

ORIGINAL RESEARCH ARTICLE

Evaluation of Anti-Diarrhoeal Activity of Stem Bark Extracts of *Ficus religiosa* Prepared by Different Methods of Extraction

Panchawat S*¹, Sisodia S.S²

¹Department of Pharmaceutical Sciences, MLSU, Udaipur (Raj.), India

²B. N. College of Pharmacy, Udaipur (Raj.), India

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ABSTRACT

Aims: The purpose of the present study was to evaluate antidiarrhoeal effect of various extracts (hydroalcoholic 60% and acetone extracts) of stem bark of *Ficus religiosa* Linn. against castor-oil-induced diarrhoeal model in rats.

Methods: Antidiarrhoeal activity of hydralcoholic 60% extract (prepared by soxhlet extraction method), acetone extract (prepared by ultrasonication assisted extraction method) and acetone extract (prepared by microwave assisted extraction method) of stem bark of *Ficus religiosa* Linn. were investigated using castor-oil-induced diarrhea model in rats. The number of faeces and total weight of faeces were studied.

Results: Standard drug Loperamide (3mg/kg, p.o.) shows significant reduction in fecal output whereas extracts (hydroalcoholic 60% and acetone extracts) at a dose of 200 mg/kg, significantly (p<0.001) reduced the castor-oil-induced frequency and consistency of diarrhoea. The hydroalcoholic (60%) extract prepared by soxhlet extraction method shows best result than other extracts.

Conclusion: The hydroalcoholic (60%) extract shows marked reduction in the number of diarrhea stools and total weight of diarrhoeal faeces. The result obtained establishes the traditional claim of plant as anti-diarrhoeal agent. Further studies are needed to understand the mechanism of anti-diarrhoeal action of *Ficus religiosa* Linn. stem bark.

Key words: Antidiarrhoeal activity, *Ficus religiosa* Linn., castor-oil, extraction.

INTRODUCTION

Ficus religiosa Linn. commonly known as peepal, belonging to family-Moraceae, attributed with several medicinal properties. *Ficus religiosa* has been widely used as a common house hold remedy from ancient times. Stem bark is used in gonorrhoea, bleeding, paralysis, diabetes, diarrhoea, bone fracture, antiseptic, astringent and antidote^[1].

Ficus religiosa has phenols, tannins, steroids, alkaloids and flavonoids, β -sitosteryl-d-glucoside, vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupen-3-one^[2].

Diarrhoea caused by intestinal pathogens is a global health concern and one of the primary causes of infant mortality especially in developing countries^[3]. According to the World Health Report, diarrhoea is the cause of 3.3% of all deaths. In young children, it can lead to death due to dehydration^[4]. A majority of diarrhoeal cases are due to bacterial enteropathogens diarrhoeagenic *E. coli*, mainly enterotoxigenic and

enteroaggregative^[5] and invasive bacterial pathogens like *Shigella*, *Campylobacter* and *Salmonella*^[6]. Amongst the viral agents, rotavirus is the most common^[7]. Oral rehydration therapy (ORT) has been the key strategy for effective case management^[8]. Moreover, there is an increasing threat of drug resistance to antibiotics^[9]. Thus there is a need to develop cost effective alternative approaches for the treatment of diarrhoea. In the present study, the antidiarrhoeal activity of different extracts of stem bark of *Ficus religiosa* Linn. prepared by various methods of extraction viz. soxhlet, ultrasonication assisted and microwave assisted extraction methods.

MATERIALS AND METHODS

Plant collection

The plant material of *Ficus religiosa* Linn. stem bark used for investigation was collected from rural areas of Udaipur (Raj.) in the month of Sep.-Oct. 2008. Plant material was identified by Dr. S.S. Katewa, College of Science, Mohanlal

Sukhadia University, Udaipur (Raj). *Ficus religiosa* Linn. was authenticated from Botanical Survey of India (BSI), Jodhpur (Raj). The Voucher specimen of the plant (BSI/AZRC/I.12012/Tech.393) was deposited at BSI, Jodhpur (Rajasthan) for further reference.

Preparation of extracts

The stem bark of *Ficus religiosa* was collected, cleaned and pulverized in a grinder mixer to obtain a coarse powder and then passed through 40 mesh sieves, weighed quantity of powdered drug was extracted with ethanol 60% (hydroalcoholic) using soxhlet extraction method, acetone using ultrasonication assisted extraction method and acetone using microwave assisted extraction method. The solvent was evaporated from extracts by distillation under reduced pressure. The dried extracts thus obtained were kept in a desiccator and used for further experiment.

