ANALGESIC AND ANTI-PYRETIC ACTIVITY OF AQUEOUS EXTRACT OF *CYNODON DACTYLON*

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Summary

Whole plant of *Cynodon dactylon* is traditionally used to treat painful and inflammatory conditions. In the present study, analgesic and anti-pyretic activities of aqueous extract of *Cynodon dactylon* at different doses was studied using hot plate, acetic acid induced writhing and yeast induced hyperthermia method. *Cynodon dactylon* showed significant analgesic and anti-pyretic activities in all models studied. Results support the traditional use of the plant in the treatment of pain and fever.

Keywords: *Cynodon dactylon*, analgesic, anti-pyretic, hot plate, acetic acid induced writhing.

Introduction

Cynodon dactylon (Poaceae), a hardy perennial grass, is one of the most commonly occurring weeds in India. In Hindi it is known as dhub, doob or harialil; other common names include durba (Bengali), garikoihallu (Kanarese), durua (Marathi), durua or haritali (Sanskrit), arugampullu (Tamil), garikagoddi (Telugu) and dhubkhabbal (Punjabi) [1]. It has a renowned position in Indian systems of medicine and is used as first aid for treatment of minor injuries, as styptik to stop bleeding [2, 3, 4] and the paste of this plant is applied on forehead in headache [5]. The roots in the form of paste with water are taken internally against fevers [6].

Materials and Methods

Experimental Animals

Healthy albino Wistar rats of both sexes weighing between 200-250g were used. Also albino mice of both sexes weighing between 20 – 25g were used. Institutional Animal Ethics Committee approved the experimental protocol**.** Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). (Approval No. 711/02/a/CPCSEA).

Plant Material

The whole plants with roots of Cynodon dactylon were collected from the local area of Meerut district and identified and authenticated by Dr. Anjula Pandey, Taxonomist, National Herbarium of Cultivated Plants, New Delhi. Voucher specimens (No. NHCP/NBPGR/2006/94/51/8929) have been kept in National Herbarium of Cultivated Plants, New Delhi and Department of Pharmaceutical Technology, MIET for future reference.

Extraction

The whole plant along with roots was dried under shade, reduced to moderately coarse powder, loaded into soxhlet extractor and was subjected to successive extraction with Petroleum Ether, Benzene, Chloroform, Ethanol and Water to get different extracts.

Preliminary Phytochemical Studies

The different extracts were then subjected to qualitative phytochemical screening for the identification of the phytoconstituents. While Petroleum Ether, Benzene, Chloroform does not show any appreciable tests for the presence of different phytoconstituents, alcoholic extract showed positive tests for the presence of glycosides, flavonoids and alkaloids. However, Water extract showed positive tests for glycosides & flavonoids only. As traditionally, the aqueous paste or the aqueous extract of the plant is used to cure pain and fever, the analgesic and anti-pyretic activities of the aqueous extract of the plant in different dose levels (200 mg/kg, 400mg/kg and 600 mg/kg) [7] is being reported here.

Antipyretic Testing

Hyperthermia was induced in rats following the method of Teotino et al., 1963. Initial rectal temperatures of rats were recorded using a six channel tele-thermometer for 1 min. Rats were made hyperthermic by subcutaneous injection of 20% yeast suspension at a dose of 1 ml/100 gm body weight. When the temperature was at peak (18 hours after yeast injection) the rectal temperature were again recorded. Those animals that showed a rise in rectal temperature of more than 1.2° C were used [8]. Different doses of aqueous extract of *Cynodon Dactylon* were given orally as a suspension prepared in 2% Tween 80 solution. Animals were divided into five groups of six animals each. First group received 1 ml of 2% Tween 80 solution orally and served as control. Second, third, fourth and fifth groups received standard antipyretic agent i.e. paracetamol suspension (100 mg/kg) [9],

aqueous extract (200 mg/kg), aqueous extract (400 mg/kg), aqueous extract (600 mg/kg) respectively. The rectal temperatures of animals were recorded at 30 minutes intervals for 4 hours following the administration of Tween 80, standard drug and plant extracts [9].

