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ANTIDIARRHOEAL ACTIVITY ON PLATYCLADUS ORIEANTALIS EXTRACT

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ABSTRACT: Platycladus is a distinct genus of evergreen coniferous tree in the cypress family Cupressaceae, containing only one species, *Platycladus orientalis*, also known as Chinese Arborvitae or Biota. It is a small, slowgrowing tree, to 15-20 m tall and 0.5 m trunk diameterThe different parts of the plant are traditionally used as a diuretic, anticancer, anticonvulsant, stomachic, antipyretic, analgesic and anthelmintic [4,5]. The plant has not been explored for its anti diarrhoeal activity so far. The bio active fraction has been prove that it contain three major iridoid glycosides. These iridoids glycoside was subjected to anti diarrhoeal activity against validated experimental models like Castor oil induced diarrhea, gastrointestinal motility tests & PGE2-induced enteropooling. The extract inhibited castor oil induced diarrhoea and PGE2 induced enteropooling in rats; it also reduced gastrointestinal motility after charcoal meal administration. The obtained data demonstrated the excellent anti-diarrhoeal activity of P.Orieantalis and thus have great potential as a source for natural health products.
Keywords: anti-diarrhoeal activity; castor oil; Platycladus Orieantalis; Gastrointestinal motility.

INTRODUCTION

Diarrhoea is a public health problem in developing countries. Acute diarrhoea is the leading cause of morbidity and mortality amongst children in developing countries [1]. Many rural dwellers in the world depend largely on medicinal herbs for the treatment of diarrhoeal conditions because these herbs are readily available, affordable and are an indispensable component of traditional medicine practice. Diarrhoea is characterized by increased frequency of bowelmovement, wet stool and abdominal pain [2]. It is a leading cause of malnutrition and death among children in the developing countries of the world today [3]. Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhoea but they have some side effects. The natural drugs are used as antidiarrhoeal drugs, which are not always free from adverse effects [5]. The natural distribution of *Platycladus orientalis* is obscured by its long history of cultivation in large parts of Asia. In Réunion the main use of *Platycladus orientalis* is as an antirheumatic..They are used to improve the circulation, to bring down fever and to treat gastric ulcers. In Mauritius tea from branches and leaves is used to cure throat inflammation, fever and influenza. In traditional Chinese medicine the leaves are credited with bitter stomachic, refrigerant, astringent, diuretic, tonic and antipyretic properties. A decoction or the juice of the leaves has been used to relieve all kinds of bleeding, gastric ulcers, gonorrhoea and colds. The seeds are prescribed as a sedative, tranquillizer, antitussive and haemostatic. In Indo-China the ground leaves are used as an emmenagogue and antitussive, the seeds as a tonic, sedative, tranquillizer and aphrodisiac. A decoction of the twigs is prescribed to treat dysentery, skin affections and cough.

MATERIAL AND METHOD

Plant material:

The leaves of *Platycladus orientalis*were collected in the month of February from the local field of Bhopal, M.P., India, and authenticated by Dr.Harish .K. Sharma, Ayurvedic Medical College, Davangere, Karnataka, India. A voucher specimen was submitted at Institute's herbarium department for future reference (AN 102). Dried leaves were ground to coarse powder. Powder was first defatted with pet.ether and then extracted with ethanol which is further evaporated to dryness to obtain alcoholic extract.

Phytochemical screening

Qualitative assay, for the presence of plant phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins were carried out on the powdered leaves following standard procedure [6,7]

Test animals: Sprague-Dawley rats (150-175g) were procured from the animal house of Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at temperature 260C, relative humidity 44 - 56%, light and dark cycles of 10 and 14 h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Dayal, India) and the food was withdrawn 18-24 h before the experiment though water was allowed *adlibitum*. All the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee.

Acute toxicity

Different doses (25-500 mg/kg, p.o.) of BPE were administered to groups of rats and observed continuously for 1 h and then at half-hourly intervals for 4 h, for any gross behavior changes further up to 72 h followed 14 days for any mortality (OECD, 425).

