EVALUATION OF WOUND HEALING POTENTIAL OF CYNODON DACTYLYN
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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 medicine10. 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 L. 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 wound1. 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 complications2. 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 alternative3. 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 Gramineae (Poaceae). It is a creeping, 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 diameter2. Cynodon dactylon aqueous extract have been evaluated for their Antioxidant4, Anti-inflammatory5 action while the fresh juice has shown the Immunomodulatory & DNA protective activity6. Phytochemical screening carried in the past has shown the presence of phenols, flavonoids, alkaloids, glycosides, proteins and amino acid in Cynodon dactylon7.

Ethnomedicinal investigation revealed the use of Cynodon dactylon L as first aid in minor injuries in traditional medicine10. 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 authentication 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 investigation12
The aqueous extract and alcoholic extract of Cynodon dactylon L were subjected to preliminary phytochemical investigation using standard method of analysis.

Identification test by TLC12
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.
tic measures were not taken and no local or systemic
environment for control group. The reference standard group was
scapular region. Rat’s wounds were left undressed to the open
All the animals were anaesthetized using ketamine
contraction and epithelialisation time. The animals were divided in
Mukherjee PK, et al. 2000. Excision wound healing model (figure:
Supervision of Experiments on Animals (CPCSEA).
NMIMS, SPTM, Dhule, Maharashtra
approved by the Institutional Animal Ethics Committee (IAEC) of
3°C, relative humidity 50%
Male wistar albino rats, weighing
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. 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.

![Image](image_url)

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

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Ingredients</th>
<th>Concentration (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbopol 940</td>
<td>10% w/w 20% w/w</td>
</tr>
<tr>
<td>2</td>
<td>Extract</td>
<td>200 mg 200 mg</td>
</tr>
<tr>
<td>3</td>
<td>Methy paraben</td>
<td>0.2ml 0.2ml</td>
</tr>
<tr>
<td>4</td>
<td>Glycerine</td>
<td>0.4 ml 0.4 ml</td>
</tr>
<tr>
<td>5</td>
<td>Triethanolamine</td>
<td>0.2 ml 0.2 ml</td>
</tr>
<tr>
<td>6</td>
<td>Water</td>
<td>q.s. q.s.</td>
</tr>
</tbody>
</table>

**Table No.2: Grouping of animals and their treatment**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group (n=6)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Paraffin wax</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>Povidone iodine</td>
</tr>
<tr>
<td>3</td>
<td>Test-1</td>
<td>Aqueous extract (10%) gel of C. dactylon</td>
</tr>
<tr>
<td>4</td>
<td>Test-2</td>
<td>Test aqueous extract (20%) gel C. dactylon</td>
</tr>
<tr>
<td>5</td>
<td>Test-3</td>
<td>Test alcoholic extract (10%) gel C. dactylon</td>
</tr>
<tr>
<td>6</td>
<td>Test-4</td>
<td>Test alcoholic extract (20%) gel</td>
</tr>
</tbody>
</table>

All the animals were anaesthetised using ketamine hydrochloride.
The rats were deprived 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
evironment for control group. The reference standard group was
treated with 0.2% w/w betadine ointment, the test groups were
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 (mm²). 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. 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
with 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 mm² 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**

<table>
<thead>
<tr>
<th>Group</th>
<th>Wound area mm²</th>
<th>Mean ± (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>494.4 ± 2.88</td>
<td>469.3 ± 3.92</td>
</tr>
<tr>
<td>Standard</td>
<td>528.6 ± 3.43</td>
<td>470.16 ± 4.39</td>
</tr>
<tr>
<td>Test aqueous 10%</td>
<td>513.2 ± 2.65</td>
<td>483.5 ± 4.39</td>
</tr>
<tr>
<td>Test aqueous 20%</td>
<td>502.6 ± 3.63</td>
<td>463.56 ± 3.71</td>
</tr>
<tr>
<td>Test alcoholic 10%</td>
<td>507.2 ± 3.45</td>
<td>473.5 ± 3.91</td>
</tr>
<tr>
<td>Test alcoholic 20%</td>
<td>504.6 ± 3.63</td>
<td>463.56 ± 3.71</td>
</tr>
</tbody>
</table>
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.

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>
<thead>
<tr>
<th>Table No 5: Tensile strength of different group.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Standard</td>
</tr>
<tr>
<td>Test Aqueous 10%</td>
</tr>
<tr>
<td>Test Aqueous 20%</td>
</tr>
<tr>
<td>Test Alcoholic 10%</td>
</tr>
<tr>
<td>Test Alcoholic 20%</td>
</tr>
</tbody>
</table>

\*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 (haemostasis) 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.

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