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Anti-Inflammatory Activity of Aqueous Extract of *Cynodon dactylon*

¹Vipin Kumar Garg and ²Sarvesh Kumar Paliwal

¹Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, NH-58, Baghpat By-pass Crossing, Delhi-Haridwar Highway, Meerut-250005, Uttar Pradesh, India

²Department of Pharmacy, Banasthali University, Niwai-304022, Rajasthan, India

Abstract: Whole plant of *Cynodon dactylon* is traditionally used to treat painful and inflammatory conditions. We have undertaken this study, since no detailed scientific study was available regarding the anti-inflammatory activity of *Cynodon dactylon*. In the present study, anti-inflammatory activity of aqueous extract of *Cynodon dactylon* at different doses was studied using carrageenan, serotonin, histamine and dextran induced rat paw edema and cotton pellet method. The study was carried out in three different doses levels of 200, 400 and 600 mg kg⁻¹ orally. The aqueous extract of *Cynodon dactylon* was found to be safe at all the doses used and there was no mortality up to the dose of 4000 mg kg⁻¹ of extract when administered orally. *Cynodon dactylon* showed significant anti-inflammatory activities in all models studied. The extract was found to reduce significantly (p<0.001) the formation of edema induced by carrageenan, serotonin, histamine and dextran after 3 and 5 h. Also, it produced maximum 46.40% inhibition in dry weight cotton pellet formation at a dose of 600 mg kg⁻¹ as compared to 46.27% inhibition produced by Indomethacin. The standard drug used was Indomethacin (10 mg kg⁻¹). The preliminary phytochemical screening has revealed the presence of glycosides and flavonoids in the aqueous extract. The presence of flavonoids might be responsible for its anti-inflammatory activity. Results support the traditional use of the plant in the treatment of inflammatory conditions.

Key words: *Cynodon dactylon*, anti-inflammatory, carrageenan, histamine, serotonin

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 harijalil; other common names include durba (Bengali), garikoihallu (Kanarese), durua (Marathi), durua or haritali (Sanskrit), arugampullu (Tamil), garikagoddi (Telugu) and dhubkhabbal (Punjabi) (Sastry and Kavathekar, 1990). *Cynodon* has a renowned position in Indian systems of medicine and many parts of the plant are assumed to have medicinal properties. Doob ghas is a valuable herbal medicine and used as first aid for minor injuries (Oudhia, 1999a, b). Farmers traditionally apply crushed leaves to minor wounds as a styptic to stop bleeding (Oudhia, 2001). The whole plant is extremely beneficial externally in wounds and the paste of the plant is applied on forehead in headache (Paranjpe, 2001). The roots in the form of paste with water are taken internally against fevers (Natarajan and Paulsen, 2000). The aqueous fluid extract of the rhizome is used as anti-inflammatory, diuretic,

anti-emetic, purifying agent and also in dysentery (Ahmed *et al.*, 1994; Kirtikar and Basu, 1980). *Cynodon* plant is useful for pains, inflammations and toothache (Mahesh and Brahatheeswaran, 2007). Various scientific studies have been carried out on *Cynodon dactylon* and various pharmacological activities have been reported. It has been reported to possess antidiabetic (Singh *et al.*, 2008a), antiulcer (Patil *et al.*, 2005), diuretic, antimicrobial (Artizzu *et al.*, 1996), hepatoprotective (Singh *et al.*, 2008b), cardioprotective (Najafi *et al.*, 2007) and immunomodulatory (Mangathayaru *et al.*, 2009) activities. Since no detailed scientific data is available regarding the anti-inflammatory activity of *Cynodon dactylon*, the present study was designed to explore the same.

MATERIALS AND METHODS

Experimental animals: Male albino wistar rats weighing between 200-250 g and albino mice (25-30 g) were used. Institutional Animal Ethics Committee approved the

Corresponding Author: Vipin Kumar Garg, Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, NH-58, Baghpat By-pass Crossing, Delhi-Haridwar Highway, Meerut-250005, Uttar Pradesh, India Tel: +91 9719564736

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).