Preliminary phytochemical screening

The freshly prepared extracts of *Ficus religiosa* were qualitatively tested for the presence of phytochemical constituents by standard methods [10].

Animals used

Albino wistar rats (175-200 g) of either sex were obtained from the animal house in B. N. College of Pharmacy, Udaipur (Raj.). The animals were maintained in a well ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet diet (Hindustan Lever Ltd., Bangalore) and water was given *ad libitum*. Animal Ethical Committee clearance was obtained from Institutional Animal Ethics Committee of B. N. Institution (Registration no. 870/ac/08/CPCSEA).

Acute toxicity studies

The acute toxicity study was carried out in adult albino rats by "fix dose" method of OECD guideline no. 420. Test procedure with a starting dose of 2000 mg/kg, body weight was adopted. Then the animals were observed continuously for three hours for general behavioral, neurological, autonomic profiles and then every 30 min for next three hours and finally for mortality after 24 hour till 14 days [11].

Castor oil induced diarrhoea

Rats were fasted 24 hr before the test with free access to water *ad libitum*. Rats were treated orally with vehicle and selected extracts of *Ficus religiosa* Linn. (stem bark extracts, 200 mg/kg, p.o.) and standard drug (loperamide, 3 mg/kg, p.o.). One hour after drug treatment, each rat

received castor oil (1 ml each orally). Each rat was then housed separately in cage over clean butter paper. Then diarrhoeal episodes were observed for a period of 4 hours. During this period, consistency of faeces, total number of diarrhoeal faeces within 4 hr and total weight of faeces after 4 hr were recorded [12,13,14].

Statistical analysis

Data were analyzed statistically by one-way ANOVA followed by Dunnett's *t*-test using computerized Graph Pad Prism version 5.4 (Graph Pad Software, U.S.A.). The results were expressed as mean \pm S.E.M. P-values less than 0.05 imply significance.

RESULTS

Preliminary phytochemical screening

The results of preliminary phytochemical screening of various extracts of *Ficus religiosa* prepared by different methods of extraction revealed that presence of alkaloid, glycoside, steroid, tannins, phenolic compounds, carbohydrates and volatile oil.

Acute toxicity studies

Acute toxicity study in which the animals treated with prepared extracts of *F. religiosa* at the higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavior changes, body weight changes at any time of observation. There was no mortality in the above mention dose at the end of the 14 days of observation. Hence, 1/10th (200 mg/kg) of this dose was selected for further study.

Castor oil induced diarrhoea

After 30 min administration of castor oil, the diarrhoea was clinically apparent in all the animals of control group, for the next 4 hour. This was markedly reduced by loperamide (3 mg/kg, p.o.) at 72.42%. A similar marked reduction in the number of defecations over four hours was achieved with *Ficus religiosa* (stem bark) extracts at the dose of 200 mg/kg, p.o (table 1, fig 1). The percentage inhibition for the number of wet faeces within 4 hours as well as total weight (gm) of wet faeces after 4 hrs indicates the presence of antidiarrhoeal activity in extracts as compared with that of control group. The average total number of wet faeces and average total weight of wet faeces in the control group were 19.67 ± 0.61 and 8.05 ± 0.27 gm respectively. The defecation after 4 hours of treatment is significantly ($p < 0.001$) inhibited by hydroalcoholic extract prepared by soxhlet method is 55.15%, acetone extract prepared by ultrasonication assisted extraction method is 40.37% and acetone extract

