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ANTI-DIARRHOEAL ACTIVITY OF METHANOLIC EXTRACT OF *PICRORRHIZA KURROA* ROYLE EX. BENTH.

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ABSTRACT

Picrorrhiza kurroa (Scrophulariaceae) is a small perennial herb growing in the hilly parts of the north-Western Himalayas region in India and Nepal. The objective of the present study was to evaluate and compare anti-diarrhoeal activity of *Picrorrhiza kurroa* royle ex. Benth using castor oil induced diarrhoea and castor oil induced enteropooling models. The test extract was assayed on the number of faecal droppings for 4hrs and weight of intestine in castor oil induced diarrhoea and castor oil induced enteropooling methods respectively. Two doses of the test extract i.e. 250mg/kg, 500mg/kg were used to evaluate the anti-diarrhoeal activity. Loperamide was used as the standard drug to compare the test results. The study concluded that the methanolic rhizome extract of *Picrorrhiza kurroa* showed significant Anti-diarrhoeal action.

Key words: *Picrorrhiza kurroa*, Anti-diarrhoeal action, Loperamide, Castor oil induced diarrhoea, Castor oil induced enteropooling.

INTRODUCTION

Picrorrhiza kurroa royle ex. Benth belonging to the family Scrophulariaceae is a small perennial herb that is widely distributed in the north – West India on the slopes of Himalayas between 3000 and 5000mts (Hooker JD, 1885; Chopra RN and Ghosh S, 1934). *Picrorrhiza kurroa* is an important herb in the traditional Ayurvedic system of medicine and has been used to treat liver and brochial problems. Other traditional uses include treatments of dyspepsia, bilious fever, chronic dysentery and scorpion sting. The most important active constituents of *Picrorrhiza kurroa*are the cucurbitacin glycosides, apocyanin, drosin, iridoid glycosides, picrosides and kutkin (Weinges K *et al*., 1972; Stuppner H, Wagner H, 1989**)**. Diarrhoeal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year (Carlos CC, Saniel MC, 1990). There are a large number of epidemiological and experimental evidence pertaining to worldwide acute diarrhoeal disease, which is one of the principle causes of death in infants (Lutterodt GD, 1989). As there is no literature available on anti-diarrhoeal action of Picrorrhiza kurroa, the present study was taken up to evaluate for Anti-diarrhoeal action of methanolic rhizome extract of *Picrorrhiza kurroa* royle ex. Benth.

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MATERIALS AND METHODS

Plant collection, identification and authentication

The plant specimen was collected from S.V University, Tirupati, India and identified as *Picrorrhiza kurroa* Royle ex. Benth. Belonging to the family Scrophulariaceae, Voucher No: SDIP, Ref No: 002 dated 26/10/2012 and authenticated by Dr.Madhavachetty, Botanist, Tirupati. The rhizomes of the plant were dried in vacuum oven at 40^{o} C.

Preparation of plant extract

Rhizomes of *Picrorrhiza kurroa* plant are coarsely powdered and are successively extracted by continuous hot percolation method using Soxhlet apparatus employing methanol followed by distillation to recover the excess solvent. Methanolic extraction yielded sufficiently good quantity of the product. The extract was later subjected to drying and stored in a dessicator for further use (Khandewl KR, 2005). The extract is soluble in water. Therefore, from the dried methanolic extract, accurately 250mg/ml and 500mg/ml solutions were prepared using distilled water.

Standard used for the activity

Loperamide was used as the solvent to compare the test results. It was prepared in the concentration of 3mg/kg in distilled water as the solvent.

Animals used for the study

Male wistar rats (150-180 gms) were used for the study and kept at the laboratory animal house of Sree Dattha Institute of Pharmacy for acclimatization to laboratory environment. They were kept in well cross ventilated room at 27±2^{o}C for 1 week before the commencement of experiment. Animals were provided with commercial rodent pellet diet and water ad libitum.

Method

Anti-diarrhoeal activity was evaluated using two methods i.e castor oil induced diarrhoea and castor oil induced enteropooling methods.

