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Antidiarrheal Activity of *Cynodon Dactylon.* pers.

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ABSTRACT

Cynodon dactylon pers (fam. Poaceae) whole plant is used in traditional system of medicine for the treatment of diarrhea. In the present investigation hexane, dichloromethane, ethyl acetate and methanol extracts of *Cynodon dactylon* whole plant were tested for anti diarrheal activity on castor oil induced diarrhea, gastro intestinal motility by charcoal meal and entero pooling models in albino rats. Methanol extract exhibited considerable reduction in inhibition of castor oil induced diarrhea. Methanol extract also showed a significant decrease in gastrointestinal motility by charcoal meal and decrease in weight of intestinal contents in enteropooling models. These results indicate that the plant possess good anti diarrheal activity.

KEYWORDS: Anti diarrheal activity, Castor oil, charcoal meal, *Cynodon dactylon.*

INTRODUCTION

Cynodon dactylon. pers (Poaceae) commonly known as Bermuda grass is a highly branched perennial grass, rooting at every node with narrow leaves, native of south Africa, south Europe and found distributed through out India (1). In traditional medicine it is claimed to be useful in diarrhea, dysentery, wounds, hemorrhages, diuresis and hyperdypsia (2). Fresh juice of plant is demulcent, astringent and useful in case of dropsy, anasarca, catarrhal opthalmia, secondary syphilis, chronic diarrhea and dysentery (3). The expressed juice of plant is used in hysteria, epilepsy and insanity (4). Phytoconstituents reported from this plant are Flavonoids- apigenin, luteolin, orientin and vitexin (5–6), Carotenoids – beta carotene, neoxanthin, violaxanthin (7), Phenolics (8), and Volatile oils (9).The alcohol extract of whole plant has been reported for anti microbial (10–11), Diuretic (12), antioxidant (13–14), anticonvulsant (15), wound healing (16), antiulcer (17), chondrioprotective (18), antiviral (19), antihyperglycemic and anti hyperlipidemic (20)activities. The present study was undertaken to verify the traditional claim of antidiarrheal activity of *Cynodon dactylon* whole plant.

MATERIALS AND METHODS

Plant material

The whole plant of C *ynodon dactylon* was collected from G. Pulla Reddy College of Pharmacy campus, Hyderabad, Andhrapradesh, India, in the month of September 2006. The Plant was authenticated by Dr. Prabhakar Reddy, taxonomist, Osmania University and a voucher specimen CD-12-06 has been deposited in Pharmacognosy and Phytochemistry Laboratory.

Preparation of extracts

Whole plant of *Cynodon dactylon* was washed, air dried and powdered. The powder was extracted successively with

n-hexane, dichloromethane, ethyl acetate and methanol using soxhlet apparatus and concentrated under vaccum.

Phytochemical screening

A preliminary phytochemical screening of all extracts was carried out to know various constituents present as per the standard procedures (21–22).

Test animals

Wistar rats (180–220 g) of either sex were procured from Mahaveera enterprises (Regn no: 146/1999/CPCSEA), Hyderabad and were housed in polypropylene cages at temperature of 25 ±2° C and relative humidity 44–56% for one week before and during experimentation. The animals were fed with standard pellet diet and water *ad libitum*. Before the experimental study the animals were fasted over night with free access to water. The animals were deprived of water during experimentation.

Acute oral toxicity

Acute oral toxicity was performed as per OECD-420 (23) guidelines. The test was performed in rats divided into different groups of 5 animals each. The animals are dosed in stepwise procedure using fixed doses of 5, 50, 300, 2000 mg/kg for each extract and observed for gross behavioural changes, body weight change and mortality. All the animals were observed to be normal compared to control group of animals and there was no mortality.

