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Evaluation of the Wound Healing Properties of *Jasminum Mesnyi* H in Diabetic Rats

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Abstract

Jasminum mesnyi H is a well known plant in the traditional medicine. Based upon the traditional use the root of the plant was selected for evaluation of its dermal wound healing potential in diabetic rats. In the present study the antidiabetic and wound healing activities of the ethyl acetate and ethanol extract of *Jasminum mesnyi* H roots were investigated. The wound healing effect was studied on the streptozotocin-induced diabetic rat model for 21 days. The glucose levels in the blood of rats were measured by using glucose oxidase method by blood glucose measuring strips. According to the obtained statistics, the ethanol and ethyl acetate extract of *Jasminum mesnyi* root at 400 mg/Kg was found to hold a high antidiabetic and wound healing potential.

Keywords: *Jasminum mesnyi*; Diabetes; Dermal wounds

Introduction

Jasminum mesnyi H (primrose jasmine**,** sansonae, peeli malati and peeli chameli) Oleaceae family is a native herb of the Himalayan region and is an evergreen shrub with long and slender arching stems that climb like a sprawling vine [1]. The crude drug is used in various antidiabetic formulations like "Pahari Butti" to lower down the blood glucose level especially in Himalayan ranges like in Solan, India. Low concentration of antioxidants may lead to Diabetes and variety of plants species show antidiabetic property due to their antioxidant potential [2-4]. *Jasminum mesnyi* Hance (*Jasminum primulinum Hemsley*) also known as "Primrose Jasmine" or "Japanese Jasmine" is found in tropical, sub-tropical and warm temperate regions of Asia continent. It is trailing evergreen shrub with long and lean arching stems that scale up like a rambling creeper. Leaves are trifoliolate, opposite and attached at the base of branchlets. Flowers are having 6-10 petals arranged in a semidouble whorl, usually axillary or rarely terminal, solitary and yellow coloured [5-7]. Leaves have been proved to have a phenolic glucoside (syringin), secoiridoids (9"- hydroxyjasmesosidic acid, 2"-hydroxyjasminin, jasmoside, , isojasminin, jasminin, jasminin 10"-O-β-D-glucoside, jasmesoside, jasmosidic acid, 9"-hydroxyjasmesoside and 4"-hydroxyisojasminin) and rutin [8-12].

Material and Methods

Plant collection

Jasminum mesnyi herb's roots were collected from the local market of Sonepat, Haryana. Taxonomic identity of the herb was confirmed at the National Institute of Science Communication and Information Resources, New Delhi. A voucher specimen (NISCAIR/RHMD/ Consult/2015/2564-143-2) has been deposited in the NISCAIR Department, New Delhi for future reference.

Plant extracts preparation

Ethyl acetate and ethanol extract were extracted from 500g of shade dried roots of *Jasminum mesnyi* by continuous hot percolation method by using soxhlet extractor. In which, ethyl acetate and ethanol were used as solvents to collect the extracts. Both ethyl acetate and ethanol extracts were evaporated to dryness under reduced pressure below 40° C to obtain a semi-solid consistency mass and were separately kept in desiccators for further use.

Animals

Wistar albino rats weighing 150 g-200 g were used for experimentation. Rats were kept under specific pathogenic-free conditions, housed, fed and treated in accordance with the international guidelines principles of laboratory animal use and care. The animals were maintained with pelleted

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food (Purina), while tap water was available ad libitum [13]. They were maintained on the standard pellet diet and water ad libitum for 2 weeks to be acclimatized prior to the investigation.

Diabetes induction

Rats were made diabetic by a single injection of streptozotocin (STZ; 50 mg/kg, i.p.) (Sigma–Aldrich Canada, Oakville, Ontario, Canada), prepared in citrate buffer (0.1 M, pH 4.5) after overnight fasting [14]. Blood was drawn from the orbital plexus 24 h after the injection and the fasting blood glucose level was estimated (with glucose oxidase reagent strips) using Glucometer (Accu-check) 7 days after streptozotocin injection, and animals with glucose levels greater than 250 mg/dl were used for the study. Blood glucose levels were estimated at the time of creation of the wounds.

Surgical procedures and treatment

After the induction of diabetes wounds were created on the 7th day. Excision wounds were created in experimental rats. Excision wounds were used for the study of biochemical parameters and of the rate of wound contraction. Animals were anesthetized with 40 mg/kg i.p. of thiopentone sodium and the right side of each rat was shaved. Excision wounds sized 4 cm^2 were made by cutting out a 2 cm×2 cm piece of skin from the shaven area.

Ethyl acetate and ethanol extracts were given orally in concentrations of 100 mg/Kg, 200 mg/Kg, and 400 mg/Kg for 21 days. The control group received an equal amount of vehicle (citrate buffer).