Analgesic Activity

Hot Plate Method

The hot plate method described by Turner (1965) was followed for the assessment of analgesic activity. Albino mice were introduced to a hot plate maintained at 55 ± 0.5°C. The reaction time to the thermal stimulus was recorded as the time interval from introduction of the animal to the plate until the first lick of the limbs or the first jump of the animals. The test groups received aqueous extract of Cynodon dactylon at different dose levels prepared as suspension in 2% Tween 80 orally, the standard group received Pentazocine (10mg/kg, i.p.) [10] and control group received only 1 ml of 2% Tween 80 solution. The reaction times were determined before and after 30 minutes, 1 hour, 2 hours and 3 hours period with reference to the control group receiving only vehicle.

Acetic Acid Induced Writhing

Acetic acid induced writhing response in mice Acetic acid solution at a dose of 10ml/kg (0.6%) was injected i.p. and the number of writhes during the following 15 minutes period was observed. The test groups received aqueous extracts of *Cynodon dactylon* at different dose levels prepared as suspension in 2% Tween 80 orally, the standard group received Aspirin (10mg/kg, i.p.) [10] and control group received only 1 ml of 2% Tween 80 solution. Significant reductions in number of writhes by drug treatment as compared to vehicle treatment animals were considered as a positive analgesic response. The percent inhibition of writhing was calculated [10].

$$\% \text{ Inhibition} = \frac{W_C - W_T}{W_C} \times 100$$

Where,
W_C = Mean number of writhes in control group.
W_T = Number of writhes in test group.
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Statistical Analysis [11]

All the results obtained from various activities, as described above, were analyzed statistically by using Student's t test and $p<0.05$ were considered significant.
The results are summarized in the tables given below.

Results

Anti-pyretic Activity

The anti-pyretic activity of the aqueous extract of *Cynodon dactylon* has been shown in table 1, which showed significant activity at 400 mg/kg and 600 mg/kg dose levels. The results were comparable to that of Paracetamol, a prototype of a anti-pyretic drug.

Table. 1. Effect of different doses of Aqueous extract of *Cynodon dactylon* and paracetamol on yeast induced hyperthermia in rats.

	Rectal Temperature (°C)						
	Initial before yeast injection	**18 Hrs. after Yeast injection**	**Time after drug administration (hrs)**				
			0.5 hrs	**1 hrs**	**2 hrs**	**3 hrs**	**4 hrs**
Control	37.10 ± 0.068	39.11 ± 0.087	39.15 ± 0.076	39.20 ± 0.085	39.18 ± 0.047	39.13 ± 0.066	38.23 ± 0.075
Paracetamol (100 mg/kg)	37.08 ± 0.047	39.18 ± 0.065	38.41 ± 0.101[d]	37.68 ± 0.087[d]	38.36 ± 0.080[d]	38.61 ± 0.104[c]	38.01 ± 0.070
Aqueous Extract (200 mg/kg)	37.15 ± 0.099	39.15 ± 0.042	39.07 ± 0.049	39.03 ± 0.071	38.91 ± 0.094[a]	39.10 ± 0.085	38.46 ± 0.088
Aqueous Extract (400 mg/kg)	37.08 ± 0.065	39.06 ± 0.095	38.63 ± 0.088[c]	37.88 ± 0.079[d]	38.61 ± 0.094[d]	38.86 ± 0.061[b]	38.05 ± 0.042
Aqueous Extract (600 mg/kg)	37.13 ± 0.061	39.18 ± 0.079	38.46 ± 0.071[d]	37.80 ± 0.085[d]	38.41 ± 0.060[d]	38.66 ± 0.111[c]	38.01 ± 0.101

Values are expressed as mean ± S.E.M. (n=6); significance at $p< 0.05^a$, $p< 0.02^b$, $p< 0.01^c$, $p< 0.001^d$ as compared to control.

Analgesic Activity

Hot Plate Method

From the result it can be deduced that the extract has shown dose dependant activity. After administration of the aqueous extracts at all the three dose levels, there is statistically significant increase in the hot plate reaction time. But the increase is comparable to the standard drug, Pentazocine only at 600 mg/kg dose level (Table 2).

