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Original article

Syzygium mundagam bark methanol extract restores skin to normal in diabetic wounded rats

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ABSTRACT

Diabetic wounds have been identified as one of the major complications associated with diabetes. This study features the use of *Syzygium mundagam* bark methanol (SMBM) extract in the treatment of wounds in Streptozotocin-Nicotinamide induced diabetic rats. The extract ointment base, at 1 and 2% respectively, was applied to the wounded areas on the rats and monitored for 21 days. The wound closure, epithelialization period and histopathology of the wounds were evaluated during the study. Both the concentrations of the extract (1% and 2%) healed the wounds even under diabetic conditions induced in rats on day 21 (99.69% and 100% respectively). The 2% SMBM treated animals showed a higher rate of epithelialization of the wound (15 ± 0.49 days). The histopathology of the wounded skin on day 10 revealed that the rats treated with SMBM extract could initiate the healing and re-epithelialization. This was evident from the migration of neutrophils and proliferation of fibroblasts. On the 21st day, complete healing of the skin could be observed in the rats treated with 2% extract which was evident from the newly formed epidermis, collagen fibers and fibroblast. The results compared well with those treated with betadine (5%). The results of this study will support the use of this plant extract for diabetic healing over the use of commercially available synthetic drugs.

1. Introduction

Incidence of Diabetes Mellitus (DM) is increasing throughout the world with an immediate appeal to researchers to find a reliable therapeutic modality. Diabetic foot ulcers is one of the major complications among diabetic patients. Wounds, under diabetic conditions show delayed healing due to microbial infection, generation of reactive oxygen species and reduced blood flow. In the past decades, an increase in such complications associated with DM is being observed all over the world [1,2]. It is predicted that under chronic conditions, patients will suffer from diabetic ulcers, especially at the proximal regions of the limb [3]. Undeniably, it can lead to limb infection, decay of tissues, limb amputation, and even death if not treated properly [4]. Diabetic wounds are also associated with multiple risk factors which have to be taken into consideration before treatment [5,6]. The risk factors include: longer duration, high Body Mass Index, ageing, and other issues such as; diabetic peripheral neuropathy, diabetic retinopathy, high glycated hemoglobin level (HbA1C), foot deformity, high plantar pressure, peripheral vascular disease etc. [7,8]. Peripheral sensorimotor and autonomic neuropathy leads to high foot pressure, foot abnormalities, and gait instability. These pathways advance foot complications in diabetic patients, which accelerates the chances of ulcer progression [9]. A control over the blood glucose along with alternative therapies would be an ideal measure to treat diabetic foot ulcers and wounds. The search for cost-effective medication with maximum healing properties and minimal to no side effect has led scientists to investigate plants as an alternative source of medicinal products.

The family Myrtaceae is estimated to contain more than 5500 species. Among them, many spp. of *Syzygium* have been studied extensively for various properties including the treatment of diabetes. The fruits of *S. mundagam* (Bourd.) Chitra are eaten by the Paniya and Kuruma tribes of Kerala, India [10]. Chandran et al. [11] have reported the anti-hyperglycaemic property of bark methanol extract of *S. mundagam*. However, earlier reports revealed that this plant was given less attention in view of its anti-diabetic properties. Hence, this study was focused on the wound healing property of *S. mundagam* in diabetic rats.

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2. Materials and methods

2.1. Collection of plant material

S. mundagam bark was collected during October 2011 from Chanthanathodu, Wayanad, Kerala, India. The authenticity was confirmed (Voucher no: CMPR 7932) by Dr. M. Prabhukumar, Scientist and Head-in-Charge, Plant Systematic and Genetic Resources Division, Centre for Medicinal Plant Research, Arya Vaidya Sala, Kottakkal, Kerala, India. The freshly collected bark was cleaned, shade dried and ground into a fine powder before extraction.

2.2. Methanol extraction of bark

The fine powders of bark were packed into thimbles (100 g) and extracted in Soxhlet apparatus with methanol (300 mL). The solvent extract was concentrated using a rotary vacuum evaporator (Equitron Ev11-ABS.051) and then air dried.

2.3. Animals and ethics

Healthy female Swiss albino mice weighing 20–25 g and Wistar albino rats (100–150 g) of approximately the same age, were used for the acute toxicity and diabetic wound studies respectively. The animals were fed with water (*ad libitum*) and a standard chow diet throughout the study period. The animals were maintained in clean polypropylene cages at room temperature. The experimental protocol was examined and approved by the institutional animal ethics committee (KMCRET/PhD/04/2012-13).

