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Research Article

In-vivo Evaluation of Antidiarrhoeal Activity of Ethanolic Extract of *Uraria lagopoides* (L.)

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Abstract: *Uraria lagopoides* (L.) (Papilionaceae), a trailing perennial herb locally known as 'Prisniparni' finds use as a remedy for several ailments. The purpose of the present study was to evaluate scientifically the anti-diarrhoeal effects of *Uraria lagopoides* used traditionally in Indian system of medicine using castor oil-induced diarrhoea and castor oil-induced enteropooling in rats. The phytochemical studies of the ethanolic extract revealed the presence of flavonoids, glycosides, proteins and phytosterols. The extract showed significant ($p < 0.001$) protection against castor oil-induced diarrhoea and castor oil-induced enteropooling at (400 mg/kg). The plant extracts exhibited dose dependent antidiarrhoeal effects in the all treated groups and the results were compared with that of loperamide (3mg/kg, p.o) as reference standard drug.
Keywords: *Uraria lagopoides*, Castor-oil, Enteropooling, Anti-diarrhoeal.

INTRODUCTION

Since the introduction of the herbal medicines, many people were impelled to consider the importance of many herbs for treating several forms of disorders. It is no wonder, during the past decade there has been an exponential rise in the application of herbal remedies and such notable increase even continues in these days. WHO report 80% of the world population relies on the drugs which are from natural origin [1]. In developing countries, the majority of people living in rural areas almost exclusively use traditional medicines in treating all sorts of disease including diarrhea. There are large numbers of epidemiological and experimental evidence pertaining to worldwide acute–diarrheal disease, which is one of the principal causes of death in the infants, particularly in malnourished and which is of critical importance in developing countries [2, 3]. Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain [4]. Diarrhoea is one of the leading causes of death among children under five globally. More than one in ten child deaths – about 800 000 each year – is due to diarrhoea. Today, only 44% of children with diarrhoea in low-income countries receive the recommended treatment, and limited trend data suggest that there has been little progress since 2000. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used antidiarrhoeal drugs, which are not always free from adverse effects [5-7].

Uraria lagopodioides (Papilionaceae), a trailing perennial herb locally known as 'Prisniparni' found in India (Bihar, Orissa, West Bengal), Nepal, China and Northern Australia. *Uraria lagopodoides* has been reported to be aphrodisiac, useful in treatment of asthma, dysentery, delirium, ulcers, malarial fevers, fractures of bones, inflammation of chest and diarrhoea. The present study was undertaken to evaluate the antidiarrhoeal potential of ethanolic extract of *Uraria lagopoides* in castor oil induced diarrhoeal rats [8, 9].

MATERIALS AND METHODS

Material

The whole pant of *Uraria lagopoides* was collected from Sathupally, Kuppam and surrounding villages of Chittoor district of Andhra Pradesh, India and authenticated by Dr. Madhava Chetty, taxonomist and HOD of Botany, Sri Venkateswara University, Thirupathi, India, loperamide (Micro labs, Bangalore). All other solvents and chemicals used were of analytical grade purchased from local source.

Extraction

After collection, the plant material was shade dried, powdered (40 mesh size) to get a coarse powder and then subjected to soxhlet extraction continued for 8 cycles (6 hrs) using methanol as a solvent. The extract was filtered and concentrated at reduced temperature on a rotary evaporator and kept in a dessicator [10].

Phytochemical screening

Ethanolic extract of *Uraria lagopoides* was subjected to preliminary phytochemical screening to test the presence of alkaloids, carbohydrates, reducing sugars, glycosides, proteins, amino acids, steroids, triterpenoids, phenolic compounds, tannins, flavonoids, fixed oils, fats, volatile oils, gums and mucilages [11].

Animals

Male wistar rats (180-200 g) were obtained from the animal house, Hindu College of Pharmacy, Guntur and kept in standard environmental conditions. They were fed with rodent diet and water ad libitum. Experiments were carried out in accordance with CPCSEA guidelines and the study was approved by Institutional animal ethical committee.

Test compound formulation

The aqueous suspension of ethanolic extract of whole plant of *Uraria lagopoides* (EEUL) was prepared in 0.5 % carboxymethylcellulose (CMC) solution in distilled water prior to oral administration to animals. It was used within seven days and stored at 8^{o}C while for further use, freshly prepared solution was used. The vehicle alone served as control.

