Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 3, 2012 ISSN - 0974-2441

Research Article

EVALUATION OF WOUND HEALING POTENTIAL OF *CYNODON DACTYLON*

PAYAL DANDE*, ANIS KHAN

SVKM's NMIMS, SPTM, Shirpur, Dhule- 425 405, Maharashtra, India. Email: payal4nmims@yahoo.com

Received:23 May 2012, Revised and Accepted:21 June 2012

ABSTRACT

Objective: To evaluate the wound healing potential of *Cynodon dactylon*.
Study design: Ethnomedicinal claim strongly suggests the use of *Cynodon dactylon L.* as first aid in minor injuries in traditional medicine[10]. To evaluate its action, aqueous and alcoholic extract of *Cynodon dactylon L.* were prepared. Preliminary phytochemical studies were performed. Wound healing potential was evaluated in different experimental model such as Excision wound healing model and Incision wound healing model in albino wistar rats by using the gel preparation of aqueous and alcoholic extract.
Results: Preliminary phytochemical studies revealed the presence of carbohydrates, tannins, phenols, flavonoids, amino acid, proteins, alkaloids and glycosides in both aqueous extract and alcoholic extract. Further, alcoholic extract & aqueous extract gel showed significant increased in the rate of wound healing in excision model ($p<0.05$) and in the incision wound healing ($p<0.01$), indicating the wound healing potential of *Cynodon dactylon*.
Conclusion: The present study revealed that the aqueous and alcoholic extract of *Cynodon dactylon L.* has a significant wound healing potential and supports its traditional claim to be used in burns & inflammation.

Keywords: *Cynodon dactylon*, excision wound healing model, incision wound healing model.

INTRODUCTION

Interruption in the cellular and anatomic continuity of a tissue leads to formation of wound[1]. Wound can be caused due to various factors such as chemical, physical, thermal, microbial or immunological harm. Such conditions lead to discomfort and are more prone to infection and other troublesome complications[2]. Wound healing encompasses a series of steps that may ultimately lead to restoration of total integrity of the damaged tissue. The current drugs available are associated with challenges of being expensive, causing allergy or leading to drug resistance. To overcome these problems, researchers are seeking the help of traditional herbs for getting a better alternative[3]. This has lead to the newer research studies that are being carried to find new drugs which will not only promise fast healing but also will reduce the complication and cost. The ayurvedic traditional practitioners from India have many such natural drugs for treating wounds and burns.

Cynodon dactylon Linn. is a member of the family Graminae (Poaceae). It is a creeping grass, very tough, drought resistant, light green in color, has a coarse texture, and fast growing. It is found in short cylindrical pieces about 3 to 20 mm long & 2 to 3 or sometimes 4 mm in diameter[5]. *Cynodon dactylon* aqueous extract have been evaluated for their Antioxidant[6], Anti-inflammatory[7] action while the fresh juice has shown the Immunomodulatory & DNA protective activity[8]. Phytochemical screening carried in the past has shown the presence of phenols, flavonoids, alkaloids, glycosides, proteins and amino acid in *Cynodon dactylon*[9].

Ethnomedicinal investigation revealed the use of *Cynodon dactylon L.* as first aid in minor injuries in traditional medicine[10]. However, there is no scientific evidence or report on the wound healing potential of the *Cynodon dactylon L*. The present study is planned to evaluate the wound healing potential of *Cynodon dactylon L*. aqueous and alcoholic extract.

MATERIAL AND METHODS

Drugs and Chemicals

Povidone-iodine ointment (Betadine), ketamine hydrochloride (Ketamine) distilled water, ethanol was used for study. All chemicals used were of AR grade.

Plant collection

Fresh herb of *Cynodon dactylon* were collected from herbal garden of SVKM's NMiMS, Shirpur campus (M.S.) and sent for authentification to Agharkar Research Institute, Pune.

Plant extraction

Aqueous extract

The aqueous extract of the herb was prepared by soaking 250 gm of herb in 2000 ml of distill water. It was further extracted in soxhlet apparatus for 12 hrs at boiling temperature. The resulting extract was filtered and concentrated in equitron rotavapour under reduced pressure. The concentrated extract was lyophilized to get a powder (yield 2.8%, w/w).

Alcoholic extract

The fresh plant upto 250 gm was cleaned with distilled water and was successfully extracted with 70:30 w/v (ethanol: water) using soxhlet apparatus. The extraction was carried out for 24 hrs at room temperature. The resulting extract was filtered and concentrated at 45°C in rotavapour under reduced pressure. The concentrated extract was lyophilized to get a powder (yield 3.2% w/w).

Phytochemical investigation[12]

The aqueous extract and alcoholic extract of *Cynodon dactylon* L. were subjected to preliminary phytochemical investigation using standard method of analysis.

