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Studies on anti-diarrhoeal activity of *Citrus sinensis* peel extract

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Abstract

The methanolic extract of the *Citrus sinensis* peel was screened for antidiarrhoeal activity using animal models against castor oil induced diarrhoea. The parameters evaluated are small intestine secretion by enteropooling assay, rate of defecation and small intestine transit. The methanolic extract showed significant activity ($p<0.05$) against castor oil induced diarrhoea and interluminal accumulation of fluid. It also reduced gastrointestinal motility after charcoal meal administration in albino mice. The results indicates that the action of *Citrus sinensis* peel extract (CSPE) could be through a combination of inhibition of elevated prostaglandin biosynthesis and reduced propulsive movement of the small intestine.

Key words: *Citrus sinensis*, Anti diarrhoeal, Albino mice, Castor oil and Charcoal meal.

1. Introduction

The aim of the therapy in diarrhoea is to treat the patient promptly to reduce the loss of electrolytes and water. There are several potent antidiarrhoeal drugs in the modern system of medicine, however on prolonged use they do have some adverse effect [1]. For this reason use of ayurvedic formulations have increased as they are devoid of any adverse effects. In the present study, anti diarrhoeal activity of *Citrus sinensis* peel methanolic extract (family: Rutaceae) has been studied using model of castor oil induced diarrhoea in mice. For comparison purpose Diphenoxylate hydrochloride, a standard drug was taken. Fresh *Citrus sinensis* peel is yellowish, bitter with a typical agreeable aroma. In our earlier studies, we found that CSPE has efficient anti oxidative and anti- inflammatory activities. Further we identified the presence of the flavonoids and free phenolic compounds [2].

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2. Materials and Methods

2.1 Plant material and extraction

Citrus sinensis peel was collected from the Chennai surrounding area of India in the month of March and April of 2007 in bulk quantities. The plant was authenticated by Dr. O.S. Vivekanandan, Botanist, S.R.M College, Chennai and specimen (441A) was deposited in Department of Pharmacognosy, Vel's College of Pharmacy, Chennai. Before drying, the materials are washed thoroughly with distilled water to remove dirt and were shade dried. They were coarsely powdered and subjected to successive solvent extraction in soxhlet apparatus using methanol at 60°C. After that, *Citrus sinensis* peel extract was concentrated at reduced temperature, pressure until a concentrated residue was obtained and the concentrated CSPE was suspended in 1% Carboxy methyl cellulose (CMC) in water for administration.

2.2 Drugs and Chemicals

Castor oil was refined pure from Paras chemical industries. Chlorpromazine HCl was purchased from Rhone poulenc (I) Ltd. Charcoal from E. Merck (I) Ltd. Atropine Sulphate was from Central drug house (P) Ltd, Diphenoxylate HCl was purchased from Searle (I) Ltd. and solvents from Qualigens fine chemicals.

2.3 Dose selection

The optimum conditions for experiments were decided on the basis of initial pilot experiments performed on three mice per treatment. *Citrus sinensis* peel extract was administered at upto 2 g/kg to an individual mouse in a group. There was no mortality due to this treatment. Hence for further studies 300 mg/kg [3] of maximum oral dose was employed.

2.4 Experimental animals

Swiss albino mice (25 – 30g) of either sex were originally obtained from the king institute, Chennai, TamilNadu, India, and have been maintained in the animal house at Vel's college of pharmacy, Chennai. They were housed in polypropylene cages under controlled environment. They were given standard pellet diet (Hindustan foods (P) Ltd, Bangalore) and water *ad libitum*. The protocol was approved by Animal Ethics Committee constituted for the purpose as per CPCSEA guideline.

2.5 Castor oil induced diarrhoea

Mice were divided into three groups as shown in table-1 for the treatment.

Group: I served as control-received vehicle only.

Group: II test group animals received 300 mg/kg of CSPE.

Group: III standard group animals received Diphenoxylate HCl. (5 mg / kg)

After 30 min each of these animals was given 0.1 ml castor oil by oral route. The number of defecations per animal was recorded up to 4h [3].

2.6 Small intestinal secretion

Intestinal secretion was indirectly analyzed by enteropooling assay. Groups of overnight fasted mice were treated with 300 mg/kg extract or vehicle (CMC) orally or chlorpromazine (30 mg/kg.,i.p.) 30 min before the oral administration of castor oil, 0.2 ml/mouse. These mice were sacrificed 30 min later, and the entire small intestine from each animal was weighed and their group average was calculated (Table-2). The difference in the weight of small intestine in control and castor oil treated groups was considered as the castor oil induced accumulation of intestinal fluid [4].

2.7 Small intestinal Transit

The effect of the extract on small intestinal transit was studied on overnight fasted mice, which

were divided in different groups. These groups were control (CMC), extract by oral treatment and 5 mg/kg Atropine Sulphate by intramuscularly. 30 min after the treatment, these mice were given 0.2 ml charcoal meal (3% charcoal in 5% gum acacia) by oral route. All animals were sacrificed after 20 min, the stomach and intestine removed, and the distance traveled by charcoal with reference to total length was calculated to express the percentage of distance traveled [4].

2.8 Statistical analysis

All results were reported as mean ± S.E.M. These results were further analysed by using Student's '*t*' – test to calculate significance of the results. 'p' – value less than 0.05 were considered significant.

3. Results

3.1 Castor oil induced diarrhoea

The methanolic extract of *Citrus sinensis* peel exhibited effective inhibition of castor oil induced diarrhoea. This effect is significant as compared with control at 300 mg/kg of CSPE and 5 mg / kg Diphenoxylate hydrochloride as shown in table - 1.