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. The study started on 02/11/2009.

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. The aqueous extract was concentrated to dryness using Rotary evaporator, giving yield as 12.10% w/v and preserved in a refrigerator. Aliquot portions of the aqueous extract of *Cynodon dactylon* (AqECD) were weighed and suspended in an appropriate volume of Tween 80 (2% v/v) for use on each day.

Acute toxicity study of the extract: Adult albino mice (25-30 g) were divided into five groups each containing ten mice. The mice were fasted for 6 h with only access to water *ad libitum* before experimental study. Group I, II, III and IV animals were administered with various doses of AqECD i.e., 1000, 2000, 3000 and 4000 mg kg⁻¹. Group V received only vehicle (Tween 80, 2% v/v in saline). All the doses and vehicle were administered orally. The animals were observed for 72 h for mortality (Ravichandran *et al.*, 2007).

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, ethanolic extract showed positive tests for the presence of glycosides, flavonoids and alkaloids. However, aqueous extract showed positive tests for glycosides and flavonoids only. As traditionally, the aqueous paste or the aqueous extract of the plant is used to cure inflammation, the anti-inflammatory activity of the aqueous extract of the plant in different dose levels (200, 400 and 600 mg kg⁻¹) is being reported here.

Anti-inflammatory activity

Acute inflammation

a) Carrageenan induced rat paw oedema: The method used was similar to that described by Muniappan and Sundararaj (2003) and Winter *et al.* (1962). The paw volume was measured initially and then at 1, 3 and 5 h after the carrageenan injection by using a mercury plethysmometer. The test groups received aqueous extract of *Cynodon dactylon* at different dose levels, standard group received Indomethacin (10 mg kg⁻¹) (Akindele and Adeyemi, 2007; Khalil *et al.*, 2006) and the control group received only the vehicle. These groups were studied with reference to the control groups. All the treatments were made orally via gavage 1 h before the injection of carrageenan.

b) Serotonin induced rat paw oedema: The method used was similar to that described by Mandal *et al.* (2000) and Maity *et al.* (1998). The treatments of test, standard and control groups were the same as above.

c) Histamine induced rat paw oedema: The method used was similar to that described by Mandal *et al.* (2000) and Maity *et al.* (1998). The treatments of test, standard and control groups were the same as above.

d) Dextran induced rat paw oedema: The method used was similar to that described by Mandal *et al.* (2000) and Maity *et al.* (1998). The treatments of test, standard and control groups were the same as above.

In all the above models, % inhibition of Oedema was calculated as follows:

$$\% \text{ Inhibition of Oedema} = (1 - V_t / V_c) \times 100$$

where, V_t is the inflammatory increase in paw volume of the rats of treated groups.

V_c is the inflammatory increase in paw volume of the rats of control groups.

Sub-Chronic inflammation

Cotton pellet granuloma: The method used was similar to that described by Mujumdar and Misar (2004) and D'Arcy *et al.* (1960).

Percentage inhibition of Granuloma Pouch in rats was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

Statistical analysis: 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 (Kulkarni, 1993).

RESULTS

Phytochemical screening: Phytochemical screening of the aqueous extract of *Cynodon dactylon* showed the presence of flavonoids and glycosides as shown in Table 1.

Acute toxicity study of the extract: The AqECD was found to be safe at all the doses used and there was no mortality up to the dose of 4000 mg kg⁻¹ of extract when administered orally. Therefore, we have taken 400 mg kg⁻¹ as the therapeutic dose and made variations by taking 200 mg kg⁻¹ as lower dose and 600 mg kg⁻¹ as higher dose.