Identification test by TLC[12]

Test solution: Dissolved test extract in 1ml of ethanol.

Solvent system: toluene: propanol (7:3)

The 20 µl test extract solution was approximately applied on a silica gel G plate of uniform thickness of 0.2 mm. The plate was developed in the given mobile phase up to a distance of 8 cm. On exposure to the iodine vapour, two brownish yellow spot at Rf value of 0.34 and 0.86 was observed.

Preparation of gel

Carbopol 940 forms very good consistent transparent gel at low concentration. Carbopol 940 is non toxic and does not cause any irritation to skin. So, carbopol 940 was selected as a gelling agent. 1% carbopol gel base was prepared by soaking carbopol 940 in hot water overnight. The formulation was made using below following formula shown in the Table 1.

Table 1: Composition of gel of aqueous and alcoholic extract of *Cynodon dactylon*

Sr.No.	Ingradients	Concentration (% w/w)	
		10% w/w	20% w/w
1	Carbopol 940	200 mg	200 mg
2	Extract	2 gm	5 gm
3	Methy paraben	0.2ml	0.2 ml
5	Glycerine	0.4 ml	0.4 ml
6	Triethanolamine	0.2 ml	0.2 ml
7	Water	q.s.	q.s.

Experimental animals

Male wistar albino rats, weighing 200-300 g, were used and housed in standard environmental conditions i.e. room temperature 22°C ± 3°C, relative humidity 50-60%. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of NMIMS, SPTM, Dhule, Maharashtra and conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Excision wound healing model

Excision wound healing model (figure: 1a) was performed as per Mukherjee PK, *et al.* 2000[14]. This model was used to monitor wound contraction and epithelialisation time. The animals were divided in six groups having six animals in each group as shown in Table 2.

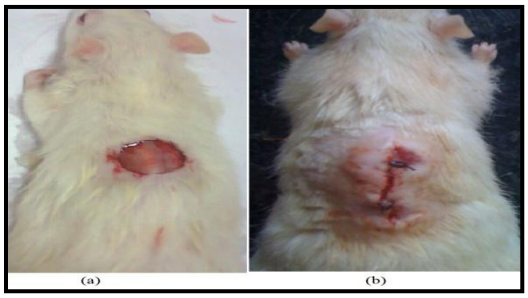

Fig 1: (a) Excision wound and (b) Incision wound model at day 0

Table No.2: Grouping of animals and their treatment

Sr. No.	Group (n=6)	Treatment
1.	Control	Paraffin wax
2.	Standard	Povidone Iodine
3.	Test -1	Aqueous extract (10%) gel of *C.dactylon*
4.	Test -2	Test aqueous extract (20%) gel *C.dactylon*
5.	Test -3	Test alcoholic extract (10%) gel *C.dactylon*
6.	Test -4	Test alcoholic extract (20%) gel

All the animals were anaesthetized using ketamine hydrochoride. The rats were depilated on the back and a predetermined area of 500 sq.mm of full thickness skin was excised in the dorsal inter scapular region. Rat's wounds were left undressed to the open environment for control group. The reference standard group was treated with 0.2% w/w betadine ointment, the test groups was treated with aqueous and alcoholic extract gel (10 % w/w and 20 % w/w) by applying the it every day till the 16th day of wound healing.

The progressive changes in wound area were monitored using vernier caliper. The measurement of wound area on graph paper was expressed as unit (mm^2). Wound contraction was expressed as percentage reduction of original wound size.

Incision wound healing model

This study was carried out (Figure: 1b), as described by James O, *et al.* 2010[15]. Tensile strength (the force required to open the healing skin) was used to measure the completeness of healing. Tensile strength of wound represents the effectiveness of wound healing. Usually wound-healing agents promote the gaining of tensile strength.

The grouping of animal was done in the same manner as excision model. Six groups of animals containing six in each group were taken. The animals were anaesthetized by ketamine injection. One full thickness paravertebral incision of 5 cm length was made including the cutaneous muscles of the depilated back of each rat. Full septic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. After the incision was made, the parted skin was kept together and stitched with sutures, 1 cm apart. The ointment of the extract, standard drug (betadine ointment) was applied to the wound of test & standard group twice daily, until complete recovery to the respective groups of animals. On 8th day after wounding the sutures were removed and the tensile strength was measured on 10th day.