Wound healing activity

Excision wound: Epithelialization time was noted as the number of days after wounding required for the scar to fall off leaving no raw wound behind. The aspect and evolution of the scratch were quantified by a blind assay [15]. To determine the rate of wound contraction, excision wounds were traced on a transparent paper having a millimeter scale, and the change in wound size was calculated as the percentage of wound area that had healed. The period of epithelialization of the wound was expressed as the number of days taken for complete epithelialization (so that no raw wound was left behind).

Grouping of animals

Animals were divided into nine groups, each consisting of six rats. The extracts were administered for 15 days. Group I: standard (Metformin 5mg/Kg), Group II: Diabetic rats with wound without treatment as normal control group., Group III: Diabetic rats without wound (for diabetes only), Group IV: Diabetic rats with wound treated with ethyl acetate extract by oral route at a dose 100 mg/Kg, Group V: Diabetic rats with wound treated with ethyl acetate extract by oral route at a dose 200 mg/Kg, Group VI: Diabetic rats with wound treated with ethyl acetate extract by oral route at a dose 400 mg/Kg, Group VII: Diabetic rats with wound treated with ethanol extract by oral route at a dose 100 mg/Kg, Group VIII: Diabetic rats with wound treated with ethanol extract by oral route at a dose 200 mg/Kg, Group IX: Diabetic rats with wound treated with ethanol extract by oral route at a dose 400 mg/Kg.

Rate of wound contraction and period of epithelialization

To determine the rate of wound contraction, excision wounds were traced on a transparent paper having a millimeter scale, at the time of wounding (0 day) before treatment with the extract and again on days 3, 6, 9, 12, 15 and18 after wounding and the change in wound size was calculated on every third day as the percentage of wound area that had healed. The period of Epithelialization of the wound was expressed as the number of days taken for complete Epithelialization (so that no raw wound was left behind). The percentage of wound contraction was calculated using:

$$\% \text{ wound contraction} = (A_0 - A_t)/A_0 \times 100.$$

where A_0 is the original wound area and A_t is the area of the wound at a specific time period after wounding [16].

Statistical analysis

All wound area measurements were expressed as percentage wound contraction size. The data were statistically analyzed by Student's t-test, using the program "GraphPad Prism 7.0". Data are significant, $P<0.05$ compared with control.

Results and Discussion

Diabetes mellitus is acknowledged to be allied with an assortment of alterations in connective tissue metabolism, as a consequence of which diabetics face the difficulty of deprived wound healing. In diabetes loss of collagen is observed, which may be due to deficit levels of production or increased catabolism of newly synthesized collagen, or both [17]. The oral dose of the ethyl acetate and ethanol root extracts of *Jasminum mesnyi* had shown the dose-dependent effect on the blood glucose level and wound healing effect on the diabetic rats. At a dose level of 400mg/Kg, the percentage area of wound contraction has increased by 30.12% to 90.14% on the 12th day for ethanol extract and the percentage area of wound contraction has increased by 24.73% to 85.81% on the 12th day for ethyl acetate extract. The lower concentration of doses of ethyl acetate and ethanol extract (100mg/Kg and 200mg/Kg) were found to be less potent in terms of percentage contraction of the wound area. (Table I) The ethyl acetate and ethanol extracts at a concentration of 400 mg/Kg showed the complete healing of wound on the 14th day (Epithelialization period) irrespective of other concentrations of 100 mg/Kg and 200 mg/Kg that had shown complete healing on 18th days.

The blood of the rats had showed the significant lowering of plasma glucose level in the ethanolic extract at a dose level of 400 mg/Kg and 200 mg/Kg on 0 days, 7 days and 14 days (Table II). The various active chemical constituents present in extracts may be responsible for this glucose lowering ability. The flavonoid present in ethyl acetate extract 400 mg/Kg and 200 mg/Kg also showed the hypoglycemic activity on 0 days, 7 days and 14 days.

The present study demonstrates that the ethyl acetate and ethanol extracts accelerate wound healing in diabetes. The results suggest that extracts treatment may have a beneficial influence on the various phases of wound healing. It is quite possible that the enhanced healing of wounds in diabetic rats by ethanol extract is a result of its hypoglycemic activity (since a control over blood glucose levels has been shown to improve wound healing in diabetics) [18] and/ or its capacity to stimulate fibroblast function during the healing process.

The results of this study seem to confirm the traditional use of *Jasminum mesnyi* for the treatment of diabetic wounds. This result encourages us to carry out a wider and more profound study to isolate responsible potent active chemical constituents to better evaluate the diabetic wound healing activity of this plant.

Conclusion

The present study demonstrated for the first time that the ethyl

acetate and ethanol extract of *Jasminum mesnyi* roots have properties to promote wound healing and antidiabetic activity when compared to normal controls. This study gives us good scientific evidence that the extract can be a promising complementary supplement in future after collecting more scientific data for diabetic patients with wound healing defect.

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