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ORIGINAL RESEARCH

Evaluation of Anti-Diarrhoeal Activity of the Leaves Extract of *Ficus Microcarpa L. (Moraceae)*

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ABSTRACT

The aim of present study was to investigate the anti-diarrheal activity of Ficus microcarpa against experimentally induced diarrhea in rats. Albino rats were used for the experiment. Anti-diarrheal activity was determined by castor oil induced diarrhea. The extract of the bark administered orally at a dose 300 mg/kg and 600 mg/kg. The extract of *Ficus microcarpa* produced a marked anti-diarrheal effect in rats. Both doses of extract significantly decreased ($P<0.05$) the total number of wet feces produced by administration of castor oil (4.31 at the dose of 300 mg/kg and 7.16 at the dose of 600 mg/kg) as compared to the castor oil treated control group (20.75). The Ficus microcarpa extract was found to possess an anti-enteropooling activity. Oral administration of castor oil produced a significant increase ($P<0.05$) in the intestinal fluid (3.14 ml) as compared to the normal rat (1.29 ml). The extract when given orally one hour before castor oil, significantly inhibited ($P<0.05$) the enteropooling; 1.48 ml (300mg/kg) and 1.68 ml (600mg/kg). The results revealed that the extract inhibited the small intestinal motility of the charcoal marker in the rats by 23.92-31.12% whereas the inhibition was noted to be 61.2% in the case of treatment by loperamide. The results of this investigation conclude that the extract contains pharmacologically active substances with anti-diarrheal properties. Further research is to be carried out to fractionate and purify the extract, in order to find the molecules responsible for the anti-diarrheal activity.

Keywords: Anti-diarrheal activity, Ficus Microcarpa, Castor oil, Methanolic extract.

INTRODUCTION

In the developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhea. Diarrhea disease is a leading cause of mortality and morbidity, especially in children in developing countries (1). According to WHO estimates for 1998, about 7.1 million deaths were caused by diarrhea (2). Therefore, there is an urgent need for the intensification of research into medicinal plants that are thought to be effective in the management of diarrheal diseases (3). In order to combat the problems of diarrhea globally, the World Health Organization in its Diarrheal Disease Control program has given a special emphasis on the use of traditional folklore medicines.diarrhea A range of medicinal plants with anti-diarrheal properties are widely used by traditional healers. However, the effectiveness of many of these anti-diarrheal traditional medicines have not been scientifically evaluated. *Ficus microcarpa* L. also known as *Ficus Retusa (Moraceae*) with common names in Chinese or Malayan banyan. It is an ever green tree reaching heights of about 15m. It is useful in conditions such as diabetes, ulcers, burning sensations, haemorrhages, leprosy, itching, liver disease, and toothaches (4). The extract was reported to have cytotoxic, antifungal, antidiabetic, antibacterial effects

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(5-7). Flavonoids, triterpenoids, acyclic compounds and steroids are the main components found in the leaves of *Ficus microcarpa*. Thus, the objective of this study was to investigate the anti-diarrheal activity of Ficus microcarpa against experimentally induced diarrhea in rats.

MATERIALS AND METHODS

Drugs and Materials:

Methanol (Research-lab fine chem. Industries, Mumbai, India), Diclofenac sodium (Ranbaxy, India), Carrageenan, acetic acid and tween 80(Acme chemicals, India) were purchase from Rajesh chemicals. Mumbai India. The standard drugs used were diclofenac sodium and histamine from Ranbaxy India Ltd. All the chemicals and drugs used were of analytical grade.

Plant collection and Preparation of the extract

The leaves of *Ficus microcarpa* was collected in November 2012 at Mahatma Phule Krushi Vidyapeeth, Rahuri, Mahrashtra State, India. The plant was authenticated at the Botanical Survey of India, Pune.