Anti-inflammatory activity

a) Carrageenan induced rat paw oedema: In carrageenan induced rat paw oedema model, the aqueous extract of *Cynodon dactylon* has shown dose dependent activity. Although the extract has shown significant activity in all the three doses used but maximum activity has been shown at a dose of 600 mg kg⁻¹ (47.69% edema inhibition at 3 h) as compared to Indomethacin (49.23% inhibition at 3 h Table 2).

b) Serotonin induced rat paw oedema: In this model also the aqueous extract of *Cynodon dactylon* has shown dose dependent activity. Although the extract has shown significant activity in all the three doses used

Table 1: Phytochemical screening of different extracts of *Cynodon dactylon*

Tests	Petroleum ether extract	Benzene extract	Chloroform extract	Ethanol extract	Aqueous extract
Tests for sterols					
Test solution+Conc.H ₂ SO ₄	-ve	-ve	-ve	-ve	-ve
Libermann buchard's					
Test	-ve	-ve	-ve	-ve	-ve
Test solution+sulphur	-ve	-ve	-ve	-ve	-ve
Salkowski test	-ve	-ve	-ve	-ve	-ve
Tests for glycosides					
Keller killiani's test	-ve	-ve	-ve	+ve	+ve
Balget's test	-ve	-ve	-ve	+ve	+ve
Bromine water test	-ve	-ve	-ve	+ve	+ve
Legal's test	-ve	-ve	-ve	+ve	+ve
Raymonds test	-ve	-ve	-ve	+ve	+ve
Test for saponins					
Haemolytic test	-ve	-ve	-ve	-ve	-ve
Foam test	-ve	-ve	-ve	-ve	-ve
Test for tannins					
Gelatin test	-ve	-ve	-ve	-ve	-ve
Ferric test	-ve	-ve	-ve	-ve	-ve
Test for alkaloids					
Dragendroff's test	-ve	-ve	-ve	+ve	-ve
Mayer's test	-ve	-ve	-ve	+ve	-ve
Hanger's test	-ve	-ve	-ve	+ve	-ve
Wagner's test	-ve	-ve	-ve	+ve	-ve
Test for carbohydrates					
Barfoed's test	-ve	-ve	-ve	-ve	-ve
Benedict's test	-ve	-ve	-ve	-ve	-ve
Molisch's test	-ve	-ve	-ve	-ve	-ve
Test for flavonoids					
Shinoda test	-ve	-ve	-ve	+ve	+ve
Alkaline reagent test	-ve	-ve	-ve	+ve	+ve
Ferric chloride test	-ve	-ve	-ve	+ve	+ve
Lead acetate test	-ve	-ve	-ve	+ve	+ve
Zn-HCl reduction test	-ve	-ve	-ve	+ve	+ve

+ve: Indicates positive result, -ve: Indicates negative result

Table 2: Effect of different doses of aqueous extract of *Cynodon dactylon* on carrageenan induced rat paw oedema

Groups	Dose (mg kg ⁻¹)	Time after carrageenan injection					
		1 h		3 h		5 h	
		EV (mL)	EI (%)	EV (mL)	EI (%)	EV (mL)	EI (%)
Control		0.21±0.0055		0.65±0.0053		0.55±0.0060	
Indomethacin	10	0.13±0.0036 ^d	38.09	0.33±0.0055 ^d	49.23	0.30±0.0074 ^d	45.45
AqECD	200	0.17±0.0085 ^c	19.04	0.50±0.0106 ^d	23.07	0.46±0.012 ^d	16.36
AqECD	400	0.15±0.0047 ^d	28.57	0.38±0.0099 ^d	41.53	0.35±0.0137 ^d	36.36
AqECD	600	0.14±0.0036 ^d	33.33	0.34±0.0057 ^d	47.69	0.31±0.0071 ^d	43.63

Values are expressed as Mean±SEM. (n = 6), ^ap<0.05, ^bp<0.02, ^cp<0.01, ^dp<0.001 as compared to control group. EV: Edema volume, EI: Edema inhibition

Table 3: Effect of different doses of aqueous extract of *Cynodon dactylon* on serotonin induced rat paw oedema

