders and as an antidote for snake bite and scorpion sting [1].The ether extract and essential oil of the leaves exhibited antibacterial activity against a number of bacterial species [2,3]. A methanol extract and an aqueous suspension of *O. sanctum* leaves were found to have anti-inflammatory, analgesic and immunostimulatory properties [4]**.** Ethanolic extract of *O. sanctum* significantly decreases the blood glucose, glycosylated hemoglobin and urea with a concomitant increase in glycogen, hemoglobin and protein in streptozotocin-induced diabetic rats [5].*O. sanctum* fixed oil and linolenic acid found to possess significant antiinflammatory activity against PGE2, leukotriene and arachidonic acid induced paw edema [6].*O. sanctum* extract protects against radiation induced lipid peroxidation and GSH and the antioxidant enzymes appear to have an important role in the protection [7].

Pharmacologically important active principles of *O.sanctum* are a large group of polyphenolic flavonoids like apigenin, vicenin-1 and vicenin-2, caffeic and ursolic acid [8].Flavonoids isolated from *O. sanctum* scavenged free radicals in vitro and showed antilipoperoxidant activity in vivo at very low concentration [9].The free radical scavenging activity of plant flavonoids helps in the healing of wounds[10].Low levels of antioxidants accompanied by raised level of markers of free radical damage play a significant role in wound healing in rats[11].Free radical scavenging activity is a major mechanism by which *O. sanctum* products protects against cellular damage[12].*O. sanctum* may act at various levels in the immune mechanism, such as antibody production, release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs in modulating the humoral immune responses [13]. Thus it appears different mechanisms like free radical scavenging, metal chelation as well as immune modulation may act at different levels individually or in combination to bring about the wound healing effects of this medicinal plant. The present study provides a scientific rationale for the traditional use of this plant in the management of skin diseases. 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 [14].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 [15].

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

MATERIALS AND METHODS

Plant material—Leaves of *O. sanctum* 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.pp531) has been deposited at the Department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal, India.

Plant Material:

Preparation of ethanol extract

Leaves of *O. sanctum* were dried in shade and powdered. The powder (75 g) 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 albino rats of either sex and of approximately the same age, weighing 150-250g 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).

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 in three different wound models, 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 everyday to the rats until the day of epithelization.

Induction of Diabetes

Animals of group II and III 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^{th} 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 [16].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-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 [17].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 [18] and protein [19].

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 *O. sanctum* 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) (Table 1 & 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) (Table 1)

Table 1: Effect of ethanolic extract of *O. sanctum* 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	21.0±1.5	242.5±20.8	30.5±2.5	290±16.0
Diabetic control	32.7±4.66^{a}	190±11.5^{b}	22±3.7	188±10.0^{a}
Diabetic + *O.sanctum*	22.5±1.5^{p}	238±18.5^{p}	31.5±4.5^{r}	270±12.0^{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 *O.sanctum*

Table 2: Effect of ethanolic extract of *O.sanctum* instreptozotocin 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	70.35±0.73	71.16 ± 0.70	47.20 ± 5.1	14.52±4.08
Diabetic control	295.83 ± 39.12^{a}	318.50 ± 35.78^{a}	43.60 ± 4.50^{b}	10.25±3.60^{b}
Diabetic + *O.sanctum*	290.00 ± 45.07	221.50 ± 28.32^{p}	45.30 ± 4.80^{q}	12.5±5.6^{q}

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 *O.sanctum*

Table 3: Effect of ethanolic extract of *O.sanctum* 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	11.47±2.406	47.43±2.34	69.4±1.74	82±4.1	96.0±4.3	99.6±1. 6				
Diabetic control	5.8±3.3^{a}	9.6±5.8^{a}	17.4 ±3.7^{a}	38.89±5.5^{a}	60±6.5^{b}		69.2±4. 6	79.3±3. 4	95.8± 4	99.6±1. 6
Diabetic + *O.sanctum*	7.5±1.51^{p}	16.5±3.6^{p}	36.5±2.8^{p}	72.6±5. 8^{p}	88±8.5^{q}		98.7±3. 6	99.8±3. 7		

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 *O.sanctum*

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 *O.sanctum* 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 (Table 2). We observed that extract treated diabetic wounds were clean, with healthy granulation tissue being produced.

DISCUSSION

Diabetes is a complex disease that impacts on acute and chronic wound management. The process of wound healing is very energy-demanding. Energy needs are increased to support the immune response and regeneration of new tissue. Impaired cell migration, inadequate leukocyte function, and insufficient collagen synthesis are the main causes for poor or delayed wound healing. In our previous study we observed that the ethanolic extract of *O. sanctum* possesses a definite prohealing action in normal healing as well as in the steroid depressed wound healing [20].

The results of the present study clearly demonstrate that the ethanolic extract of *O. sanctum* 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 [21]. 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 [22]. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *O. sanctum* has been found to be largely responsible for the therapeutic potential of Tulsi (*O. sanctum)* [23].

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 [24]. Oxygen free radicals play a important role in the failure of ischemic wound healing and antioxidants improve the healing in ischemic skin wounds [25].

Phytochemical screening revealed the presence of flavonoids in the ethanol extract of *O. sanctum*. Better collagenation, seen under the influence of this plant extract, may be because of the presence of flavonoids, which is responsible for the free radical scavenging activity. Researcher also found that *O. sanctum* leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic β-cells [26].

O. sanctum 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 [27]. This could be the reason for the faster healing in the extract treated rats. Since *O. sanctum* 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 *O. sanctum* 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 *O. sanctum* may be useful in the management of diabetic wounds due to its positive wound healing activity and hypoglycemic effects

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