The leaves were air dried at room temperature and were later ground to powder. The coarsely powdered plant leaves (200gm) were extracted with methanol for 5 h. After filtration using Whatman No.1 filter paper, the methanolic extract was evaporated in vaccum below 50^{o}C. The yield of evaporation and solvent removal of methanolic extract of *Ficus microcarpa* was 7.40 % w/w, which was stored in refrigerator for further use (8).

Animals

Institutional Animal Ethical Committee, reg. no- MESCOP-1211/ac/08/CPCSEA approved the protocol. The Albino rats were obtained from National Toxicology Center, Pune, India and kept in animal house in standard environmental condition of temperature (22+ 3^{0}C), humidity (60+ 5^{0}C) and at 12 hr light/dark cycle. During experimental time rats were given standard pellet diet (Prashant Enterprises, Pune, India) and water ad libitum. Albino rats (150-200g) of 2-3 months were used.

Phytochemical screening

The methanolic extract was subjected to qualitative phytochemical screening according to standard methods (9).

Acute toxicity test:

The acute toxicity of *Ficus microcarpa* methanol extract was determined in rats according to the method of Hilaly *et al.* with slight modifications. Rats fasted for 16h were randomly divided into groups of six rats per group. Graded doses of the extract (200,400,800, 1600 and 3200mg/kg p.o) were separately administered to the rats in each of the groups. All animals were then allowed free access to food and water and observed over a period of 48h for signs of acute toxicity. The number of deaths within this period were recorded. The number of deaths within this period were recorded (10).

Castor oil-induced diarrhea

Animals were fasted for 18h but allowed free access to water. They were randomized into four groups of six rats each. All Groups received castor oil at a dose of 1ml/animal orally (p.o.)

30 min after castor oil administration, the first group (control) received vehicle (0.5% Tween 80 in distilled water), the second and third group received extract 300mg/kg and 600mg/kg body weight, respectively (11). The fourth group received the reference drug loperamide (3mg/kg orally). After this administration, the animals were placed separately in metabolic cages with filter paper, which was changed every hour. The severity of diarrhoeadiarrhea was assessed each hour for 6h. The total number of feces, diarrhea feces excreted, and the total weight of feces were recorded within a period of 24h and compared with the control group. The total number of diarrhea feces of the control group was considered to be 100%. The results were expressed as a percentage inhibition of diarrhea(12).

Enteropooling assay

Intraluminal fluid accumulation was determined by the method of Robert et al. (13). The rats were divided into four groups of six animals each. Group 1 received 2 ml of normal saline and group 2 received 2 ml of castor oil. Group 3and 4 received extracts of 300 and 600 mg/kg p.o. respectively, one hour before the oral administration of castor oil. Two hours later, the rats were anesthetized with ether and sacrificed. The edges of the small intestine were tied with the thread and the intestine was removed and weighed. The intestinal content was collected by squeezing it into a graduated tube and the volume was measured. The intestine was reweighed and the difference between the full and empty intestines were calculated.

Gastrointestinal transit test

Four groups of six animals each, fasted overnight were used. The test extract was given orally to group 2 and group 3 (300 and 600 mg/kg, respectively), while group 1 was used as a control; the 4th group received loperamide (5mg/kg) as a standard. Five minutes later, 0.5 ml of a 3% charcoal suspension in 5% suspension of tragacanth powder was administered orally to each animal. All the animals were killed by cervical translocation 30 min later and the distance travelled by the charcoal plug from pylorus to cecum was determined and expressed as a percentage of the total length of the small intestine (14).

Statistical analysis

Results were expressed as mean +S.D. Statistical analysis of the data was done using one–way analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS

Phytochemical analysis

The preliminary phytochemical analysis of the methanolic extract of Ficus microcarpa revealed the presence of alkaloids, steroid terpenoids, and tannins.

