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RESEARCH ARTICLE

Wound Healing Potential of *Paspalum scrobiculatum* Linn. in Streptozotocin-induced Diabetic Rats

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ABSTRACT

Aim of the Study: The aim is to study the evaluation of the wound healing properties of ethyl acetate and ethanolic extracts of roots of *Paspalum scrobiculatum* in diabetic rats. **Materials and Methods:** Ethyl acetate and ethanolic extracts (100, 200, and 400 mg/Kg body weight) were administered orally to male Wistar albino rats. Streptozotocin was used to induce irreversible diabetes mellitus and excision wound healing method for determining wound healing activity for 21 days. **Results:** Ethyl acetate and ethanolic extracts at different dose levels produced the decrease in fasting blood glucose in a dose-dependent manner. After 14 days, the maximum reduction in fasting blood glucose (211.00 mg/dl and 224.50 mg/dl) was observed in diabetic rats treated with ethanolic extract at 400 and 200 mg/kg dose, respectively. A momentary decrease in blood glucose level was also observed in the ethyl acetate extract-treated rats at a dose of 400 mg/kg. The maximum percentage contraction in wound area (27.60–98.51% and 24.13–93.96%) was observed in diabetic rats treated with ethanolic extract at 400 and 200 mg/kg dose, respectively, on the 15th day of 21 days' study. **Conclusion:** The study reveals that *P. scrobiculatum* has wound healing activity along with antidiabetic activity, thereby mitigating its conventional uses and amplify it into an allopathic system of medicine.

Keywords: Dermal wounds, diabetes, ethyl acetate and ethanolic extracts, *Paspalum scrobiculatum*

INTRODUCTION

Paspalum scrobiculatum Linn. (Poaceae) commonly named as Kodo millet is cultivated almost all the way throughout India (tribal, dryland, and hilly area). For diabetic persons, it is recommended as a replacement for rice and has exceptional medicinal properties and insecticidal properties, relatively unidentified to modern societies.[1] *P. scrobiculatum* L. is a top-knotted evergreen meadow, with 120–150 cm in height, stalk stem plump glabrous, a bit curled at the bottom, spread evenly in Central India and Karnataka in India.[2] The other plant part (grains) is a tonic, sweet, hemostatic and is used as hemorrhages, anti-inflammatory, congestive hepatomegaly, and general debility.[3] Historic Vedas such as Sushruta Samhita and Charak Samhita have proclaimed this plant to cure the diabetes mellitus.[4,5] Plant and extracts proved to have an excellent anti-inflammatory and wound healing activity due to the existence of dynamic terpenes, alkaloids, and flavonoids as chemical constituents.[6-8] Apart from this, the plant has also shown medicinal activities such as antirheumatic, antidiabetic, tranquilizing, and wound healing. Due to these medicinal activities, the plant is recommended as food for diabetic patients with wound.[9] Ethanolic and aqueous extracts of grains lead to a significant increase in serum insulin level and the decrease in fasting blood glucose in a dose-dependent manner. This indicates that *P. scrobiculatum* retains a comprehensive antidiabetic activity.[10]

MATERIALS AND METHODS

Plant material

The roots of *P. scrobiculatum* were collected during July 2014 from Andhra Pradesh, India, and identified by Dr. Madhav Chetty in Sri

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Venkateswara University, Andhra Pradesh, India. A voucher is also retained in the university department for future reference.

Plant extract preparation

Ethyl acetate and ethanolic extracts were extracted from 500 g of shade-dried roots of *P. scrobiculatum* by the continuous hot percolation method using Soxhlet extractor, in which ethyl acetate and ethanol were used as solvents to collect the extracts. Both ethyl acetate and ethanolic extracts were dried under vacuum <40°C to obtain a semi-solid consistency mass and were separately kept in desiccators for further use.