Castor oil-induced diarrhoea in rats

A total of 24 male wistar rats were divided into four groups of six animals in each group. All rats were fasted for 18 h and received castor oil at a dose of 1 ml/animal orally (p.o.) using orogastric cannula for induction of diarrhea (Doherty, 1981). Thirty minutes after castor oil administration, rats of group I (control) received 1.0 ml/100 g of 0.9% NaCl in distilled water (normal saline) and rats of groups II, received 250 mg/kg of EEUL p.o., rats of group III received 500 mg/kg of EEUL and group IV received standard drug, loperamide (3 mg/kg p.o.), respectively. The animals were placed separately in metabolic cages over white clean Whatman filter paper which was changed every hour. The severity of diarrhoea was assessed each hour for 4 h. The total number of diarrhoea feces of the control group was considered 100% [12, 13]. Anti-diarrhoeal activity was determined in terms of percentage of protection, which will be calculated by following formula

$$\text{Percentage of protection (\%)} = \frac{\text{Mean weight of stool of Control animals} - \text{Mean weight of stool of drug / extract treated animals}}{\text{Mean weight of stool of control animals.}} \times 100$$

Castor oil-induced enteropooling

Castor oil-induced enteropooling was determined by the method of Robert et al. (1976). The adult rats were fasted for 18 h and divided into four groups of six animals each. Castor oil (1 ml) was administered orally to these animals. One hour later, Group I received 1 ml/100 g of normal saline solution and rats of groups II received 250 mg/kg EEUL p.o, rats of group III received 500 mg/kg EEUL and rats of group IV received standard drug, loperamide (3 mg/kg orally), respectively. After 2 h of treatment, the rats were sacrificed by ether anesthesia. The edges of the intestine from pylorus to ceacum were tied with thread and the intestine was removed and weighed. Intestinal content was collected by measuring cylinder, and volume measured [14, 15].

Statistical analysis

Results are presented as means ± standard deviation (SD) and simple percentages. The student 't' test was used to determine the significant difference between two groups ($p < 0.001$).

RESULTS

The phytochemical results confirm the presence of flavonoids, glycosides, proteins and phytosterols in extracts (Table 1). These are the phytochemicals which are essential in many medicinal plants responsible for the anti-diarrhoeal activity. The reported medicinal property of the plant might be due to the presence of these bioactive components *in Uraria lagopoides*. Phytochemicals such as alkaloids and tannins were found to be absent in the extract.

In the castor oil-induced diarrhoea experiment, ethanolic extract of *Uraria lagopoides* produced a markedly antidiarrhoeal effect in the rats (Table 2, Fig. 1 and 2). At dose of 200 mg/kg, the extract significantly decreased ($p < 0.05$) the total number of watery stool and mean weight of fecal drops produced upon administration of castor oil (12.0±0.58 and 4.28±0.23 respectively) where as at dose of 400 mg/kg, the extract significantly decreased ($p < 0.001$) the total number of watery stool and mean weight of fecal (6.83±0.31 and 2.38±0.13 respectively) compare to the control group (20.83±0.47 and 9.80 ± 0.17). The effect of the dose of the extract was similar to that of the standard drug, loperamide (3 mg/kg). *Uraria lagopoides* significantly ($p < 0.001$) inhibited castor oil-induced enteropooling in rats at oral dose of 400 mg/kg (Table 3 and Figure 3). The intestinal fluid in control animals was 3.06 ± 0.19 ml .The inhibition of intestinal accumulation was 57.19% ($p < 0.05$) at a dose of 200mg/ml and 75.16% ($p < 0.001$) at a dose of 400 mg/kg of the drug. The standard drug, loperamide (3 mg/kg) also significantly inhibited ($p < 0.001$) intestinal fluid accumulation 86.93%.

Table 1. Qualitative Phytochemical analysis of *Uraria lagopoides*

Chemical Constituents	Tests	Hexane Extract	Choroform Extract	Ethylacetate Extract	Ethanol Extract	Water Extract
Alkaloids	Mayer's	-	-	-	-	-
	Dragendroff	-	-	-	-	-
	Wagners	-	-	-	-	-
	Hager's	-	-	-	-	-
Carbohydrates	Molisch	-	-	-	-	-
	Benedict	-	+	-	-	+
	Fehling	-	+	+	+	+
Glycosides	Libermann Burchard	-	-	-	+	-
	Modified Borntrager	-	-	-	+	-
	Legals	-	-	-	-	-
Saponins	Foam test	-	-	-	-	+
Phytosterols	Salkowski	+	+	-	+	-
	Libermann Burchard	-	+	+	-	-
	Tshugajeu	-	-	-	-	-
Fixed oils and Fats	Stain test	-	-	-	-	-
Resins	Acetone-water test	-	-	-	-	-
Phenolic acids and Tannins	Ferric chloride	-	-	-	-	-
	Gelatin	-	-	-	-	-
Proteins and Amino acids	Xanthoproteic	-	-	+	+	-
	Ninhydrin	-	-	-	+	-
	Biuret	-	-	-	-	-
Flavonoids	Alkaline reagent	-	-	+	+	-
	Lead acetate	-	-	+	-	-
	Shinoda	-	-	-	-	-
Gums and Mucilage	Alcohol precipitation	-	-	-	-	-

(+) Detected(-) Not detected

Table 2: Antidiarrhoeal effect in the rats

Groups	Treatment	Dose mg/kg	Mean no. of fecal drops	Mean wt. of feaces (g)	% protection
I	Control	-	20.83 ± 0.47	9.80 ± 0.17	-
II	Standard (Loperamide)	3	$3.50 \pm 0.22^{***}$	$0.93 \pm 0.12^{***}$	90.51 %
III	EEUL	200	$12.0 \pm 0.58^{**}$	$4.28 \pm 0.23^{**}$	56.33 %
IV	EEUL	400	$6.83 \pm 0.31^{***}$	$2.38 \pm 0.13^{***}$	75.71 %

Values are expressed as mean ± SD, n = 6, $^{**}p < 0.05$ and $^{***}p < 0.001$ compared with control.