For measuring the tensile strength the rats were again anaesthetised and each rat was placed on a stack of towels on the middle of the board. The amount of the towels could be adjusted in such a way so that the wound was on the same level as the tips of the arms. The clamps were then carefully clamped on the skin of the opposite edges of the wound at a distance of 0.5 cm away from the wound. The longer pieces of the fishing line were placed on the pulley and finally to the polyethylene bottle. The position of the board was adjusted so that the bottle receives a rapid and constant rate of water from a large reservoir, until the wound began to open. The amount of water in the polyethylene bag was weighed and equated as the tensile strength of the wound. The tensile strength induced by the extract and by betadine ointment treated wounds was compared with the control.

Evaluation was done by measuring wound area in mm^2 in excision wound healing model and tensile strength measurement in incision wound healing model.

Statistical evaluation

Data obtained are presented as means ± standard error of mean (S.E.M.) for the number of animals in each group (n = 6). The groups were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test, $P<0.05$ was considered significant in excision wound healing model and $P<0.01$ was considered significant in incision wound healing model.

Table No 3: Effect of *Cynodon dactylon* extract and betadine on wound contraction in excision wound model

Group	Wound area mm^2 Mean (± SEM)						
	0th day	4th day	8th day	10th day	12th day	14th day	16th day
Control	496.4 (± 2.88)	469.13 (± 3.92)	417.63 (± 2.75)	333.13 (± 2.94)	271.6** (± 2.28)	198.36* (± 3.57)	170.66* (± 4.10)
Standard	528.26 (± 3.43)	470.16 (± 2.65)	310.66** (± 4.39)	238.46** (± 3.63)	149.43** (± 4.35)	89.3** (± 1.26)	26.23** (± 1.44)
Test aqueous 10%	513.26 (± 2.66)	483.5 (± 3.45)	333.8** (± 2.45)	249.5** (± 4.56)	170.46* (± 4.23)	102.53** (± 2.63)	74.56** (± 1.70)
Test aqueous 20%	502.6 (± 2.63)	463.56 (± 3.71)	315.13** (± 0.99)	229.5** (± 4.65)	157.4** (± 3.40)	89.53* (± 2.18)	52.23** (± 3.61)
Test alcoholic 10%	507.26 (± 2.66)	473.5 (± 3.45)	323.8** (± 2.45)	239.5** (± 4.56)	160.46* (± 4.23)	96.2** (± 2.63)	54.56** (± 1.70)
Test alcoholic 20%	504.6 (± 2.63)	463.56 (± 3.71)	315.13** (± 0.99)	229.5* (± 4.65)	154.4** (± 3.40)	92.53** (± 2.18)	41.23** (± 3.61)

RESULTS

Preliminary phytochemical screening

The preliminary screening of aqueous extract and alcoholic extract showed the presence of phenols, alkaloids, glycosides, flavonoids, tannins, carbohydrate, proteins and amino acid.

Wound healing results analysis

In the study of wound healing potential, the aqueous extract and alcoholic extract showed prominent increase in the rate of wound healing of rats when compared to the control group. In the excision wound healing model, the test extract gel of *Cynodon dactylon* L. showed significant wound healing rate when compared to control group ($p < 0.05$) as shown in Table 4.

Table No 4: % wound area healed on different days in excision wound model

Group	% wound area healed on different days						
	0th day	4th day	8th day	10th day	12th day	14th day	16th day
Control	0.00	6.92	32.15	44.97	60.09	72.41	87.52
standard	0.00	8.99	38.83	52.70	69.37	83.86	94.49
Test aqueous 10%	0.00	7.85	34.87	50.95	66.98	79.57	89.37
Test aqueous 20%	0.00	8.25	36.44	52.73	68.24	80.77	90.03
Test alcoholic 10%	0.00	8.13	35.17	53.21	68.73	81.13	91.19
Test alcoholic 20%	0.00	8.87	37.17	54.73	69.59	82.23	92.57

In the incision wound healing model, the aqueous extract & alcoholic extract of *Cynodon dactylon* at 10th day was measured and found to be increase the wound healing rate in a manner, and the results were statistically significant ($p < 0.01$) as given in Table 5. The wound healing of alcoholic extract was well comparable to the standard drug. There was less effect with the aqueous extract when compared with alcoholic extract.

Table No 5: Tensile strength of different group.

Group	Breaking Strength (gm) Mean (±SEM)
Control	184.33* (±1.20)
Standard	289.00** (±4.48)
Test Aqueous 10%	272.33** (±4.26)
Test Aqueous 20 %	277** (±1.15)
Test Alcoholic 10%	277 **(±2.464)
Test Alcoholic 20 %	287 **(±2.08)

***P < 0.01 and **P< 0.0001 when compared to control**

DISCUSSION

The body's natural defense mechanism responds to any injury and help in regulation of repair mechanism. The repair of wounds involves different phases including contraction, the formation of epithelialisation and fibrosis. However, it has to be accelerated in order to prevent from any infection and to relieve the pain & inflammation. The wound healing potential of *Cynodon dactylon* was studied in rats. The results showed that the topical application of gel of the aqueous extract and alcoholic extract of *Cynodon dactylon* produced prominent increase in wound healing area of rats as compared to control group. These effects were as good as seen with that of Povidone -Iodine (standard drug) ointment.