Table 1 Effect of Ficus microcarpa leaves methanolic extract on castor oil-induced diarrhoea

Group	Total number of faeces	Total number of diarrhoeal faeces	Percentage of inhibition	Total weight of faeces (g)	Percentage of inhibition
Castor oil (1ml) +Vehicle (0.5% Tween 80)	24.31 ± 4.37	20.75 ± 5.11	0%	7.35 ± 2.20	0
Loperamide (3mg/kg) + Castor oil (1ml)	9.30 ± 0.80*	6.15 ± 1.56*	70.37	1.76 ± 0.85*	76.81
Extract (300mg/kg) + Castor oil (1ml)	21.40 ± 2.31	4.31 ± 2.52**	79.25	5.02 ± 1.42	31.67
Extract (600mg/kg) + Castor oil (1ml)	20.49 ± 4.32	7.16 ± 1.32*	65.55	5.02 ± 0.56	31.72

Values of Mean + S.E.M. (n=4), *P<0.05, **P<0.01, Dunnett Multiple comparison test

Acute toxicity test

Oral administration of graded doses (200, 400, 800, 1600 and 3200 mg/kg p.o.) of the methanolic extract of *Ficus microcarpa* to rats did not produce any significant change in behavior, breathing, cutaneous effect, sensory nervous system responses or gastrointestinal effect during the observation period. No mortality was recorded in any group after 72h of administering the extract to the animals.

Effect of *Ficus microcarpa* extract and Castor oil-induced diarrhea

In the castor oil induced diarrhea experiment where the rats that did not receive the plant extract, the rats showed typical diarrhea sign such as watery and frequent defecation. The extract of *Ficus microcarpa* produced a marked anti-diarrheal effect in rats. Both the doses of extract significantly decreased (P<0.05) the total number of wet feces produced by administration of castor oil (4.31 at the dose of 300 mg/kg and 7.16 at the dose of 600mg/kg) as compared to the castor oil treated control group (20.75). The percentage of inhibition of castor oil induced diarrhea in extract treated rats was 79.25 and 65.55% respectively at 300 and 600mg/kg dose. The effect of Ficus microcarpa extract was similar to that of the standard drug, loperamide (3 mg/kg) which produced an inhibition of 70.37% (Table 1). The average weight of feces in the control group was 7.35 g. Treatment with both doses of extract significantly reduced (P<0.05) the weight of feces to 5.02 g (Table 1).

Enteropooling assay

The Ficus microcarpa extract was found to possess an anti-enteropooling activity. Oral administration of castor oil produced a significant increase (P<0.05) in intestinal fluid (3.14 ml) as compared to the normal rat (1.29 ml). The extract when given orally one hour before the castor oil administration, significantly inhibited (P<0.05) the enteropooling; 1.48 ml (300mg/kg) and 1.68 ml (600mg/kg). The volume of intestinal fluid was similar to that obtained in the normal group (1.29 ml) (Table 2). The weight of intestinal content also significantly decreased following treatment with castor oil (from 3.55 to 1.04 g in the normal rats). However, the extract produced a marginal decrease in the weight of the intestinal content.

Effects of Ficus microcarpa extract on charcoal-induced gut transit changes

The result revealed that the extract inhibited the small intestinal motility of the charcoal marker in the rat by 23.92-31.12%, whereas the inhibition was noted be 61.2% in the case of treatment by loperamide (Table 3).

Table 2 Effect of Ficus microcarpa leaves methanolic extract on Enteropooling assay in rat

Group	Volume of intestinal content (ml)	Weight of intestinal content (g)
Normal saline (2ml)	1.29 ± 0.25	1.04 ± 0.26**
Castor oil (2ml)	3.14 ± 0.38**	3.55 ± 0.63**
Extract (300mg/kg)+ Castor oil (2ml)	1.48 ± 0.29	2.45 ± 0.26**
Extract (600mg/kg) + Castor oil (2ml)	1.68 ± 0.38	2.41 ± 0.32**

Values of Mean + S.E.M. (n=4), *P<0.05, **P<0.01, Dunnett Multiple comparison test

Table 3 Effect of Ficus microcarpa leaves methanolic extract on Gastrointestinal transit in rat.