Animals

About 150–200 g weighing Wistar strain albino rats used for experimentation were procured from a disease-free small animal house from LUVAS, Hisar, Haryana, with the prior approval of the Institutional Animal Ethics Committee with regd no. 360–73 dated May 05, 2016. Pathogenic-free conditions were provided to the rats. The rats were housed, fed, and treated as per the international guideline principles of laboratory animal use and care. The animals were maintained in polypropylene cages under standard conditions (25 ± 2°C, 12 h light and dark cycle) with pelleted food (Purina), while tap water was available *ad libitum*.[11]

Diabetes induction

Streptozotocin (STZ; 50 mg/kg, i.p.) (Sigma-Aldrich Canada, Oakville, Ontario, Canada), prepared in citrate buffer (0.1 M, pH 4.5), was used to make rats diabetic by a single dose after overnight fasting.[12] Blood was taken out from the rat's orbital plexus 24 h after the injection, and the fasting blood glucose level was estimated after the 7th day of streptozotocin injection (with glucose oxidase reagent strips) using Glucometer (Accu-check®) and animals with glucose levels preeminent than 250 mg/dl were used for the study. At the time of creation of the wounds, blood glucose levels of rats were studied.

Surgical procedures and treatment

Wounds were created on the 7th day after the induction of diabetes. Excision wound healing method was used to calculate the wound healing potential of different extracts in experimental rats. Different biochemical parameters and the rate of wound contraction were studied. Rats were anesthetized with thiopentone sodium in a dose level of 40 mg/Kg i.p. and each rat was shaved on the right side. 4 cm^2-sized excision wounds were created 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, 200, and 400 mg/Kg for 21 days. Citrate buffer was given in an equal amount to the control group.

Wound healing activity

Excision wound

When no raw wound is left behind and when the scar falls off, time period in days was noted as epithelialization time.[13] Excision wounds on a transparent paper having a millimeter scale were traced to determine the rate of wound contraction, and the percentage of wound area healed was calculated using change in wound size.

Grouping of animals

Animals were divided into nine groups. Each group consists of six rats. The extracts were administered for 15 days. Group I: Standard (Metformin 5 mg/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, and Group IX: Diabetic rats with wound treated with ethanol extract by oral route at a dose 400 mg/Kg.

The rate of wound contraction and period of epithelialization

At 0 day, before extract treatment and after wounding of 3, 6, 9, 12, 15, and 18 days, excision wounds were traced on a transparent paper having mm scale, and on every 3rd day, change in wound size was calculated as a percentage of wound area that has healed. The percentage contraction of the wound was calculated using

% wound contraction = (A0−At)/A0 ×100.

Where A0 is the original wound area and At is the area of the wound at a specific time period after wounding.[14]

Epithelialization period

Epithelialization period is the days in the number required for scar falling without any raw wound left behind. The epithelialization period of the wound was expressed as days in number taken for complete epithelialization (when no raw wound was left behind).[15]

Statistical analysis

Wound area was measured as percentage contraction in wound size. Analysis of data was statistically done by Dunnett's *t*-test using GraphPad prism 7.0. When $P < 0.05$ compared with control, the data are considered to be statistically significant.

RESULTS

When extracts of *P. scrobiculatum* L. were administered to glucose-loaded normal rats fasted for 18 h, there is a reduction in blood glucose level. In our study, the difference observed between the initial and final fasting blood glucose levels of different groups under investigation revealed a significant elevation in blood glucose in the diabetic control group at the end of the 14th day experimental period along with healing of wounds by excision wound healing method observed for 21 days. There is a significant decrease in the fasting blood glucose level and an increase in the percentage of contraction of the wound area when extracts were administered to diabetic rats.

In 0 days, 7th day and the 14th day significant decrease in the level of plasma glucose in the ethanolic extract at the dose of 400 mg/kg and 200 mg/kg was observed as illustrated in Table 1. The decrease in the blood glucose level may be due to active constituents present in the extract. The flavonoids present in ethyl acetate extract 400 mg/Kg and 200 mg/Kg showed the hypoglycemic activity on 0, 7th, and 14th days.