The aerial part extract of *Cynodon dactylon* has been shown to possess alkaloids, phenols, tannins and flavonoids on preliminary screening.

These phenolic compounds and flavonoids may have either individual or additive effects that have helped to fasten wound healing process. It is also reported that *Cynodon dactylon* L. possess antioxidant (free radical scavenging), anti-inflammatory (prevent inflammation of wounded cells) and Immunomodulatory (haemostatis) activity. The antioxidant potential of *Cynodon dactylon* reduces the chances of cell damage which may have fastened the wound healing process. The anti-inflammatory action of *Cynodon dactylon* prevented the inflammation of cells during wound healing process. This confirms that *Cynodon dactylon* extract has potential to fasten the wound healing process.

CONCLUSION

The present study revealed that the aqueous extract and alcoholic of *Cynodon dactylon L.* has a significant wound healing potential and supports its traditional claim to be used in burns & inflammation.

ACKNOWLEDGMENTS

The authors wish to thank Mustafa Pata and Ravikumar Sinojia for their technical help, and Associate dean with all Management members of NMIMS, School of Pharmacy and Technology Managment, Shirpur, for providing the necessary facilities to carry out the research work.

REFERENCES

1. Bennet RG. Fundamentals of cutaneous surgery. St. Louis C. V. Mosby , 1988; p.78.
2. Ingold WM. Wound therapy growth factors as agents to promote healing. Trends in Biotechnol, 1993; 11: 387-392.
3. Sai KP and Babu M. Traditional medicine and practices in burn care need for newer scientific perspectives. Burns, 1998; 24:387-388.
4. Kumara PD, Jayawardane GL and Aluwihare AP. Complete colonic duplication in an infant Ceylon. Med. Med. J. 2001; 46: 69-70.
5. Purna SK and Babu M. Collagen based dressing- a review. Burns, 2000; 26:54-62.
6. Kumar A, Sawarkar HA, Deshmukh VS, Mishra KK, SinghM, Verma T and Kashyap P. *Cynodon dactylon (L.)* Pers. Pharmacological actions and medicinal applications. International Journal of Herbal Drug Research, 2011; Vol I, Issue 1, 1-7.
7. Santhi R, Kalaiselvi K and Annapoorani S. Antioxidant efficacy of *Cynodon dactylon* leaf protein against ELA implanted swiss albino mice. Journal of Pharmacy Research, 2010; 3(2), 228-230.
8. Garg VK and Paliwal SK. Anti-nflammatory activity of aqueous Extract of *Cynodon dactylon*. International Journal of Pharmacology, 2011; 7: 3, 370-375.
9. Mangathayaru K, Umadevi M, Reddy UC. Evaluation of the immunomodulatory and DNA protective activities of the shoots of *Cynodon dactylon*. Journal of Ethnopharmacology, 2009; 123: 181–184.
10. Suresh K, Deepa P, Harisaranraj R, Vaira AV. Antimicrobial and Phytochemical investigation of the leaves of *Carica papaya L., Cynodon dactylon (L.) Pers., Euphorbia hirta L., Melia azidarach L., Psidium guajava L.* Ethnobotanical leaflets, 2008; 12:1184-91.
11. Balasubramanian G, Sarathi M, Venkatesan C, Thomas J, Sahul Hameed AS. Studies on the immunomodulatory effect of extract of *Cynodon dactylon* in shrimp, *Penaeus monodon*, and its efficacy to protect the shrimp from white spot syndrome virus (WSSV). Fish Shellfish Immunol, 2008a; 25:820-828.
12. Khandelwal KR Practical Pharmacognosy Techniques and Experiments. Nirali Prakashan, 2005; 19th edition, Page No. 149-156.
13. The Ayurvedic Pharmacopoeia of India. Government of India Ministry of Health & Family Welfare, 1st ed. Part-1, Vol.- II, p. 62.
14. Mukherjee PK, Verpoorte R, Suresh B. Evaluation of *in-vivo* wound healing activity of *Hypericum patulum* (Family: Hypericaceae) leaf extract on different wound model in rats. Journal of Ethnopharmacology, 2000; 70 (3): 315-321.

15. James O and Ayide I. Victoria excision and incision wound healing potential of *Saba florida* (benth) leaf extract in Rattus novergicus. International Journal on Pharmaceutical and Biomedical Research, 2010; 1:4,101-107.