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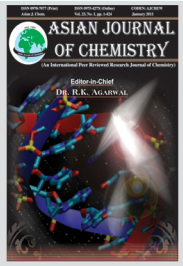

Antidiarrhoeal Activity of Ethanolic Extract of Leaf Galls 'Kakadshringi' in Swiss Albino Mice

ANURADHA S. UPADHYE*, ANAGHA A. RAJOPADHYE and ARVIND M. MUJUMDAR

Agharkar Research Institute, G.G. Agarkar Road, Pune-411 004, India

*Corresponding author: Fax: +91 20 25651542; Tel: +91 20 25654357; E-mail: upadhye.anuradha@gmail.com

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The ethnobotanical and ayurvedic literature indicated that 'Kakadshringi' leaf galls are used for the treatment of diarrhoea. During survey it was observed that source of 'Kakadshringi' is leaf galls on botanically three different plant species *viz.*, *Pistacia integrrima* Stew. ex Brindis, *Terminalia chebula* Retz. and *Garuga pinnata* Roxb. In present work, the study intended to investigate the antidiarrhoeal activity of ethanolic extract of leaf galls used as 'Kakadshringi' in mice. The authenticated resources of 'Kakadshringi' were powdered, defatted and then HPTLC profile of potent extract was carried out using well established protocols as a part of standardization. Based on exploratory studies on petroleum ether and ethanolic extracts of all three samples using castor oil induced diarrhea further studies were carried out on *Pistacia integrrima* Stew. ex Brindis ethanolic extract. The ethanolic extract of *Pistacia integrrima* Stew. ex Brindis and loperamide showed significant reduction in fecal output in castor oil and magnesium sulphate induced diarrhoea and castor oil induced intraluminal fluid accumulation. The extract also inhibited dose dependently (100-500 mg/kg) the intestinal propulsion of charcoal meal in normal and barium chloride induced changes in gastrointestinal tract. The data obtained indicate that the ethanolic extract of *Pistacia integrrima* Stew. ex Brindis has antidiarrhoeal, antisecretory and antipropulsive activities. Based on these studies *Pistacia integrrima* Stew. ex Brindis can be equated to 'Kakadshringi' and its indication for the treatment of diarrhoea.

Key Words: 'Kakadshringi' complex, Ethanol extract, Antidiarrhoeal activity, Swiss albino mice.

INTRODUCTION

Diarrhoea is one of the leading causes of morbidity and mortality, especially in children in the developing countries. The World Health Organization (WHO) has constituted a diarrhoeal disease control program which includes studies of traditional medicinal practices, together with the health education and preventive approaches[1,2]. In alternative systems of medicine many plant resources are being practiced by traditional practitioners for the treatment of diarrhoea.

The ethnobotanical and Ayurvedic literature indicated that 'Kakadshringi' leaf galls are used for the treatment of diarrhoea. It is constituent of Ayurvedic preparations like 'Shringydichurna', 'Karkatadi Churna', 'Balachatubhradra', 'Brihattalishadi Churna', 'Balachaturbhadra'[3,4].

During crude drug market survey, it was observed that 'Kakadshringi' is leaf galls obtained from botanically different locally available plant species. The leaf galls on *Pistacia integrrima* Stew. ex. Brandis, Anacardiceae (PIK) and *Terminalia chebula* Retz., Combretaceae (TCK) are commonly marketed in north and south India, respectively[5]. The galls on *Garuga pinnata* Roxb., Burseraceae (GPK) are being used by local people in western ghats of Maharashtra and observed as mixture with others in market samples[6]. It was proposed to evaluate efficacy and potency of these galls for its use in diarrhoea by using suitable animal models.

EXPERIMENTAL

All extracts were suspended in 1 % w/v carboxymethylcellulose (SD Fine, Mumbai); castor oil (refined pure) - Paras Chemical Industries, Mumbai, India; activated charcoal - E.Merck (India) Limited; magnesium sulphate and barium chloride - SD Fine Chemicals, Mumbai, India; loperamide hydrochloride - Cipla Pharmaceuticals Ltd., Mumbai, India; solvents AR grade-SD Fine Chemicals, Mumbai, India.