ANTI DIARRHEAL ACTIVITY

Castor oil induced diarrhea (24–26)

The animals were divided into 10 groups of 6 animals in each. Group I served as control and received 1 % aqueous Tween solution. Group II–IX received hexane, dichloromethane, ethyl acetate and methanol extract at dose level of 200 and 300 mg/kg as fine suspension in Tween 80 orally (25). Group X received standard drug Atropine sulphate 5mg/kg orally (25–31).

After one hour, all the groups received 1ml of castor oil orally and each rat was then housed separately in the cages provided with a clean plastic sheet at the bottom. The total weight of feces was determined every hour up to 4 hours and compared with control group

The mean weight of feces was calculated and expressed as % inhibition of diarrhea using formula.

Convert this Microsoft equation

$$\%\,Inhibition\ of\ diarrhea = \frac{Mean\ weight\ Of\ defecation\ in\ control\ group - Mean\ weight\ of\ defecations\ in\ testgroup}{Mean\ weight\ of\ defecations\ in\ control\ group} * 100$$

Gastrointestinal transit by charcoal meal test (25–26)

The activity was evaluated by measuring the length of distance travelled by charcoal meal from pyloric end of stomach in rats. The rats were divided into 4 groups of 6 animals in each. Group I served as control and received 1 % aqueous Tween solution orally. Group II and III received methanol extract orally at dose of 200 and 300 mg/kg respectively .Group IV received standard drug Atropine sulphate 5 mg/kg orally (29–31). After one hour, each animal was given 1 ml of charcoal meal (5 % charcoal in 10 % aqueous acacia) orally. All the animals were sacrificed one hour after administration of charcoal meal, peritoneal cavity was cut open and distance travelled by charcoal meal was measured from pylorus to ceacum.

Convert this Microsoft equation

% Intestinal transit was calculated using formula.

$$\%\ \text{Intestinal transit} = \frac{\text{Distance traveled by the charcoal}}{\text{Total length of intestine}} * 100$$

Castor oil induced enteropooling (25–26)

In entero pooling method animals were divided in to 5 groups of six animals each. Group I received 1 % aqueous Tween solution served as control. Group II received standard atropine sulphate 5 mg/kg orally. Group III and IV received methanol extract 200 and 300 mg/kg orally respectively. One hour later all groups received 1 ml castor oil orally. Two hours later rats were sacrificed and whole length of intestine from pylorus to ceacum was removed by tying the ends with threads. Intestinal fluid was collected and weighed. The percentage inhibition of weight of intestinal contents was calculated as

Convert this Microsoft equation

$$\%\ \text{Inhibition of Contents} = \frac{\text{Mean weight of intestinal fluid in Control grou} - \text{Mean weight of intestinal fluid in test group}}{\text{Mean weight of intestinal fluid in control group}} * 100$$

Statistical analysis

The results are expressed as mean ± S.E.M. The statistical differences between the means were determined by performing the one-way ANOVA followed by Dunnet's post hoc test. $P < 0.01$ were considered significant.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical screening of *Cynodon dactylon* extracts showed the presence of flavonoids,

Table 1. Phyto chemical screening of different extracts of Cynodon dactylon.

Phytoconstituents	Hexane extract	Dichloro methane extract	Ethyl acetate extract	Methanol extract
Steroids/Triterpenoids	+	+	–	–
Flavonoids	–	–	+	+
Tannins & glycosides	–	–	–	+
Cardiac glycosides	+	+	–	–
Carbohydrates	–	–	–	+
Proteins	–	–	+	+
Fixed oils and fats	+	+	–	–

"–" *Absent.*; "+"*Present.*

Table 2. Effect of hexane, dichloromethane, ethyl acetate and methanol extracts of Cynodon dactylon on castor oil induced diarrhea.