Increase in the percentage area of wound contraction from 27.60% to 98.51% and 22.18% to 96.90%, respectively, on the 15th day in ethanolic and ethyl acetate extract was observed at the dose of 400 mg/Kg. There is no much increase in the percentage contraction in the wound area in the lower doses (100 and 200 mg/Kg) in ethyl acetate and ethanol extract as illustrated in Table 2. Complete wound healing is shown by ethyl acetate and ethanol extract at the dose level of 400 mg/Kg on the 17th day. 100 and 200 mg/Kg showed complete healing of wounds on the 18th day.

Table 1: Antidiabetic activity of *Paspalum scrobiculatum* L. in Streptozotocin-induced diabetes mellitus

Group	Plasma glucose level (mg/dl)		
	0 day	7th day	14th day
Standard (metformin)	275.83±4.945	151.66±3.626*	160.33±2.21*
Diabetic control with wound	285.16±2.072	296.33±3.412	304.16±6.263
Diabetic control without wound	281.66±5.420	284.16±4.490	285.00±5.721
Ethyl acetate extract 100 mg/Kg	286.00±7.000	200.50±4.500	221.50±8.500
Ethyl acetate extract 200 mg/Kg	282.50±5.500	181.5±4.500*	201.00±5.100
Ethyl acetate extract 400 mg/Kg	285.50±4.500	165.50±7.500*	192.50±4.500*
Ethanol extract 100 mg/Kg	279.50±12.500	226.50±4.500	235.00±7.000
Ethanol extract 200 mg/Kg	284.50±11.500	185.02±3.000*	224.50±6.500*
Ethanol extract 400 mg/Kg	283.50±9.500	163.00±7.000*	211.00±9.000*

Values are expressed as mean±SEM, n=6, P<0.05 versus diabetic control group (Dunnett's *t*-test after analysis of variances)

Table 2: Wound healing activity of *Paspalum scrobiculatum* L. in diabetic excision model

Group	Percentage contraction in wound area							Epithelization period (in days)
	3rd day	6th day	9th day	12th day	15th day	18th day	21st day	
Standard (metformin)	33.30±0.304	56.34±0.432*	78.71±0.354*	96.96±0.692*	100	100	100	14.86±0.307*
Control with wound	15.54±0.164	35.06±0.284	48.37±0.189	67.19±0.276	78.62±0.392	95.45±0.761	100	20.50±0.365
Ethyl acetate extract 100 mg/Kg	19.09±0.180	38.98±0.075	56.50±0.250	81.43±0.185	92.82±0.395	100	100	17.50±0.50
Ethyl acetate extract 200 mg/Kg	19.49±0.020	39.26±0.735	60.50±0.020	85.31±0.050	94.92±0.195	100	100	17.50±0.50
Ethyl acetate extract 400 mg/Kg	22.18±0.380	42.81±0.260*	67.09±0.075*	95.29±0.085*	96.90±0.035*	100	100	16.50±0.50*
Ethanol extract 100 mg/Kg	20.66±0.145	39.73±0.315	62.52±0.420	81.91±0.190	90.68±0.685	100	100	17.50±0.50
Ethanol extract 200 mg/Kg	24.13±0.335	43.56±0.185*	68.50±0.250*	86.87±0.100*	93.96±0.310*	100	100	17.00±0.00*
Ethanol extract 400 mg/Kg	27.60±0.495	54.69±0.105*	73.74±0.305*	91.15±0.195*	98.51±0.225*	100	100	16.00±0.00*

Values are expressed as mean±SEM, n=6, P<0.05 versus diabetic control group (Dunnett's t-test after analysis of variances)

DISCUSSION

The present study is the preliminary assessment of the antidiabetic and wound healing activity of the ethyl acetate and the ethanolic root extracts of *P. scrobiculatum*. There is a dose-dependent fall in blood glucose level in streptozotocin-induced diabetic rats and a decrease in wound size in both the extracts. Diabetic patients have difficulty in wound healing due to alteration in connective tissue metabolism. A decrease in the level of production or increase in the catabolism of newly synthesized collagen causes loss of collagen in diabetic patients.[13]