The galls on TCK and GPK were collected from Satara and Pune, Maharashtra, India, respectively in winter season of 2006-2007. The PIK were purchased from Pune crude drug market, Maharashtra, India. The galls were authenticated and deposited in the crude drug repository of Agharkar Research Institute, Pune-411 004; vide voucher specimen numbers AO 013, AO 018 and AO 020, respectively.

Extraction: The samples were shade dried and coarsely powdered. The powders were successively extracted with petroleum ether (60-80 ºC) and ethanol using Soxhlet apparatus. These extracts were concentrated under reduced temperature and pressure using rotary evaporator. Yields for petroleum ether extracts of PIK, TCK and GPK were 3.69, 2.43 and 1.65 % w/w and that of ethanol extracts were 13.79, 15.5 and 13.45 % w/w, respectively.

Experimental animals: Swiss albino mice of either sex weighing between 25-30 g were used for all experiments. They

were originally obtained from the National Institute of Virology, Pune, India and have been inbred in the animal house facility at the Agharkar Research Institute, Pune. They were housed in polypropylene cages in an air-conditioned area at 25 ± 2 °C with 10:14 h light and dark cycle. They were given Amrut brand balanced animal feed and water *ad libitum*. The optimum conditions for experiments were decided on the basis of initial pilot experiments using at least 3 mice. In final studies for each treatment overnight fastened at least 6 animals were used. The Institutional Animal Ethics Committee of Agharkar Research Institute 101/1999/CPCSEA, Pune, India approved the experimental protocols used in the present study.

Toxicity study: Initially all extracts were studied for acute oral toxicity as per revised OECD guidelines No. 423 using limit dose protocol. These extracts were devoid of any toxicity up to 2000 mg/kg in albino mice by oral route.

Assessment of antidiarrhoeal activity

Pilot studies of antidiarrhoeal activity: For the explorative study 500 mg/kg dose of petroleum ether and ethanolic extract of each sample was tested against castor oil induced diarrhoea (details are given at respective place). The ethanolic extract of TCK and GPK showed less than 10 % activity where as PIK showed more than 45 % antidiarrhoeal activity and petroleum extract of all samples were devoid of any activity. Hence, for further studies 100, 250, 500 mg/kg doses of ethanolic extract of PIK were used.

Castor oil induced diarrhoea: The mice were divided into different groups as shown in Table-1 for the treatment either with doses of extract, vehicle or standard loperamide hydrochloride (5 mg/kg). After 0.5 h each of these animals was given 0.1 mL of castor oil by oral route. The number of defecations per animal was recorded hourly up to 4 h[7].

Magnesium sulphate induced diarrhoea: The mice were divided into different groups as shown in Table-1 for the treatment either with doses of extract, vehicle or standard loperamide hydrochloride (5 mg/kg). After 0.5 h, each of these animals was given 2 g/kg magnesium sulphate by oral route. The number of defecations per animal was recorded hourly up to 6 h[8].

Small intestinal secretions study: Effect of extract on intestinal secretion was indirectly studied by enteropooling assay. The mice were divided into different groups as shown in Table-2 for the treatment either with doses of extract, vehicle or standard loperamide hydrochloride (5 mg/kg) before the oral administration of castor oil 0.2 mL per mouse. These mice were sacrificed 0.5 h later and entire small intestine from each animal was weighed and their group average was calculated. The difference in the weight of intestine in control and castor oil treated group was considered as the castor oil induced accumulation of intestinal fluid[9].

Effect on gastrointestinal motility: Effect of extract on small intestinal transit was studied in different groups of mice in normal and barium chloride treated animals as follows:

(I) In normal group 0.5 h after treatment of extract or vehicle, mice were administered with 0.2 mL of charcoal meal (3 % charcoal in 5 % gum acacia) by oral route. All animals were sacrificed after 20 min and distance traveled by charcoal with reference to total length of small intestine was calculated for each mouse to express percentage of distance traveled[9] as shown in Table-3.