3.2 Small intestinal secretion

The castor oil induced intra luminal accumulation of fluid inhibited 51.44 % at a dose of 300 mg / kg extract. The reference drug chlorpromazine at a dose of 30 mg / kg reduced intestinal secretion by 96.29 % both these values were significant as compared with control as shown in table - 2.

Table 1. Effect of CSPE on Castor oil induced diarrhoea in albino mice.

Group	Number of mice	Mean number of defecations
I Control	10	12.40 ± 0.48
II CSPE (300 mg/kg)	10	6.0 ± 0.37*
III Diphenoxylate HCl (5 mg/kg)	10	4.8 ± 0.33*

Values are as mean ± S.E.M. *Significant as compared with control $P<0.05$.

Table 2. Effect of CSPE on Castor oil stimulated intraluminal fluid accumulation in the small intestine of mice gut.

Group	Weight of small intestine (mg/20 g ± S.E.M.)	Castor oil induced intraluminal fluid (mg)
I Control (CMC)	948 ± 9.12	-
II Castor oil	$1412 \pm 5.93_a$*	486 ± 5.30
III CSPE (300 mg/kg)	$1134 \pm 6.32_b$**	236 ± 3.61**
IV Chlorpromazine (30 mg/kg)	$1012 \pm 8.28_b$**	18 ± 1.03***

Significant as compared with control P<0.05, **Significant as compared with castor oil treated group P<0.05, a-Compared between control and castor oil treated group, b-Compared between castor oil and drug treated group.

Table 3. Effect of CSPE on gastro intestinal transit in mice

Group	Number of mice used	Distance traveled by charcoal marker as % of total length of small intestine (Mean ± S.E.M.)	% Inhibition
Control (CMC)	10	92.21 ± 5.25	-
CSPE (300 mg/kg)	10	81.42 ± 4.62*	11.70
Atropine sulphate (5 mg/kg)	10	42.38 ± 7.32*	54.03

*Significant as compared with control. $P < 0.05$

3.3 Small intestinal transit

The results of the present study revealed that the extract of *Citrus sinensis* peel at 300 mg/kg and Atropine sulphate at 5 mg/kg significantly inhibited the gastrointestinal transit of Charcoal in mice by 11.70 and 54.03% respectively, as compared with control as shown in table - 3.

4. Discussion

In the present investigation, methanolic extract of Citrus sinensis peel has shown anti diarrhoeal activity in a castor oil induced model in albino mice. This activity is significant at a dose of 300 mg/kg and this effect was also substantiated by significant action or castor oil induced intra luminal accumulation of fluid by indirect entero pooling assay in mice. The experiments conducted on gastro intestinal motility after charcoal meal administration have shown a reduction in the propulsive movement of small intestine after pre-treatment with methanolic *Citrus sinensis* peel extract or atropine. These results demonstrate the inhibitory effect of CSPE on castor oil induced diarrhoea, intra luminal fluid accumulation and peristaltic activity in small intestine. Prostaglandin contributes to the pathophysiological functions of the gastro intestinal tract, and also on the local electrical and mechanical activities of ileal circular muscles [5]. Castor oil increases peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and water [6]. Induction of diarrhoea by castor oil is through elevated prostaglandin biosynthesis [3]. From these results, it can be concluded that the extract may act through inhibition of prostaglandin and reduction in propulsive movement of small intestinal tract.

Preliminary phytochemical investigation of CSPE revealed the presence of flavanoids [2]. The antidiarrhoeal activity of flavanoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion [1, 7 and 8], which are known to be altered in this intestinal condition. *In vitro* and *in vivo* experiments have shown that flavanoids are able to inhibit the intestinal secretary response, induced by prostaglandins E_2 [9]. Flavanoids inhibit several enzymes, including those involved in the arachidonic acid metabolism [10]. Further detailed investigations are underway to determine the exact phytoconstituents which are responsible for the antidiarrhoeal activity.

References

1. Friedi G Hengle. (1980) *Lancet*. 1: 1413.
2. Ramachandran S, Anbu J, Saravanan M, Kishore Gnanasam S, Sridhar SK. (2002) *Indian J. Pharm. Sci*. Jan – Feb: 66 – 68.
3. Awouters F, Niemegeers CJE, Lenaerts FM, Janssen PAJ. (1978) *J. Pharm. Pharmacol*. 30: 41-45.
4. Rao VSN, Santhos FA, Sobreira TT, Souza MF, Melo CL, Silveira ER. (1997) *Planta medica*. 63: 146.
5. Sanders KM. (1984) *American J. Physiol*. 246: G361.
6. Bruton LL. (1985) In: *The pharmacological basis of therapeutics*, Vol. 2, VIII Edn, McGraw-Hill, New York; 914.
7. Dicarlo G, Autore G, Izzo AA, Maibline P, Mascolo N, Viola P, Diurno M V, Capasso F. (1993) *J. Pharm. Pharmacol*. 45: 1054-1059.
8. Galvez J, Crespo ME, Jimenez J, Suarez A, Zarzuelo A.(1993) *J. Pharm. Pharmacol*. 45: 157-159.
9. Sanchez de Medina F, Galvez J, Gonzalez M, Zarzuelo A, Barrett KE. (1997) *Life Sci*. 61: 2049-2055.
10. Mora A, Paya M, Rios JL Alcaraz MJ. (1990) *Biochem. Pharmacol*. 36: 317-322.