The rich fiber content of *P. scrobiculatum* L. may be responsible for the antidiabetic activity. Dietary fibers lower the level of blood glucose by decreasing the rate of absorption of carbohydrate from the intestine and so beneficial for type-II diabetic patients.[16] Phenolic compounds present in plant extract may be beneficial in diabetes and many other diseases as reported in earlier studies. Therefore, the activity of the plant may be due to these phenolic compounds.[17,18]

CONCLUSION

The present study demonstrated, for the 1st time, that the ethyl acetate and ethanol root extract of *P. scrobiculatum* 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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REFERENCES

1. Roy B, Pal DC. Abstracts: Fourth International Congress of Ethnobiology. Lucknow: National Botanical Research Inst; 1994. p. 17-21.
2. Singh C, Singh P, Singh R. Modern Techniques of Raising Field Crops. New Delhi: Oxford and IBH Publishing Co Pvt Ltd.; 1999.
3. Council of Scientific and Industrial Research. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. New Delhi: Publications and Information Directorate; 2003.
4. Kumar SP, Ayurveda S. Ayurved Drug Plants. Patna: Shri Baidyanath Ayurved Bhavan Pvt Ltd.; 2004.
5. Ahlawat PS, Prakash O, Saini GS. Scientific Crop Production in India. Meerut: Aman Publishing House; 2000.
6. Porras-Reyes BH, Lewis WH, Roman J, Simchowitz L, Mustoe TA. Enhancement of wound healing by the alkaloid taspine defining mechanism of action. Proc Soc Exp Biol Med 1993;203:18-25.
7. Samuelsen AB. The traditional uses, chemical constituents and biological activities of *Plantago* major L. A review. J Ethnopharmacol 2000;71:1-21.
8. Mensah AY, Sampson J, Houghton PJ, Hylands PJ, Westbrook J, Dunn M, *et al.* Effects of *Buddleja globosa* leaf and its constituents relevant to wound healing. J Ethnopharmacol 2001;77:219-26.
9. Murty AV, Subramanyam NS. Textbook of Economic Botany. New Delhi: Wiley Eastern Limited; 1989.
10. Jain S, Bhatia G, Barik R, Kumar P, Jain A, Dixit VK, *et al.* Antidiabetic activity of *Paspalum scrobiculatum* linn. In alloxan induced diabetic rats. J Ethnopharmacol 2010;127:325-8.

11. Hedrich HH, Bullock G. Laboratory Mice: The Handbook of Experimental Animals. United Kingdom: Elsevier Academic Press; 2006.
12. Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. J Clin Invest 1969;48:2129-39.
13. Villegas LF, Fernández ID, Maldonado H, Torres R, Zavaleta A, Vaisberg AJ, *et al.* Evaluation of the wound-healing activity of selected traditional medicinal plants from perú. J Ethnopharmacol 1997;55:193-200.
14. Yates CC, Whaley D, Babu R, Zhang J, Krishna P, Beckman E, *et al.* The effect of multifunctional polymer-based gels on wound healing in full thickness bacteria-contaminated mouse skin wound models. Biomaterials 2007;28:3977-86.
15. Dinesh M, Manjunath C, Joshi VG, Karigar AA, Sikarwar MS. Wound healing property of ethanolic extract of *Crataeva nurvala* root bark. J Pharm Res 2010;3:2788-9.
16. Khan A, Safdar M. Role of diet, nutrients, spices and natural products in diabetes mellitus. Pak J Nutr 2003;2:1-12.
17. Vasco C, Ruales J, Eldin A. Total phenolic compounds and antioxidant capacity of major fruits from Ecuador. Food Chem 2008;111:816-23.
18. Ahmad N, Mukhtar H. Green tea polyphenols and cancer: Biologic mechanism and practical implications. Nutr Rev 1999;57:78-3.