Treatment (Dose)	Mean weight of wet faeces (gms)	% Inhibition of Diarrhea
Control (1% Tween solution)	6.1275 ± 0.81	–
Atropine sulphate (5 mg/kg)	1.1340 ± 0.51	81.76**
Hexane extract 200 mg/kg	4.5400 ± 0.76	31.25
Hexane extract 300 mg/kg	4.2280 ± 0.53	31.00
Dichloromethane extract 200 mg/kg	5.2250 ± 0.52	14.51
Dichloromethane extract 300 mg/kg	4.4850 ± 0.47	25.91
Ethyl acetate extract 200 mg/kg	5.4323 ± 0.71	12.63
Ethyl acetate extract 300 mg/kg	4.3450 ± 0.61	30.11
Methanol extract 200mg/kg	1.5150 ± 0.35	75.63**
Methanol extract 300mg/kg	1.2750 ± 0.47	80.66**

Results are expressed as mean ± S.E.M from 6 animals.;
***P < 0.001 compared to control group.*

steroids/ triterpenoids, carbohydrates, fixed oils, fats and phenolic glycosides (Table 1).

Castor oil induced diarrhea

In this method methanol extract reduced the weight of faeces considerably and almost equal to that of the standard drug atropine sulphate. At doses of 200 mg and 300 mg/kg the percentage of inhibition of diarrhea was 73.89% and 82.76% respectively as compared to atropine sulphate (86.94%). However hexane, dichloromethane and ethyl acetate extracts did not show considerable activity (Table 2).

Gastrointestinal motility by charcoal meal test

Methanol extract was tested on gastrointestinal motility by charcoal meal test. The results shown that methanol extract at doses of 200mg/kg and 300mg/kg caused profound decrease in intestinal transit time by 55.67% and 49.73% respectively compared to control.Under similar experimental conditions the percentage decrease in intestinal transit time caused by Atropine sulphate was 39.93% at a dose 5mg/kg (Table 3).

Castor oil induced enteropooling

In enteropooling method also the methanol extract showed 48.25% inhibition of weight of intestinal contents at a

Table 3. Effect of methanol extract of cynodon dactylon on gastrointestinal motility.

Treatment	Total length of intestine (cms)	Distance traveled by charcoal meal (cms)	% Intestinal transit
Control (1% aqueous Tween solution)	76±2.88	49±1.65	64.73±2.39
Standard (Atropine sulphate 5 mg/kg)	80±2.46	31.5±3.15	39.93±4.73**
Methanol extract 200 mg/kg	75±3.25	42±2.98	55.67±1.59*
Methanol extract 300 mg/kg	81±2.96	42±2.98	51.72±2.82

Results are expressed as mean ± S.E.M from 6 animals.;
***P < 0.01 compared to control group.;*
**P < 0.05 compared to control group.*

Table 4. Effect of methanol extract of Cynodon dactylon on castor oil induced enteropooling.

Treatment	Weight of intestinal Contents (gms)	% Inhibition of weight of Intestinal contents
Control (1% aqueous Tween solution)	1.58±0.14	–
Standard (Atropine sulphate 5 mg/kg)	1.08±0.22	33.54**
Methanol extract 200 mg/kg	1.26±0.36	20.25**
Methanol extract 300 mg/kg	0.8175±0.03	48.25**

Results are expressed as mean ± S.E.M from 6 animals.;
***P < 0.001 compared to control group.*

dose of 300 mg/kg, compared to that of 33.54% shown by atropine sulphate (Table 4).

DISCUSSION

It is known that castor oil or its component recinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that result in hyper secretary response in the intestines leading to diarrhea (32–33). The experimental studies in rats demonstrated a significant increase in the portal venous PGE2 concentration and increase in secretion of water and electrolytes into small intestine following oral administration of castor oil (34–35). The ricinoleic acid from castor oil results in irritation and inflammation of intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion (36). Inhibitors of prostaglandins biosynthesis delayed castor oil induced diarrhea (37).

In the present study, extracts of *Cynodon dactylon* were evaluated for anti diarrheal activity against castor oil induced diarrhea in wistar rats. Methanol extract exhibited significant anti diarrheal activity against castor oil induced diarrhea in rats and is equal to the standard drug atropine sulphate 5 mg/kg (24–31). Where as hexane, dichloromethane and ethyl acetate extracts did not show considerable activity.