2.4. Acute dermal toxicity

Acute dermal toxicity was performed as per the OECD guidelines 402 (1987) to determine any allergic reaction and adverse effects after applying a concentration of the test substance on the skin. Here, the test was done using *Syzygium mundagam* bark methanol (SMBM) extract to determine the therapeutic dose. The ointment containing SMBM extract with the highest concentration of 5% (w/w) was uniformly applied on the shaved dorsal skin of a rat and observed for any sign of toxic or allergic reactions for 48 h.

2.5. In vivo *diabetic wound healing activity*

To induce diabetes, rats were administered intraperitoneally (*i.p.*) with 120 mg/kg nicotinamide. After 15 min, 60 mg/kg streptozotocin (STZ) was injected (*i.p.*) and monitored. The elevated blood glucose and hyperglycemia were confirmed in rats at 72 h and on 10th day after injection. The rats with no change in blood glucose level (>250 mg/dL) on the 10th day were used for the diabetic wound study. Animals were divided into five groups of six rats each and treated for 21 days. Group I: Untreated diabetic control rats with wound; Group II: Diabetic control rats with wound (ointment base alone); Group III: Diabetic wound rats with SMBM extract (1%); Group IV: Diabetic wound rats with SMBM extract (2%); Group V: Diabetic wound rats with standard drug betadine (5%).

A standardized wound area (2 cm^2 and 0.2 cm depth) was created on the shaved dorsal skin of the diabetic rats under anesthesia with diethyl ether. The therapeutic property was analysed by percentage wound shrinkage on 5th, 10th, 15th and 21st day and period of epithelialization. The granulomatous tissue from the wounded area was taken for histopathological analyses [12,13]. The wound tissues from the treatment groups were cut into 2 mm sections using a microtome. The sections were then fixed in 10% formalin and stained with haematoxylin and eosin.

2.6. Ointment preparation

The ointment was prepared by the trituration method as mentioned in British Pharmacopoeia [14]. Briefly, cetostearyl alcohol (0.5 g) and hard paraffin (0.5 g) were melted and was stirred well with yellow soft paraffin (8.5 g) and wool fat (0.5 g) until all the ingredients were melted. The mixture was stirred until uniform mixing and cold. The unwanted particles were removed by decantation. Then, 100 and 200 mg/kg of SMBM extract were added to the ointment base to get 1 and 2% respectively. 5% betadine was used as a standard.

2.7. Statistical analyses

The results were analysed and shown as a mean ± SEM. The data obtained from the study were analysed statistically using one-way ANOVA followed by Dunnett's *t*-test (SPSS version 17.0). Values at $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered statistically significant.

3. Results

3.1. Dermal toxicity study

SMBM extract was found to be non-toxic, and treated rats did not show any sign of allergic reactions when applied with 5% extract. Based on the safety test, 1% and 2% of the extract were fixed for the diabetic wound study.

3.2. Diabetic wound

The topical application of SMBM extracts (1% and 2%) demonstrated a significant reduction in the wound areas. Both the concentrations of the extract (1% and 2%) could treat the wound completely under severe diabetic conditions of the rats on day 21 (99.69% and 100% respectively). The wound contraction and healing were evident from day 10. Table 1 and Fig. 1 depicted that 2% SMBM treated animals have faster epithelialization at the wound site (15.49 days) than the animals treated with betadine (5%) (17.40 days).

Table 1
Diabetic wound healing activity of SMBM extract.

Groups and Dose	Wound contraction (%)				Epithelialization period
	Day 5	Day 10	Day 15	Day 21	(Days)
Ointment base only	28.31 ± 8.38	67.19 ± 10.15	74.81 ± 14.28	89.50 ± 6.10***	23.60 ± 6.34
Control	23.94 ± 6.06	52.31 ± 6.91	64.06 ± 3.36	69.12 ± 2.21	23.61 ± 1.69
SMBM (1%)	24.56 ± 8.35	84.44 ± 1.10*	94.94 ± 0.74*	99.69 ± 0.31***	15.80 ± 0.12
SMBM (2%)	25.88 ± 8.37	83.13 ± 2.17*	96.88 ± 0.83**	100.00 ± 0.00***	15.49 ± 0.13
Betadine (5%)	22.81 ± 11.69	57.56 ± 10.03	86.94 ± 4.51*	99.25 ± 0.48***	17.40 ± 0.95

The data represents mean ± SEM (n = 6). Significantly different at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to control. SMBM- *S. mundagam* bark methanol extract.