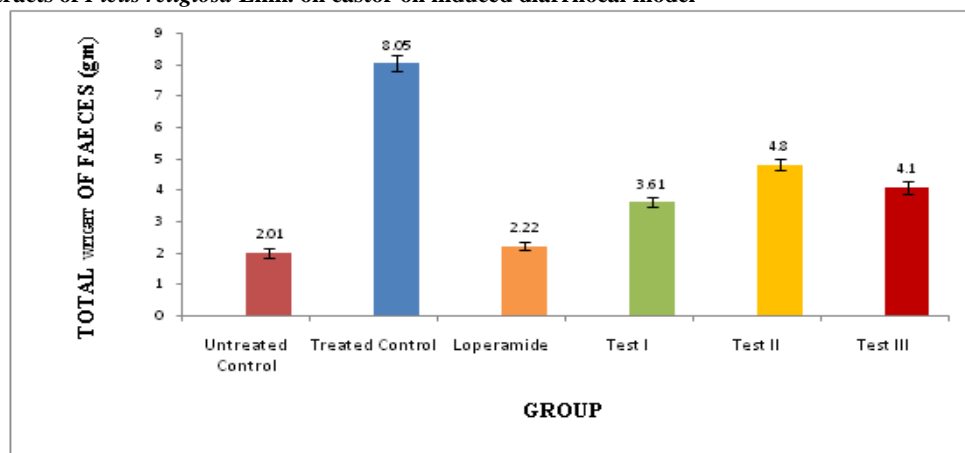
prepared by microwave assisted extraction method is 49.06%.

Table 1: Effect of *Ficus religiosa* Linn. extracts prepared by different extraction methods in castor oil induced diarrhoea

Group and Treatment	Total no. of diarrhoeal faeces (within 4 hr)	Consistency of faeces	Total weight (gm) of faeces (after 4 hr)	Percentage protection
I (Normal treated with vehicle only)	7.00 ± 0.36	Solid	2.01 ± 0.16	-
II (Control treated with castor oil + vehicle)	19.67 ± 0.61	Semisolid with excess of water	8.05 ± 0.27	-
III (Standard treated with castor oil + loperamide)	7.33 ± 0.33	Solid	2.22 ± 0.13***	72.42%
IV (Test-I treated with castor oil + HA extract prepared by soxhlet extraction)	9.16 ± 0.40	Semisolid lumps	3.61 ± 0.15***	55.15%
V (Test-II treated with castor oil + acetone extract prepared by ultrasonication extraction)	13.00 ± 0.63	Semisolid with excess of water	4.80 ± 0.18***	40.37%
VI (Test-III treated with castor oil + acetone extract prepared by microwave extraction)	11.50 ± 0.22	Semisolid	4.10 ± 0.21***	49.06%

Values are mean ± SEM, (n=6); p<0.05 considered as significant, *** p<0.001, One- way ANOVA followed by Dunnett's test as compared to control.

Fig 1: Effect of extracts of *Ficus religiosa* Linn. on castor oil induced diarrhoeal model



Values are mean ± SEM, (n=6); p<0.05 considered as significant, *** p<0.001, One- way ANOVA followed by Dunnett's test as compared to control.

DISCUSSIONS

In developing countries, a quarter of infants and childhood mortality is related to the diarrhoea. Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by an excess loss of fluid in the faeces. During the past decade oral rehydration therapy has reduced mortality from acute diarrhoeal disease, whereas chronic diarrhoea remains a life threatening problem. Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhoea and dysentery.

Oral administration of castor oil (1ml/animal) to the control group causes significant diarrhoea (8.05 ± 0.27). Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid [15] which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin [16,17].

The weight of intestinal content was reduced (2.22 ± 0.13) significantly (p<0.001) with the standard drug loperamide (3mg/kg, p.o.). The therapeutic

effect of loperamide is believed to be due to its antimotility and antisecretory properties [18]. The stem bark extract (hydralcoholic extract prepared by soxhlet extraction method) of *Ficus religiosa* (200 mg/kg) produced a significant (p<0.001) reduction of weight of intestinal content 3.61 ± 0.15 and percentage protection was found to be 55.15% in the severity and frequency of diarrhoea produced by castor oil.

Antidiarrhoeal properties of medicinal plants might be ascribed to tannins, alkaloids, saponins, flavonoids, sterols and reducing sugars [19]. The antidiarrhoeal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic conditions [20]. Flavonoids present in the plant extracts are reported to inhibit release of autocoids and prostaglandins, thereby may inhibit motility and secretion induced by castor oil [21]. Tannins and tannic acid present in antidiarrhoeal plants denature proteins in the intestinal mucosa by forming protein tannates which make the intestinal mucosa more resistant to chemical alteration and reduce secretion [22]. In phytochemical screening of extracts of *Ficus religiosa* showed the presence of alkaloids,

tannins, flavonoids, phenolic compounds and sterols. The presence of these constituents may mediate the antidiarrhoeal property of the extract.

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