The GI motility test with activated charcoal meal was carried out to find out the effect of methanol extract on peristaltic movement. Methanol extract reduced the intestinal propulsive movement in the charcoal meal treated model. This action enhances the reabsorption of water and electrolytes and thus prevents the diarrhea

In enteropooling test also methanol extract significantly inhibited the weight of intestinal contents.

The preliminary phytochemical screening of the extracts revealed the presence of flavonoids, steroids/triterpenoids, carbohydrates, fixed oils, fats and phenolic glycosides. Isolation of flavonoids has been reported from plant (5–6). Previous reports have demonstrated that flavonoids (38), tannins (39), sterols and/or terpenes (40) possess antidiarrheal activity.

The anti diarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion (41–42), which are known to be altered in diarrhea. *Invitro* and *in vivo* experiments have shown that flavonoids are able to inhibit the intestinal secretory response, induced by PGE_2 (43). There are reports that flavonoids also modify mucosal permeability and inhibit intestinal peristalsis (44), there by helpful in controlling diarrhea. The presence of Tannins (phenolic glycosides) may also contribute to the anti diarrheal activity of methanolic extract, since tannins may precipitate the proteins of enterocytes; reduce peristaltic movement and intestinal secretions (45–46). As a consequence it's possible to suggest that anti secretory and antimotility properties of flavonoids along with tannins may contribute to the observed antidiarrheal activity of methanolic extract.

The results indicate that methanolic extract of *Cynodon dactylon* possess significant antidiarrheal activity due to their inhibitory effect on gastrointestinal propulsion and fluid secretion. This study has validated the use of Cynodon *dactylon* for the treatment of diarrhea in traditional medicine.

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REFERENCES

1. Sudarshan S. R., *Encyclopaedia of Indian Medicine* vol 4 (Popular Prakashan Pvt. Ltd, Bombay, 2006) 73.
2. Warrier P.K, Nambiar V.P.K and Raman Kutty C, *Indian Medicinal Plants* Vol **2**, (Orient Longman Limited, Hyderabad, 1994) 290.
3. Dr Nadkarni K.M. and Nadkarni A.K., *Indian Materia Medica* vol 1, (Popular Prakashan Pvt Ltd, Bombay, 1995) 425.
4. Caius Jean Ferdinand, *The Medicinal and Poisonous Plants of India* (Scientific publishers, Jodhpur, 1986) 71.
5. Johnson A W, Snook M E. and Wiseman B R. Green Leaf Chemistry of Various turfgrasses Differentiation and Resistance to Fall Army worm. *Crop science*. **42**: 2004(2002).
6. Nair G A. Flavonoids of cynodon dactylon. *Journal of medico ethno botanical research*. **16**(3-4): 153(1995).
7. Chen B H. and Bailey C A. Seperation of carotenoids in turfgrass bermudagrass by high performance liquid chromatography. *Journal of chromatography*. **393**(2): 297(1987).
8. Chou Chang -Hung and Young Chiu -Chung. Phytotoxic substances in twelve subtropical grasses. *Journal of Chemical Ecology*. **1**(2): 21, (1975).
9. Chapman G W., Burdick D., Higman H C. and Robertson JA. Steam volatiles from coastal Bermuda grass. *Jour. Sci. Fd. Agric*. **9**(4): 312 (1978).
10. Ahmed S S, Reza M S, Haider S S and Jabbar A. Anti microbial activity of *Cynodon dactylon* (linn).pers. *Fitoterapia*. **65**(5): 463–464 (1994).
11. Prem kumar V. and Shyam sunder D. Evaluation of anti microbial activities of *Cynodon dactylon*. *Indian drugs*. **41**(12): 748 (2004).
12. Artizzu, Bonsignore, cottiglia G. Diuretic and anti microbial activity of *Cynodon dactylon* (linn).pers essential oil. *Fitoterapia*. **67**(20): 174 (1996).
13. Auddy B., Ferreira M., Blasina F., Lafon L., Arredondo F., Dajas F., Tripathi P.C., Seal T., and Mukherjee B. Screening of anti oxidant activity of three Indian medicinal plants, traditionally used for the management for neurodegenerative diseases. *J Ethnopharmacol*. **84**(2): 131 (2003).
14. Jagetia, Chandra Ganesh and baliya Manjeswar. The evaluation of nitric oxide scavenging activity of certain medicinal plants. *Journal of medicinal food*. **7**(3): 343 (2004).
15. Odenigbo G., Obi P. and Awachi Ifeachoo. *Anti convulsant activity of aqueous ethanolic extracts of Cynodon dactylon Fitoterapia*. **64**(5): 447(1993).
16. Subramanian S. and Nagarajan S. Wound healing activity of *Pongamia pinnata* and *Cynodon dactylon* (linn). *Fitoterapia*. **59**(1): 43–47 (1988).
17. Patil M., Jalalpure S.S., Prakash N.S and Kokate C.K. Anti ulcer properties of *Cynodon dactylon* extracts in rats. *Acta Hort. (ISHS)*. **680**: 115 (2005).
18. Poonguzhali K P, Hariprasad C, Chandrashekharan A N, Gowri C and Ganesan N, Effect of *Cynodon dactylon* and tenoxicam on the lysosomal