Table 3: Inhibited castor oil-induced enteropooling in rats

Groups	Treatment	Dose mg/kg	Mean volume of intestinal fluid (ml)	% inhibition
I	Control	-	3.06 ± 0.19	-
II	Standard (Loperamide)	3	$0.47 \pm 0.09^{***}$	86.93 %
III	EEUL	200	$1.31 \pm 0.12^{**}$	57.19 %
IV	EEUL	400	$0.76 \pm 0.14^{***}$	75.16 %

Values are expressed as mean ± SD, n = 6, $^{**}p < 0.05$ and $^{***}p < 0.001$ compared with control.

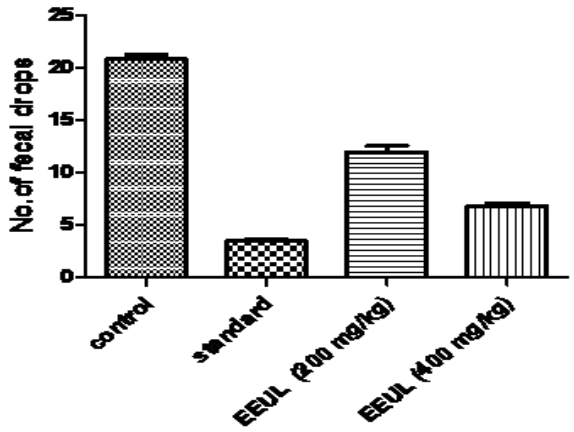

Fig. 1: A graph of the effect of ethanolic extract of *Uraria lagopoides* on castor oil induced diarrhoea

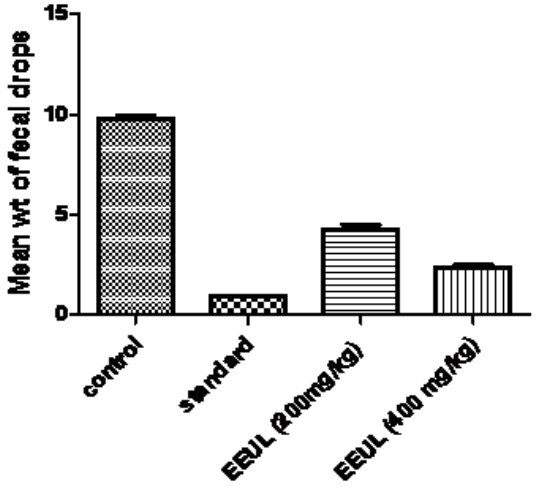

Fig. 2: A graph of effect of ethanolic extract of *Uraria lagopoides* on castor oil-induced diarrhoea

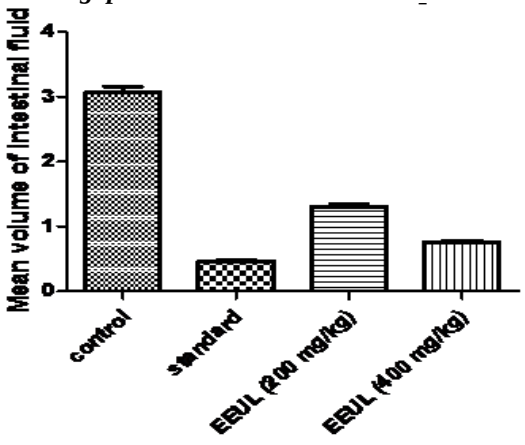

Fig. 3: A graph of effect of ethanolic extract of *Uraria lagopoides* on castor oil-induced enteropooling

DISCUSSION

Diarrhea results from imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry, resulting in an excess loss of fluid as faeces. In some diarrhoeas, the secretory component predominates, while other diarrhoeas are characterized by hyper-motility [16, 17]. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion. The use of castor oil induced diarrhea model in our study is logical since the autocoids and prostaglandins can cause diarrhoea in man [18, 19]. The significant reduction of frequency of defecation, number of fecal dropping and mean weight of stool demonstrates the efficacy of *Uraria lagopoides* as antidiarrhoeal agent. The extract significantly inhibited the castor oil induced intestinal fluid accumulation. These observations tend to suggest that extract reduced diarrhoea by inhibiting castor oil induced intestinal accumulation of fluid.

CONCLUSION

Although the exact mechanism of action is not known, the present work confirms the antidiarrhoeal properties of ethanolic extract of *Uraria lagopoides* which supports the traditional use of *Uraria lagopoides* in the treatment of diarrhoea. Further studies are required to identify and isolate the active principles to establish the exact mechanism of action of the test extract.

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