EVALUATION OF EFFECT OF THE NORMAL DEFECATION

Five groups of six mice each were placed individually in separate cages with filter papers at the bottom. The doses (25, 50 and 100 mg dry extract per kg body mass) of *extract* were administered orally to different groups. The nonspecific antidiarrhoeal reference drug diphenoxylate HCl (5.0 mg/ kg, p.o.) and aqueous acacia suspension 5 ml/kg were administered to two groups and they later served as controls [14]. The total number of faecal droppings in each group was assessed every hour for the next 4 h. Percent reduction in the total number of faeces in the treated groups was obtained by comparison with control animals.

Castor oil-induced diarrhoea in Rats

The method reported by with modifications, has been used in the present study [15]. Rats of either sex (210-235 g) were fasted for 18 h; they were then divided into five groups of five individuals. The butanol extract of *Platycladus Orieantalis* was administered orally at doses of 25, 50 and 100 mg/kg by gavage as suspension to the first three groups of animals. The fourth group received loperamide (3 mg/kg) orally as suspension (positive control). The fifth group, which served as the blank, was administered with aqueous acacia suspension. After 60 min of treatment, the animals of each group received 1ml of castor oil orally, by gavage, and the consistency of faecal material and the frequency of defecation were noted up to 4 h in the transparent plastic dishes placed beneath the individual rat cages [16].

Gastrointestinal motility tests

Rats were fasted for 18 h and then placed in five cages containing five individuals in each cage. Each animal was administered orally with 1ml of charcoal meal (5% deactivated charcoal in 10% aqueous tragacanth), followed by oral administration of *extract* suspension to three groups of animals in doses of 25, 50 and 100 mg/kg. The fourth group received atropine (0.1 mg/kg,i.p.), the standard drug for comparison and the fifth group was treated with aqueous acacia suspension (vehicle control). Thirty minutes later, each animal was sacrificed and the intestinal distance moved by the charcoal meal from the pylorus was cut, measured, and expressed as a percentage of the distance from the pylorus to caecum for each animal [17].

PGE2-induced enteropooling

In this method, rats were deprived of food and water for 18 h and placed in five cages, with five animals per cage. The first three groups were treated with 25, 50 and 100 mg/kg doses of *extract*. The fourth group was treated with 1ml of a 5% (v/v) ethanol in normal saline (i.p.) and then it was treated with aqueous acacia suspension, which served as vehicle control. Immediately after the extract administration PGE2 (Astra Zeneca, India) was administered orally to each rat (100mg/kg) in the first three groups.

The fifth group was treated with PGE2 (100mg/kg) as well as with aqueous acacia suspension and served as the PGE2 control group. After 30 min following administration of PGE2, each rat was sacrificed and the whole length of the intestine from the pylorus to the caecum was dissected out, its content collected in a test tube, and the volume measured [17].

RESULTS

The extractive value of *P.Orieantalis* leave in n-Hexane (7.5%), Chloroform (15.35%) in butanol (29.2%) and in aq. Portion (55.10%). The preliminary phytochemical studies on the BPE demonstrate the presence of alkaloids, flavonoids, glycosides, tannins, saponins, steroids and triterpenoids. On chemical analysis, *extract* was found to be a mixture of iridoid glucosides. In the acute toxicity study, no deaths were observed during the period at the doses tested. In the present investigation, the butanol extract of *P.Orieantalis* showed dose dependent antidiarrhoeal activity in various validated models in rats. Castor oil produced characteristic semisolid diarrhoea droppings in all animals of the control group. The effect of the *extract* at the dose of 25-100 mg/kg caused a dose dependent decrease in the total faecal matter (12.72% and 61.81%). Loperamide, a standard antidiarrhoeal d inhibited the diarrhoea by 69.09% (Table 1). The *extract* at doses of 25 and 100 mg/kg decreased the propulsion of charcoal meal through the gastrointestinal tract, as compared with the control group ($p<0.05$ - $p<0.001$). Atropine (0.1 mg/kg) reduced the motility of the intestine to a greater extent ($p < 0.001$) (Table 2). The *extract* significantly inhibited PGE2 induced enteropooling in rats in higher dose levels compared with PGE2 treatment ($p<0.001$) (Table 3). PGE2 induced a significant increase in the fluid volume of the rat intestine when compared with control animals, received ethanol in normal saline.