enzyme activities in the cartilage tissue of osteoarthritic guinea pigs. *Journal of clinical biochemistry and nutrition*. **24**(3):141(1998).

19. Balasubramanian G, Sarathi M, Rajesh kumar S and Sahul hameed A S, Screening the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp. *Aquaculture*. **263**(1–4): 15 (2007).
20. Mahesh N and Brahateewaran. Anti-hyperglycemic activities of aqueous and ethanolic extracts of *Cynodon dactylon* (linn) in Streptozotocin induced diabetic rats. *Asian journal of biochemistry*. **2**(1): 66 (2007).
21. Dr. Ansari, Essentials *of pharmacognosy*, (Birla Publications, New Delhi, 2005–6) 585.
22. Wagner H. and Bladt S., *Plant drug analysis*, (Springer, 1996) 349.
23. OECD Guideline for testing of chemicals-Acute oral toxicity -Fixed dose procedure, assessment number 420, adopted 17th December, 2001. Organization for economic co-operation and development. OECD Environment, health and safety publications, Paris(www.oecd.org)
24. Gerhadt Vogel H.*Drug discovery and evaluation: pharmacological assays assays*, (Springer, 2nd Edition, 2006) 874–875.
25. Venkatesan N. et al.Anti diarrheal potential of *asparagus recemosus* wild root extract in laboratory animals. *J Pharm Pharmaceut Sci*. **8**(1): 39 (2005).
26. Chitme H. R. and chandra Ramesh. Studies on anti diarrheal activity of *calotropis gigantica* R.BR in experimental animals. *J Pharm pharmaceut sci*. **7**(1): 70 (2004).
27. Patel N J, Gujarati V B, Gouda T S, Venkatrao N, Nanda kumar K and Shantha kumar S M, Anti diarrhoeal activity of alchoholic and aqueous extracts of roots of *tylophora indica* (wight & Arn) in rodents. *Pharmacologyonline*. **1**: 19 (2006).
28. Bhyrapur Mathad Vrushabendra Swamy, Chandanam Sridhar, Srinivasrao, Ramaiayan Dhanpal, Veeranna Balamuralidhar and Lakshinarayanashetty Ashokababu Veccham, Anti diarrheal evaluation of *Benincasa hispida* (Thunb) Cogn. Fruit extracts. *Iranian journal of pharmacology and therapeutics*. **4**: 24 (2005).
29. Sagar Lenika, Sehgal Rajesh and Ojha Sudarshan. Evaluation of anti motility effect of *lantana camara* L.var acuelata constituents on neostigmine induced gastrointestinal transit in mice. *BMC complement altern Med*. **5**(5): 18 (2005).
30. Vrushabendra Swamy B.M., Jayaveera K.N., Ravindra Reddy K. and Bharathi T. Anti-diarrhoeal activity of fruit of *Mormordica cymbalaria* Hook. F. *The Internet Journal of Nutrition and Wellness*. **5**(2: (2008).
31. Aschenbrenner Diane S. and Venable Samantha J.. *Drug Therapy in Nursing* ((Lippincott Williams and Wilkin, 3rd Edition, Philadelphia, USA, 2005) 1035.