		Time after serotonin injection					
		1 h		3 h		5 h	
Groups	Dose (mg kg ⁻¹)	EV (mL)	EI (%)	EV (mL)	EI (%)	EV (mL)	EI (%)
Control		0.24±0.0062		0.64±0.0057		0.51±0.0075	
Indomethacin	10	0.15±0.0068 ^d	37.50	0.30±0.0049 ^d	53.12	0.26±0.0080 ^d	49.01
AqECD	200	0.18±0.0095 ^d	25.00	0.41±0.0094 ^d	35.93	0.36±0.0081 ^d	29.41
AqECD	400	0.16±0.0101 ^d	33.33	0.35±0.0055 ^d	45.31	0.30±0.0047 ^d	41.17
AqECD	600	0.15±0.0072 ^d	37.50	0.32±0.0089 ^d	50.00	0.27±0.0106 ^d	47.05

Values are expressed as Mean±SEM (n = 6), ^ap<0.05, ^bp<0.02, ^cp<0.01, ^dp<0.001 as compared to control group. EV: Edema volume, EI: Edema Inhibition

Table 4: Effect of different doses of aqueous extract of *Cynodon dactylon* on histamine induced rat paw oedema

		Time after histamine injection					
		1 h		3 h		5 h	
Groups	Dose (mg kg ⁻¹)	EV (mL)	EI (%)	EV (mL)	EI (%)	EV (mL)	EI (%)
Control		0.22±0.0057		0.60±0.0097		0.53±0.0073	
Indomethacin	10	0.13±0.0072 ^d	40.90	0.30±0.0055 ^d	50.00	0.27±0.0102 ^d	49.05
AqECD	200	0.20±0.0094	9.09	0.43±0.0169 ^d	28.33	0.38±0.0094 ^d	28.03
AqECD	400	0.18±0.0083 ^c	18.18	0.37±0.0145 ^d	38.33	0.33±0.0096 ^d	37.73
AqECD	600	0.15±0.0081 ^d	31.81	0.33±0.0095 ^d	45.00	0.30±0.0102 ^d	43.39

Values are expressed as Mean±SEM (n = 6), ^ap<0.05, ^bp<0.02, ^cp<0.01, ^dp<0.001 as compared to control group. EV: Edema volume, EI: Edema inhibition

Table 5: Effect of different doses of aqueous extract of *Cynodon dactylon* on dextran induced rat paw oedema

		Time after dextran injection					
		1 h		3 h		5 h	
Groups	Dose (mg kg ⁻¹)	EV (mL)	EI (%)	EV (mL)	EI (%)	EV (mL)	EI (%)
Control		0.20±0.0070		0.64±0.0096		0.56±0.0093	
Indomethacin	10	0.14±0.0057 ^d	30.00	0.34±0.0060 ^d	46.87	0.31±0.0060 ^d	44.64
AqECD	200	0.18±0.0094	10.00	0.56±0.0097 ^d	12.50	0.54±0.0070	3.57
AqECD	400	0.16±0.0106 ^b	20.00	0.42±0.0127 ^d	34.37	0.45±0.0105 ^d	19.64
AqECD	600	0.15±0.0093 ^c	25.00	0.37±0.0106 ^d	42.18	0.33±0.0106 ^d	41.07

Values are expressed as Mean±SEM (n = 6), ^ap<0.05, ^bp<0.02, ^cp<0.01, ^dp<0.001 as compared to control group. EV: Edema volume, EI: Edema inhibition

Table 6: Effect of different doses of aqueous extract of *Cynodon dactylon* on cotton pellet granuloma pouch in rats

Groups	Dose (mg kg ⁻¹)	Dry Wt. of cotton pellet induced granuloma (mg)	Inhibition (%)
Control		37.88±0.0440	
Indomethacin	10	20.35±0.0449 ^d	46.27
AqECD	200	32.91±0.0636 ^d	13.12
AqECD	400	21.95±0.0650 ^d	42.05
AqECD	600	20.30±0.0344 ^d	46.40