Table1. Effect of butanol extract of *P.Orieantalis* on castor oil induced diarrhoea in rat

Treatment	Dose (mg/kg)	Total no. of fecal droppings	Reduction (%)
Control (acacia suspension 5 ml/kg)	-	55	-
P.Orieantalis	25	48	12.72
P.Orieantalis	50	32	41.8
P.Orieantalis	100	21	61.81
Loperamide	3	17	69.08

** Values are presented as mean values of six rats in each group.*

Table 2. Effect of butanol extract of *P.Orieantalis* on charcoal-induced gut transit changes

Treatment	Dose (mg/kg)	Total no. of fecal droppings	Reduction (%)
Control (acacia suspension 5 ml/kg)	-	60	0.00
P.Orieantalis	25	57	5
P.Orieantalis	50	50a	16.6
P.Orieantalis Atropine	100	35b	41.6
sulphate	0.1	27b	55

Values are expressed as mean + S.E.M. (n=6).
ap<0.05, bp<0.001 compared to respective control group.

Table 3. Effect of butanol extract of *P.Orieantalis* on PGE2-induced enteropooling

Treatment	Volume of intestinal fluid (ml)	Inhibition (%)
Ethanol in saline	1.24±0.21	0.00%
P.Orieantalis (25mg/kg)	3.50±0.23a	3.04%
P.Orieantalis (50mg/kg)	3.01±0.17a	16.60%
P.Orieantalis (100mg/kg)	1.92±0.14b,x	46.81%
PGE2 in ethanol(100mg/kg)	3.61±0.25a	0.00%

Values are expressed as mean + S.E.M. (n=6).
a p<0.001 compared with respect to ethanol in saline treatment.
b p<0.05 compared with respect to ethanol in saline treatment.
x p<0.001 compared with respect to PGE2 treatment.

DISCUSSION AND CONCLUSIONS

In the present study, the butanol extract of *P.Orieantalis* exhibited significant anti-diarrhoeal activity against castor oil induced diarrhoea in rats. The *extract* had a similar activity as loperamide, when tested at 50 and 100 mg/kg and inhibited the frequency of faecal droppings. Castor oil releases ricinoleic acid which induces changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoea [18-19]. The experimental studies in rats demonstrated a significant increase in the portal venous PGE2 concentration following oral administration of castor oil [20]. Ricinoleic acid markedly increased the PGE2 content in the gut lumen and also caused an increase of the net secretion of the water and electrolytes into the small intestine [21]. Inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhoea [15]. The extract appears to act on all parts of the intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model; at 100 mg/kg *extract* showed activity similar to that of atropine. The *extract* at different dose levels 50 and 100 mg/kg significantly inhibited the PGE2 induced intestinal fluid accumulation (enteropooling). These observations tend to suggest that the *extract* at different dose levels 50 and 100 mg/kg greduced diarrhoea by inhibiting gastrointestinal motility and PGE2 induced enteropooling. The present results indicate that the butanol extract of *P.Orieantalis* possesses significant antidiarrhoeal activity due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion. The inhibitory effect of the extract justifies the use of the plant as a non-specific antidiarrhoeal agent in folk medicine.

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REFERENCES

1. Hardman JG, Limberd LE. The Pharmacological basis of therapeutics. In: Goodman and Gilman.s (Eds), 10th edition, Mac Graw Hill, New York, 1992. pp. 914- 931.
2. Nadkarni AK. Barleria prionitis.Linn. In Dr. KM.Nadkani.s. Indian Material Medica, 3rd edn, Reprint Vol.1.Popular Book Depot, Bombay. 1994.
3. Kiritkar KR, Basu BD. Barleria prionitis Linn. In Indian Medicinal Plant 3rdedn, Blatter E, Caicus JF, MhaskarKS (eds).Sri Satguru Publication, Delhi, 2000. 8, 2587-2590.
4. Rao ChV, Jaiswal SK, Dubey MK, das S, Verma AR, Vijayakumar M. Evaluation of flower of Barleria prionitis for anti-inflammatory and anti nociceptive activity, International Journal of Pharma and Bio Sciences 2010; 1 (2): www.ijpbs.net.
5. Singh B, Chandan BK, Prabhakar A, Taneja SC, Singh J. Chemistry and Heapatoprotective Activity of an Active Fraction from Barleria prionitis Linn.In experimental animals. Phytother Res. 2005; 19, 391- 404.

