

**IN VIVO EVALUATION OF ANTIDIARRHOEAL
ACTIVITY OF AERIAL PARTS OF *CYNODON
DACTYLON* LINN. PERS. (POACEAE)**

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Summary

The aerial parts as well as rhizomes of *Cynodon Dactylon* are used in traditional medicine for the treatment diarrhea. Thus the aqueous extract of aerial parts *Cynodon dactylon* (poaceae) was investigated for its anti-diarrhoeal property in Wistar rats to substantiate folkloric claim.

Aq. extract of aerial parts of *Cynodon dactylon*, at graded dose (250 & 500mg/kg body weight) was investigated for anti-diarrhoeal activity in term of reduction in the rate of defecation and consistency of faeces in castor oil induced diarrhea. To understand the mechanism of its anti-diarrhoeal activity, its effect was further evaluated on intestinal transit and castor oil induced intestinal fluid accumulation (enteropooling).

At various doses (250 & 500mg/kg body weight) the extract showed a remarkable anti-diarrhoeal activity evidenced by the reduction of the rate of defecation and consistency of faeces. Results are comparable to that of standard drug loperamide (50 mg/kg body weight). A single oral dose of Cynodon dactylon extract of 250mg/kg body weight produced a significant decrease in the severity of diarrhea. Extract produced profound decrease in intestinal transit (39.66%) also significantly inhibited castor oil induced enteropooling comparable to that of intraperitoneal injection of standard drug atropine sulphate at a doses of 0.1mg/kg body weight and 3 mg/kg body weight respectively. Experimental findings showed that aqueous extract of aerial parts of Cynodon dactylon possess significant anti-diarrhoeal activity and may be a potent anti-diarrhoeal drug in future.

Key words: Cynodon dactylon, anti-diarrhoeal activity, castor oil, atropine sulphate.

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Introduction

Diarrhoea is characterized by increased frequency of bowel movement, wet stool, and abdominal pain¹. It is a leading cause of malnutrition and death among children in the developing countries of the world today². Many governments and international organizations are trying to control this disease but the rate of incidence is still high, about 7.1 million per year³. Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhea but they have some side effects⁴. The natural drugs are used as anti-diarrhoeal drugs, which are not always free from side effects. Therefore, the search for safe and more effective agents has continued to be important areas of active research. Since ancient times, diarrhea has been treated orally with several medicinal plants or their extracts based on folklore medicines.

The grass *Cynodon dactylon* (Family: Poaceae) grows throughout India up to a height above sea level of 8,000ft. The plant is also known by the name of Bermudagrass. A hardy perennial grass with creeping culms, rooting at nodes and forming spreading mats on the surface of the soil⁵. It is abundant on road sides and paths, and readily takes possession of any uncultivated area. It flowers nearly throughout the year. The aerial parts and rhizomes of *Cynodon dactylon* was reported for its cardioprotective action⁶, antibacterial, antimicrobial⁷, antioxidant, wound healing, anti diabetic, diuretic effects. *Cynodon dactylon* is reported to contain cynodin, hydrocyanic acid and triticin. It also contains proteins, carbohydrates, fibers, Ca, Fe, K and β -carotene. The plant is traditionally used for jaundice, diuretics, and astringent, to stop bleeding in piles, skin infections in India at West Bengal, Assam, Manipur, and Mizoram parts⁸. The present study was undertaken to evaluate the antidiarrhoeal potential of aqueous extract of aerial parts of *Cynodon dactylon* in normal and castor oil induced diarrhoeal rats.

Materials and Methods

Plant material

Aerial parts of *Cynodon dactylon* pers. (Family: Poaceae) were collected from the hilly region of Pune, India. The plant was authenticated by Agharkar Research Institute, Pune⁹.

Preparation of extract

The fresh aerial parts of *Cynodon dactylon* was collected for extraction. The green plant up to 500 g was extracted with boiling water for 48 hr. The resulting extract was filtered and concentrated by evaporating the solvent. The concentrated extract was then used for further activity¹⁰.