Values are expressed as Mean±SEM (n = 6), ^ap<0.05, ^bp<0.02, ^cp<0.01, ^dp<0.001 as compared to control group

but maximum activity has been shown at a dose of 600 mg kg⁻¹ (50.00% edema inhibition at 3 h) as compared to Indomethacin (53.12% inhibition at 3 h Table 3).

c) Histamine induced rat paw oedema: In this model also the aqueous extract of *Cynodon dactylon* has shown dose dependent activity. Although the extract has shown significant activity in all the three doses used but maximum activity has been shown at a dose of 600 mg kg⁻¹ (45.00% edema inhibition at 3 h) as compared to Indomethacin (50.00% inhibition at 3 h Table 4).

d) Dextran induced rat paw oedema: In this model also the aqueous extract of *Cynodon dactylon* has shown dose dependent activity. Although the extract has shown significant activity in all the three doses used

but maximum activity has been shown at a dose of 600 mg kg⁻¹ (42.18% edema inhibition at 3 h) as compared to Indomethacin (46.87% inhibition at 3 h) as shown in Table 5.

Cotton pellet granuloma: The % inhibition in the granuloma weight shown by the extract at different doses was 13.12% at 200 mg kg⁻¹, 42.05% at 400 mg kg⁻¹ and 46.40% at 600 mg kg⁻¹ as compared to 46.27% shown by the standard drug, Indomethacin as shown in Table 6.

DISCUSSION

The present study establishes the anti-inflammatory activity of the aqueous extract of *Cynodon dactylon* in the models used. It is evident that carrageenan induced

oedema is commonly used as an experimental animal model of acute inflammation and it is believed to be biphasic of which the first phase is mediated by release of histamine and serotonin in the early phase followed by kinin release and then by prostaglandin in the later phase (Castro *et al.*, 1968; Jothimamivannan *et al.*, 2010). Histamine induced inflammation model is used to study the anti-inflammatory activity of various agents. Histamine is one of the most important mediators of inflammation. Histamine increase vascular permeability and act with prostaglandins to induce edema (Jain and Bari, 2010).

The extract effectively suppressed the inflammation produced by histamine and serotonin. So it may be suggested that its anti-inflammatory activity is possibly backed by its anti-serotonin activity which is responsible for the same. Also as seen from the results the extract has suppressed the inflammation till 5 h in all the models used. This shows its efficacy to suppress the later phase of inflammation produced by kinins and prostaglandins. The extract also reduced the oedema produced by dextran which is known to be mediated both by histamine and serotonin (Ghosh *et al.*, 1963). So, we can say that the extract is effective in suppressing both acute and later phases of inflammation mediated by histamine, serotonin, kinin and prostaglandins.

The extract exhibited significant anti-inflammatory activity in the cotton pellet test. The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation. Monocyte infiltration and exudation takes place in chronic inflammation. This proliferation becomes widespread by proliferation of small vessels or granuloma. NSAIDs decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration/inflammation, preventing generation of collagen fibres and suppressing mucopolysaccharides. The extract exhibited significant anti-inflammatory activity in the cotton pellet induced granuloma pouch method. This may be due to its efficacy to inhibit the increase in number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation (Narendhirakannan, 2007).

From the above results, it can be deduced that aqueous extract has shown dose dependent activity. Phytochemical screening has shown the presence of flavonoids and glycosides in aqueous extract. The potent activity may be attributed to the presence of these phytoconstituents. The ability of the extract to cause edema inhibition produced by these inflammatory mediators suggests that it contains phytochemically active constituent (s) with anti-inflammatory properties

(Umukoro and Ashorobi, 2006). Anti-inflammatory activity of flavonoids has been reported in many animal models (Jadhav and Kharya, 2005). Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediators of inflammation (Sawadogo *et al.*, 2006). More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism of action.

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