Castor oil induced diarrhoea

The anti-diarrhoeal activity of methanolic extract was evaluated according to the method described by Teke *et al*., (2007) Male wistar rats were fasted for 18hrs and divided into four groups of four animals each. Castor oil in the dose of 1ml was given orally to all groups of animals to induce diarrhoea. One hour prior to castor oil administration various treatments were given, Group I (Control) were given distilled water, Group-II (Standard) was treated with Loperamide (3mg/kg p.o.), Group-III was administered 250mg/ml of test extract and Group-IV was treated with 500mg/ml of test extract by oral route. Animals were placed separately in individual cages lined with filter papers which were changed for every hour and number of times of faecal droppings were assesses for 4hrs. The results are interpreted in Table 1.

Castor oil induced enteropooling

The anti-diarrhoeal activity of methanolic extract was evaluated according to the method described by Inayathulla *et al*., (2010) Male wistar rats were fasted for 18hrs and divided into five groups of four animals each. Group I (Control) were given distilled water, Group-II (Standard) was treated with Loperamide (3mg/kg p.o.), Group-III was administered 250mg/ml of test extract and Group-IV was treated with 500mg/ml of test extract by oral route, one hour before the oral administration of castor oil (2ml v/v). One hour later, the rats were sacrificed and the small intestine was removed after tying the ends with threads and weighed. The intestinal contents were collected into a graduated cylinder and its volume measured. The intestine was reweighed and the differences between the full and empty weights are calculated. The results are interpreted in Table 2.

Statistical Analysis

Data was expressed as Mean ± Standard error of mean (SEM) and statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's t test.

Table 1. Effect of methanolic rhizome extract of *Picrorrhiza kurroa* on castor oil induced diarrhoea

Group	Treatment	No. of feacal droppings in 4 hrs	% Inhibition
Control	Castor oil+ Distilled water	22.0 ± 2.35*	--
Standard	Castor oil+ Loperamide(3mg/kg)	4.5 ± 1.25*	79.5
MRPK	Castor oil+Test 1(250 mg)	10.83 ± 1.64*	50.7
MRPK	Castor oil+Test2(500mg)	6.33 ± 0.88*	71.2

Results are expressed as Mean ± SEM; n=5 in each group; *p<0.05, MRPK is methanolic extract of *Picrorrhiza kurroa*

Table 2. Effect of methanolic rhizome extract of *Picrorrhiza kurroa* on castor oil induced enteropooling

Treatment	Dose (mg/kg)	Volume of fluid (ml)	Weight of intestinal content (gms)	% inhibition
Control	--	2.85 ± 0.29	3.91 ± 0.69	--
Loperamide	3	1.87 ± 0.53*	0.98 ± 0.33*	75.87
MRPK	250	1.65 ± 0.02*	01.41 ± 0.45*	64.46
MRPK	500	1.49 ± 0.05*	0.56 ± 0.31*	72.67

Results are expressed as Mean ± SEM; n=5 in each group; *p<0.05, MRPK is methanolic extract of *Picrorrhiza kurroa*

RESULTS AND DISCUSSION

The % yield of methanolic extract of rhizomes of *Picrorrhiza kurroa* after 24hrs of hot percolation was found out to be 34%. The preliminary phytochemical screening showed the presence of carbohydrates, glycosides, saponins, steroid like phytochemical constituents. Cucurbitacins, Phenolic, Iridoid glycosides are some of the principle constituents responsible for various pharmacological activities. Iridoid glycosides like kutkin, Picroliv, Picrisides I, II, III & IV, Kutkosides are the chemical moieties that may be responsible for Antidiarrhoeal activity (Ghisalberti EL, 1998). Table 1 & 2 reveals that, the methanolic extract of *Picrorrhiza kurroa* rhizomes showed significant anti-diarrhoeal activity in a dose dependent manner. In Castor oil induced diarrhoeal method, there is a decrease in the number of times of faecal droppings when compared to the control. 500 mg/kg dose showed a significant decrease in the number of faecal droppings when compared to the standard. In castor oil induced enteropooling method, it revealed that the test extract showed a marked reduction in the volume of the intestinal contents and weight of the intestine.

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