Animals

Wister albino rats (180-220g) of either sex were selected for the experiments. Animals were allowed to be acclimatized for a period of 2 weeks in our laboratory environment prior to the study. Animals were housed in polypropylene cages (4 animals per cages), maintained under standard laboratory conditions (i.e.12:12 hour light and dark sequence; at an ambient temperature of 25±2.c; 35-60%humidity); the animals fed with standard rat pallet diet and water ad libitum. The principles of Laboratory Animal Care (NIH, 1985) were followed and instructions given by our institutional animal ethical committee were maintained throughout the experiment.

Chemicals and Reagents

Atropine sulphate and loperamide (standard reference antidiarrhoeal drugs), castor oil(laxative agents), normal saline solution (0.9%Nacl), charcoal meal(10%activated charcoal in 5%gum acacia) and vehicle(2%v/v Tween 80 in distilled water) were used.

Castor oil induced diarrhea

Rats were fasted for 18 h and divided into four groups of six animals per group. Castor oil at a dose of 1 ml/animal orally, was given to all groups of animals for the induction of diarrhoea¹¹.

Thirty minutes after castor oil administration, the first group (control group) received vehicle (0.5%v/v Tween 80 in distilled water), while second and third group were given aqueous extract at doses of 250 and 500mg/kg body weight respectively by oral route. Loparamide (50mg/kg body weight)¹². Animals of groups were placed separately in individual cages lined with filter paper. The filter papers were changed every hour and the severity of diarrhoea was assessed hourly for six hours. The total number of faeces were recorded within a period of six hour and compared with the control group. The total number of diarrhoeal faeces of the control group was considered 100%. The results were expressed as percentage of inhibition of diarrhea¹³.

Gastrointestinal motility test

This test was done by using charcoal meal as a diet¹⁴. The rats were divided into 5 groups of six animals each and fasted for eighteen hours before the experiment. The first group (the control group) was orally administered the vehicle (0.5% Tween 80 in distilled water). The second and third group orally receives Aq. Extract of *C. dactylon* at doses of 250 and 500gm/kg respectively. The fourth group received the standard drug, atropine sulphate (0.1 mg/kg body weight i.p.). Thirty min later each animal was given 1 ml of charcoal meal (10% activated charcoal in 5% gum acacia) orally. Each animal was sacrificed thirty min after administration of charcoal meal. The distance covered by the charcoal meal in the intestine was expressed as a percentage of the total distance traveled from the pylorus to the caecum¹⁵.

Castor oil –induced enterpooling

Intraluminal fluid accumulation was determined by the method of Boominathan et al. 2005. Over night fasted rats were divided into five groups of six animals each. Group 1 which received normal saline (2 ml/kg i.p.) served as control group. Group 2 received atropine 3mg/kg i.p.) And group 3 & 4 received extract of 250 and 500 g/kg i.p. respectively, one hour before the oral administration of castor oil (1 ml).

Two hours later, the rats were sacrificed: the small intestine contents was collected after tying the ends with threads and weighed. The intestinal content was collected by milking into a graduated cylinder and their volume was measured. The intestine was reweighed and the difference between the full and empty was calculated.

Statistical analysis

Data were analyzed by one way ANNOVA followed by Dunnett's t-test using computerized GraphPad I stat version 3.

Results

The Aq. Extract of *C. dactylon* was found to be effective against castor oil induced diarrhea on experimental rats at various doses of 250 & 500mg/kg bw has been shown in table 1. A single oral dose of Aq. extract 250 mg/kg body weight produced significance decrease in the severity of diarrhea in terms of reduction in the rate of defecation and consistency of faeces on Wistar albino rats. The percentage inhibition for the number of wet faeces as well as wet mass indicates the presence of antidiarrhoeal activity in the extract as compared with that of control group. Experimental results reflect the activity is more pronounced at the dose of 250 mg/kg than 500 mg/kg body weight. The percentage inhibition of number of wet faeces as well as mass found 54.98% and 70.11% respectively at the dose of 250 mg/kg bw very much comparable to that of standard drug loperamide (50mg/kg bw). The Aq. Extract produced profound decrease in intestinal transit of 39.66% at the dose of 250 mg/kg bw and significantly inhibited castor oil induced enterpooling in terms of volume and weight of intestinal content comparable to that of intraperitoneal injection of standard drug atropine sulphate at a dosed of 0.1 mg/kg bw respectively as indicated in tab 2 & 3.