(II) In second group 20 min after treatment of extract or loperamide hydrochloride or vehicle, mice were administered with barium chloride 5 mg/kg by oral route. Activated charcoal 0.2 mL (3 % charcoal in 5 % gum acacia) was administered by oral route to each mouse after 10 min. After 20 min each mouse was sacrificed and distance traveled by charcoal with respect to the total length of intestine was measured to calculate percentage of distance traveled in each group[10]. The percentage of inhibition compared with the control group was determined[11] as shown in Table-3.

Phytochemical analysis and chromo-profile of ethanolic extracts: The total tannin content[12] and protein perceptible tannin[13] of the three samples were determined. As a part of standardization, the high performance thin layer chromatography (HPTLC) study of potent ethanolic extract of PIK was carried out on instrument comprising of Linomat IV for spotting, using Densitometer-TLC scanner III with "CATS" software (Camag, Switzerland). HPTLC pre-coated silica gel F_{254} (Merck, Darmstadt, Germany) plates were developed up to 8.0 cm in a Camag Twin chamber previously saturated with mobile phase toluene:ethyl acetate:formic acid (5:4:1, v/v/v) and scanned at 254 and 365 nm to study their behaviour. Only under 254 nm distinguishing bands were observed (Fig. 1).

Statistical analysis: The results of all experiments were reported as mean ± SEM. These results were further analyzed by using Student's t-test to calculate significance of the results. *p* values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Effect of extract on castor oil and magnesium sulphate induced diarrhoea: In initial exploratory studies petroleum ether extracts of all samples and ethanolic extracts of TCK and GPK were not showing significant antidiarrhoeal activity against castor oil induced diarrhoea. In main experiment of castor oil induced diarrhoea the ethanolic extract of PIK (100-500 mg/kg orally) significantly ($p < 0.05$-0.001) reduced the fecal output produced by castor oil as compared to the control. However, this activity was compared to standard drug loperamide hydrochloride as shown in Table-1.

The ethanolic extract of PIK showed dose dependent inhibition on magnesium sulphate induced diarrhoea. This effect was significant at dose 100, 250, 500 mg/kg of PIK extract over 6 h as compared to the control. However, this activity was as compared to standard drug loperamide hydrochloride as shown in Table-1.

Effect of extract on small intestinal secretion: The castor oil induced intraluminal accumulation of fluid was inhibited by extract in a dose-dependent manner. However, the activity was compared to loperamide hydrochloride as shown in Table-2.

Effect of extract on gastrointestinal transit: The results of present study revealed that the ethanolic extract of PIK inhibited the gastrointestinal transit of charcoal meal in dose dependent manner in normal as well barium chloride treated animal and significant at 250 and 500 mg/kg doses. Loperamide