Table. 2. Effect of different doses of aqueous extract of *Cynodon dactylon* on Hot Plate reaction time in mice

Groups	Dose (mg/kg)	Reaction Time (Seconds)				
		Initial	Time after drug administration (Hrs)			
			0.5 hrs	1 hr	2 hrs	3 hrs
Control		9.16 ± 0.0270	9.18 ± 0.0228	9.21 ± 0.0432	9.22 ± 0.0142	9.20 ± 0.0106
Pentazocine	10	9.21 ± 0.0144	25.17 ± 0.0876[d]	32.25 ± 0.0234[d]	37.08 ± 0.0273[d]	31.15 ± 0.0536[d]
Aqueous Extract	200	9.20 ± 0.0107	12.88 ± 0.0413[d]	15.68 ± 0.0275[d]	17.38 ± 0.0239[d]	14.00 ± 0.0382[d]
Aqueous Extract	400	9.21 ± 0.0218	19.95 ± 0.0506[d]	23.08 ± 0.0224[d]	28.03 ± 0.0234[d]	21.13 ± 0.0315[d]
Aqueous Extract	600	9.22 ± 0.0176	23.03 ± 0.0310[d]	30.23 ± 0.0197[d]	35.18 ± 0.0445[d]	28.48 ± 0.0238[d]

Values are expressed as mean ± S.E.M. (n=6); significance at $p< 0.05^a$, $p< 0.02^b$, $p< 0.01^c$, $p< 0.001^d$ as compared to control.

Acetic Acid Induced Writhing

The aqueous extracts at dose levels of 200, 400 and 600 mg/kg exhibited 30.55, 68.55 & 82.10 % inhibition of writhing as compared to that of 82.96% inhibition shown by Aspirin. It is quite evident from the result that the extract at 600 mg/kg showed comparable activity to that of Aspirin (Table 3).

Table. 3. Effect of different doses of aqueous extract of *Cynodon dactylon* on Acetic acid induced writhing in mice

S. No.	Groups	Dose (mg/kg)	No. of Writhings (Mean ± SEM)	% Inhibition
1	Control		38.16 ± 1.3522	
2	Aspirin	10	6.5 ± 0.4282[d]	82.96
3	Aqueous Extract	200	26.5 ± 0.5628[d]	30.55
4	Aqueous Extract	400	12.00 ± 0.5774[d]	68.55
5	Aqueous Extract	600	6.83 ± 0.4773[d]	82.10

Values are expressed as mean ± S.E.M. (n=6); significance at $p< 0.05^a$, $p< 0.02^b$, $p< 0.01^c$, $p< 0.001^d$ as compared to control.

Discussion

The present study establishes the anti-pyretic and analgesic activities of the aqueous extract of *Cynodon dactylon* in the models used. Since antipyretic and analgesic activities are commonly mentioned as characteristic of drugs or compounds which have an inhibitory effect on prostaglandin biosynthesis [12], the yeast induced hyperthermia in rat model was, therefore, employed to investigate the antipyretic activity of this plant. It was found that the aqueous extract at the dose of 600 mg/kg showed a significant decrease in rectal temperature similar to that shown by the standard drug, paracetamol. This result seems to support the view that the extract has some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature [13].

Likewise, the analgesic activity of aqueous extract of the plant was evaluated using the hot plate method and writhing test in mice. The hot plate method is useful in detecting centrally acting analgesics [14] whereas acetic acid induced writhing method is useful to detect peripheral analgesic effects. Acetic acid, which is used as an inducer for writhing syndrome, causes algesia by liberation of endogenous substances, which then excite the pain nerve endings [8]. The fact that aqueous extract of *Cynodon dactylon* showed analgesic activity in both the models studied, indicate that this effect could be due to the presence of two components; one acting centrally and the other via peripheral route [14].

From the above results, it can be deduced that aqueous extract has shown dose dependent activity. As the phytochemical screening has shown the presence of flavonoids and glycosides in aqueous extract, its potent activity may be attributed to the presence of these phytoconstituents. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism of action.

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