6. Singh B, Bani S, Gupta DK. Anti-inflammatory activity of TAF an active fraction of Barleria prionitis Linn. J.ethanopharmacol. 2003; 85, 187-193.
7. Amoo SO, Finnie JF, Staden JV. In vitro pharmacological evaluation of three Barleria species. Journal of Ethnopharmacol. 2009; 121(2): 274-277.
8. Coker MF, Berky S, Pandou C. New development in acute diarrhoea current problem. Paediatrics 1998; 24: 15-17.
9. Ezekwesili CN, Obiora KA, Ugwu OP. Evaluation of Anti-Diarrhoeal Property of Crude Aqueous Extract of Ocimum gratissimum L. (Labiatae) In Rats. Biokemistr. 2004; 16(2): 122-131.
10. Victoria CG, Bryce J, Fontaine O, Monasch R. Reducing deaths from diarrhoea through oral rehydrationtherapy. Bulletin of World Health Organization. 2000; 78, 1246 .1255.
11. Park K, Park.s Textbook of preventive and social medicine. Jabalpur, India, M/S Banarsidas Bharat Publishes, 2000. pp. 172-175.
12. Ata A, Kalhari KS, Samarasekera R. Chemical constituents of Barleria prionitis and their enzyme inhibitory and free radical scavenging activities. Phytochemistry Letters 2009; 2 (1): 37-40.
13. KoKate CK, Purohit AP, GoKhle SB. Phrmacognosy, 5th Ed., Nirali Prokashan, Pune, 1997, pp. 109.137.
14. Melo L, Thomas G, Mukherjee R. Antidiarrhoeal activity of bisnordihydrotoxiferine isolated from root bark of Strychonus trinervis (Vell.) Mart. (Longaniaceae), J. Pharm. & Pharmacol. 1988; 40, 79.82.
15. Awouters F, Nimegeers CJE, Lenaerts FM, Janssen PAJ. Delay of castor oil diarrhoea in rats; a new way to evaluate inhibitors of prostaglandin biosynthesis. Journal of Pharmacy and Pharmacology 1978; 30, 41.45.
16. Agunu, A., S. Yusuf, G.O. Andrew, A.U. Zezi and E.M. Abdulrahman, 2005. Evaluation of five medicinal plants used in diarrhoeal treatment in Nigeria. J. Ethnopharmacol., 100: 27-30.
17. Atta, A.H. and S.M. Mouneir, 2004. Antidiarrhoeal activity of some Egyptian medicinal plant extracts. J. Ethnopharmacol., 92: 303-309.
18. Ezekwesili CN, Obiora KA,Ugwu OP. Evaluation of Anti- Diarrhoeal Property of Crude Aqueous Extract of Ocimum gratissimum L. (Labiatae) In Rats. Biokemistr 2004; 16(2): 122-131.
19. Victoria CG, Bryce J, Fontaine O, Monasch, R. Reducing deaths from diarrhoea through oral rehydration therapy. Bulletin of World Health Organization 2000; 78: 1246 –1255.
20. Fauci AS, Bravnwold E, Isselpacker K, Wilson JD, Kasper DL, Hauser SL, Longo DL. Harrison's Principles of Internal Medicine. New York, McGraw Hill Company. 1993; Vol (1): 236 -242.
21. Bhaduri SK, Chanda S, Majumdar P. Chemical characterization of the stem of Cyperus tegetum - A semi-aquatic plant of economic importance. Bioresource Technology 1998; 63:279-281.
22. Johnson T. Ethnobotany Desk References. CRC Press, Boca Raton London, New York, Washington1999, P256.