Table 1-Effect of the Aqueous extract of *C. dactylon* at different dose levels on castor oil induced diarrhea.

<i>Group</i>	<i>Total number of diarrhoeal faeces (g)</i>	<i>Inhibition (%)</i>	<i>Total weight of faeces(g)</i>	<i>Inhibition (%)</i>
Castor oil(1ml)+ vehicle(0.5%Tween 80)	13.33±3.41	0.00	3.38±0.32	0.00
Loparamide(50mg/kg)+ castor oil(1ml)	3.66±1.89	72.46	0.98±0.17	71.83
Aq.extract(250mg/kg)+ castor oil(1ml)	6.00±1.46	54.98**	1.04±0.15	70.11
Aq.extract(500mg/kg)+ castor oil(1ml)	7.83±2.42	41.26*	1.20±0.07	65.22

Values are expressed as mean ± S.E.M (n=6). **p≤0.01, *p≤0.05 when compared with control.

Table 2- Effect of aqueous extract of *C. dactylon* at different dose levels on charcoal-induced gut transit changes.

Group	Distance travelled by charcoal meal	Inhibition (%)
Vehicle(0.5% Tween 80)(control)	69.33±2.44	0.00%
Atropin sulphate (0.1mg/kg)	29 ±1.65	58.17%
Aq.extract(250mg/kg)	41.83±2.77	39.66%
Aq.extract (500mg/kg)	51.66±1.70	25.47%

Values are expressed as mean ±S.E.M (n=6) ** p≤ 0.01 when compared with vehicle-control

Table 3- Effect of Aqueous extract of *C. dactylon* at different dose level on castor oil enterpooling.

Group	Volume of intestinal content(ml)	Weight of intestinal content(g)
Normal saline +castor oil(1ml)	4.10 ±0.18	3.935 ±0.45
Atropin sulphate +castor oil(1ml)	1.59 ±0.17	1.89 ±0.16
Aq. extract(250mg/kg) +castor oil(1ml)	1.94 ±0.14	2.09 ±0.15
Aq. extract(500mg/kg) +castor oil(1ml)	2.10±0.11	2.50 ±0.16

Values are expressed as mean ± S.E.M (n=6). p≤0.01 when compared with control.

Discussion

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry resulting in the excess loss of fluid in the faeces. In some diarrhea the secretory components predominates while other diarrhoea is characterized by hyper motility¹⁶. Castor oil causes diarrhea due to its active metabolites, ricinoleic acid^{17, 18}, which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin¹⁹. Castor oil reported to induce diarrhoea by increasing the volume of intestinal contents by preventing the reabsorption of water. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin²⁰. In this study, Aq. Extract of aerial parts of *C. dactylon* exhibited a significant dose dependant antidiarrhoeal activity. The results were comparable to that of the standard drug loparamide (50mg/kg) with regard to the severity of diarrhoea.

Aqueous extract of aerial parts of *C. dactylon* also significantly reduced intestinal transit as observed by decrease in transit motility of charcoal meal. This may be due to the fact that the extract may increase the reabsorption of water by decreasing intestinal motility as observed in the decreasing intestinal motility as observed in the weight and the volume of intestinal contents on castor oil induced enterpooling. Above observation suggest that the extract at 250 g/kg body weight reduces the diarrhoea by inhibiting peristalsis, gastrointestinal motility and castor oil induced enterpooling. It is equally effective in the prevention and curing of diarrhoea. Phytochemical screening revealed the presence of steroid (Liebermann-Burchard test showed positive results) ,glycosides (Borntragers & modified Borntragers test both showed positive test) and alkaloids (Wagner's test showed positive) in the Aq. Extract²¹. Steroids and glycosides are useful for the treatment of diarrhoea and also may enhance intestinal absorption of Na and water .Hence, steroid or glycosides ay be responsible for the antidiarrhoeal activity.

Conclusion

In conclusion, the results of this investigation revealed that Aq. Extract of aerial parts of *C. dactylon* contains active substances with antidiarrhoeal properties. This provides the rationale for the use of extract of aerial parts of *C. dactylon* as an anti-diarrhoeal drug by traditional healers. Further research is to be carried out to fractionate and purify extract, in order to find out the molecule responsible for the anti-diarrhoeal activity observed.

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