TABLE-1
EFFECT OF ETHANOLIC EXTRACT OF PIK ON CASTOR OIL AND MAGNESIUM SULPHATE INDUCED DIARRHOEA IN ALBINO MICE

No.	Treatment	No. of defection at various hours (mean ± SEM)					
		1	2	3	4	5	6
1	Castor oil	5.00 ± 0.33	7.33 ± 0.51	8.66 ± 0.51	10.33 ± 0.38	–	–
2	Extract PIK 100 mg/kg	3.16 ± 0.49[a]	3.83 ± 0.55[a]	5.16 ± 0.55[a]	6.03 ± 0.52[a]	–	–
3	Extract PIK 250 mg/kg	2.00 ± 0.33[b]	3.83 ± 0.44[b]	5.10 ± 0.44[b]	6.00 ± 0.65[b]	–	–
4	Extract PIK 500 mg/kg	1.33 ± 0.45[b]	2.00 ± 0.52[b]	2.50 ± 0.46[b]	3.33 ± 0.38[b]	–	–
5	Loperiamide 5 mg/kg	1.66 ± 0.51[e]	2.66 ± 0.19[e]	3.66 ± 0.51[e]	5.33 ± 2.18[e]	–	–
6	Magnessium sulphate	5.00 ± 0.33	5.66 ± 0.19	7.66 ± 0.19	8.66 ± 0.19	9.33 ± 0.19	11.00 ± 0.33
7	Extract PIK 100 mg/kg	5.83 ± 0.34[c]	6.83 ± 0.28[c]	7.33 ± 0.45[c]	8.50 ± 0.39[c]	9.16 ± 0.28[c]	10.00 ± 0.33[c]
8	Extract PIK 250 mg/kg	5.33 ± 0.19[d]	6.66 ± 0.30[d]	6.83 ± 0.30[d]	7.83 ± 0.36[d]	8.66 ± 0.3[d]	9.16 ± 0.28[d]
9	Extract PIK 500 mg/kg	4.50 ± 0.39[d]	5.83 ± 0.28[d]	7.00 ± 0.23[d]	7.83 ± 0.28[d]	8.00 ± 0.23[d]	9.16 ± 0.28[d]
10	Loperiamide 5 mg/kg	4.16 ± 0.28[e]	4.66 ± 0.45[e]	6.50 ± 0.39[e]	6.33 ± 0.51[e]	7.16 ± 0.55[e]	8.50 ± 0.39[e]

$n = 6$ in each group; [a] Significant reduction as compared to castor oil group $p < 0.05$; [b] Significant reduction as compared to castor oil group $p < 0.001$; [c] Significant reduction as compared magnesium sulphate group $p < 0.05$; [d] Significant reduction as compared magnesium sulphate group $p < 0.001$; [e] Significant reduction as compared to respective control group $p < 0.001$.

TABLE-2
EFFECT OF ETHANOLIC EXTRACT OF PIK ON CASTOR OIL INDUCED INTRALUMINAL FLUID ACCUMULATION IN ALBINO MICE

No.	Treatment	Weight of small intestine g/20g of mice (mean ± S.E.M.)	Castor oil induced intraluminal fluid (mg)
1	Control	1.123 ± 0.024	–
2	Castor oil	1.808 ± 0.066*	515
3	PIK Extract 100 mg/kg	1.452 ± 0.038[a]	317
4	PIK Extract 250 mg/kg	1.367 ± 0.024[a]	204
5	PIK Extract 500 mg/kg	1.213 ± 0.034[b]	149
6	Loperiamide 5 mg/kg	1.245 ± 0.026[b]	103

$n = 6$ in each group; *Significant reduction in fluid accumulation as compared to control group $p < 0.001$; [a]Significant reduction in fluid accumulation as compared to castor oil group $p < 0.02$; [b]Significant reduction in fluid accumulation as compared to castor oil group $p < 0.001$.

hydrochloride significant ($p < 0.001$) reduced the mean length traveled by charcoal meal and propulsion inhibited in normal and barium chloride treated by 51.33 and 58.24 %, respectively. However, this activity was compared to standard drug as shown in Table-3.

Phytochemical analysis and chromo-profile: The tannin content of PIK, TCK, GPK were 24.44 ± 0.62, 19.75 ± 0.32, 15.67 ± 0.67 %, respectively and protein perceptible tannins in PIK, TCK, GPK were 17.89 ± 0.025, 10.91 ± 0.036, 5.12 ± 0.028 %, respectively. Thus the percentage of tannin and protein perceptible tannin were higher in PIK than other two samples. HPTLC profile of the PIK ethanol extract showed distinguishing 7 bands only under 254 nm at R_f 0.05, 0.22, 0.38, 0.56, 0.66, 0.81, 0.93 (Fig. 1).