32. Ammon H V, Thomas P J and Philips S, Effect of oleic and recinoleic on net jejenal water and electrolyte movement. *J clin inves*. **53**: 374 (1974).
33. Gaginella T S, Stewart J J, Olson W A and Bass P. Actions of recinoleic and structurally related fatty acid on the gastro intestinal tract II, effects on water and electrolyte absorption in vitro. *J Pharmacol Exp Ther*. **195**: 355 (1975).
34. Luderer JR, Dermer IM and Hayes AT. Advances in prostaglandin and thromboxane research. (Raven press, New York, 1980).
35. Beubler E and Juan H. Effect of recinoleic acid and other laxatives on net water flux and prostaglandin E release by the rat colon. *J Pharm Pharmacol*. **31**: 681 (1979).
36. Pierce N F, Carpenter C C J, Elliot H Z and Greenough W B. Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement i canine jejunum. *Gastroenterology*. **60**:22(1971).
37. Awouters F, Nimegeers C J E, Lenaerts F M and Janssen P A J. Delay of castor oil diarrhoea in rats: a new way to evaluate inbhibitors of prostaglandin biosynthesis. *J Pharm Pharmacol*. **30**: 41 (1978).
38. Mukherjee P K, Saha K and Murugesan T. Screening of anti diarrhoeal profile of some plant extract of a specific regions of west bengal, India. *J Ethnopharmacol*. **60**:85 (1998).
39. Galvez J., Crespo M E, Jimenez J, Suarez A and Zarzuelo A. Anti diarrhoeic activity of qurcitrin in mice and rats. *J Pharm Pharmacol*. **45**: 157 (1993).
40. Otshudi A L, Vercruysee A and Foriers A. Contribution to the ethnobotanical, Phytochemical and pharamcological studies of traditionally used medicinal plants in the treatment of dysentry and diarrhea in Lomela area (DRC). *J Ethnopharmacol*. **71**: 411 (2000).
41. Di Carlo G, Autore G, Izzo A A, Maibline P, Mascolo N, Viola P, Diurno M V and Capasso F. Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure activity relationships. *J Pharm Pharmacol*. **45**: 1054 (1993).
42. Rao V S N, Santos F A, Sobreika T T, Souza M F., Melo L L and Silveira E R. Investigations on the gastroprotective and antidiarrhoeal properties of ternatin, a tetramethoxyflavone from egletes viscose. *Planta Med*. **63**: 146 (1997).
43. Sanchez de medina F, Galvez J, Gonalez M, Zarzuelo A and Barret K E, Effects of quercetin on epithelial chloride secretion. *Life Sci*. **61**: 2049 (1997).
44. Gharzouli Kamel and Holzer Peter. Inhibition of Guinea Pig Intestinal Peristalsis by the Flavonoids Quercetin, Naringenin, Apigenin and Genistein. *International Journal of experimental and clinical pharmacology*. **70**:1 5 (2004).
45. Okudo T, Yoshoda T and Hatano T. New methods of analyzing tannins. *J Nat Prod*. **52**:1 (1989).
46. Evans WCEd. *Pharmacognosy*, Singapore: Harcourt brace and Co. Pvt. Ltd. 1997.