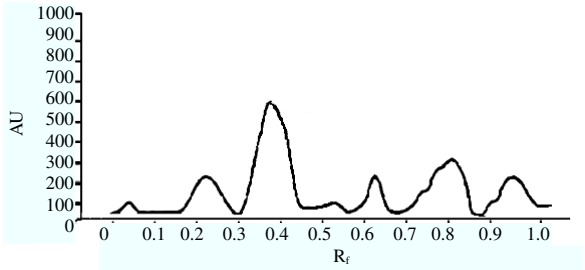

Fig. 1. HPTLC profile of active ethanol extract of PIK scanned at 254 nm; mobile phase, toluene:ethyl acetate:formic acid (5:4:1, v/v/v)

TABLE-3
EFFECT OF ETHANOL EXTRACTIC OF PIK ON GASTROINTESTINAL TRANSIT IN ALBINO MICE

No.	Treatment	Distance traveled by charcoal (as % of total length of small intestine) mean ± SEM	Inhibition (%)
1	Normal control	71.50 ± 1.78	–
2	PIK Extract 100 mg/kg	65.00 ± 2.81[a]	9.09
3	PIK Extract 250 mg/kg	55.66 ± 5.08[b]	17.48
4	PIK Extract 500 mg/kg	51.00 ± 1.08[b]	28.5
6	Loperiamide 5 mg/kg	31.00 ± 1.87[c]	51.33 %[e]
7	$BaCl_2$ control	87.37 ± 1.79	–
8	PIK Extract 100 mg/kg	68.87 ± 4.76[d]	11.87
9	PIK Extract 250 mg/kg	57.02 ± 3.95[d]	33.14
10	PIK Extract 500 mg/kg	46.73 ± 1.97[d]	46.89
11	Loperiamide 5 mg/kg	48.00 ± 1.27[c]	58.24 %[e]

$n = 6$ in each group; [a]Significant reduction in gastrointestinal motility as compared to normal group $p < 0.05$; [b]Significant reduction in gastrointestinal motility as compared to normal group $p < 0.001$; [c]Significant reduction in gastrointestinal motility as compared to respective group $p < 0.001$; [d] Significant reduction in gastrointestinal motility as compared to barium chloride control $p < 0.001$; [e]% Inhibition of gastrointestinal motility as compared to respective group.

The use of plant resources for the treatment of diarrhoea are commonly practiced in traditional Indian system of medicine. As mentioned earlier, leaf galls on three different plant species *viz.*, *Pistacia integrrima* (PIK), *Terminalia chebula* (TCK) and *Garuga pinnata* (GPK) are used as 'Kakadshringi' for treatment of diarrhoea.

In the present work, comparative studies of three samples was undertaken to evaluate antidiarrhoeal activity. The petroleum ether extract of all samples and ethanolic extract of TCK and GPK have not shown significant antidiarrhoeal activity. The ethanol extract of PIK profoundly inhibited castor oil and magnesium sulphate induced diarrhoea in dose dependent manner. However, this activity was less as compared to standard drug loperamide hydrochloride. Castor oil is known to hydrolyze in the upper small intestine to ricinoleic acid, a local irritant, which irritates the mucosa of the gastrointestinal tract resulting in fluid accumulation and a watery luminal content that flows rapidly through the intestine[14]. On the other hand, magnesium sulphate has been reported to induce diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been demonstrated that it

promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water[15,16]. Furthermore, the extract is also showing inhibition of peristalsis activity in normal as well as in barium chloride induced increase peristalsis as evident from distance traveled by charcoal meal. The significant reduction in peristaltic activity is one of the important factors contributing to antidiarrhoeal activity of extract. It is probable that the ethanol extract of PIK may be exerting its antidiarrhoeal and antipropulsive activities by slowing intestinal motility.

Conclusion

Based on these results, it can be suggested that the use of galls on *Pistacia integerrima* (PIK) may be justified for the treatment of diarrhoea. However, further detailed investigations and chronic studies on the three samples known as 'Kakadshringi' are warranted for pharmaceutical purpose.

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