Treatment group	Distance travelled by marker as % of total length of small intestine	% inhibition
control	73.51 ± 9.8	0
Extract (300mg/kg)	55.92 ± 7.15	23.92
Extract (600mg/kg)	48.59 ± 4.31*	31.12
Loperamide (5mg/kg)	28.52 ± 2.57 **	61.2

Values of Mean + S.E.M. (n=4), *P<0.05, **P<0.01, Dunnett Multiple comparison test

DISCUSSION

In traditional medicine, many plants are claimed to have antidiarrheal efficacy without any scientific basis. Castor oil causes diarrhea due to its active metabolite, ricinolic acid, which stimulate peristaltic activity in the small intestine, leading to changes in electrolyte permeability of the intestinal mucosa (15). The aim of the present study was to evaluate antidiarrheal effects of the leaves extracts of Ficus microcarpa. The extract exhibited a significant anti-diarrheal activity. The results were similar to that

of the standard drug loperamide (3mg/kg) with regards to the severity of the diarrhea. The extract significantly reduced intestinal transit as observed by the decrease in intestinal motility of the charcoal meal. The extract also leads to a marked reduction in the weight and the volume of the intestinal contents. Phytochemical screening revealed the presence of alkaloids, steroids, terpenoids, and tannins. Earlier studies showed that the anti-diarrheal properties of medicinal plants were due to tannins, alkaloids. The anti-diarrheal activity of this extract may also be due to the presence of the terpenoids and tannins. The tannins make the intestinal mucosa more resistant and hence reduce secretion (16). This can be due to the fact that the extract increased the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal. The standard drug, Loperamide, regulates the gastrointestinal tract as well as reported to slow down transit in the small intestine, reduce colon flow rate, and consequently reduce any effect on colonic motility.

CONCLUSION

In conclusion, the results of this investigation revealed that the extract contains pharmacologically active substances with anti-diarrheal properties. These properties confirm the use of Ficus microcarpa as an anti-diarrheal drug as proposed by traditional healers. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecules responsible for the anti-diarrheal activity observed.

Ficus Microcarpa L. (Moraceae) Yapraklarının Ekstresinin Anti-diyareik Etkinliğinin Değerlendirilmesi

ÖZET

Bu çalışmanın amacı, sıçanlarda deneysel olarak oluşturulan diyareye karşı Ficus microcarpa ekstresinin antidiyareik etkinliğini araştırmaktır. Deneylerde Albino sıçanlar kullanıldı. Antidiyareik aktivite hint yağı ile oluşturulan diyare modelinde çalışıldı. 300 mg/kg ve 600 mg/kg dozlarında Ficus microcarpa ekstresi sıçanlarda anlamlı bir anti diyareik etki gösterdi. Toplam ıslak dışkı sayısı hint yağı grubuna (20.75) göre ekstre tedavisi uygulanan gruplarda her iki dozda önemli ölçüde (P <0.05; 300 mg/kg 'de 4.31 ve 600 mg/kg dozunda 7.16) azaldı. Ficus microcarpa ekstresinin anti- enteropooling etkinliğe sahip olduğu bulundu. Kontrol grubu sıçanlarda 1.29 ml olan barsak sıvısı oral hint yağı uygulaması sonrasında anlamlı olarak yükseldi (P <0.05 ,3.14 ml). Hintyağı öncesinde oral uygulanan ekstre ise barsak sıvısındaki artışı anlamlı olarak inhibe etti (P <0.05; 300mg/kg ile 1.48 ml ve 600mg/ kg ile 1.68 ml). İntestinal motilitenin inhibisyonu aktif kömür deneyi ile çalışıldı; ekstre ile inhibisyon % 23.92-31.12 ve loperamid ile % 61.2 olarak belirlendi. Bu çalışmanın sonuçları, Ficus microcarpa ekstresinin antidiyareik özelliklere sahip farmakolojik olarak aktif maddeler içerdiğini düşündürmektedir. Antidiyareik etkiden sorumlu moleküllerin belirlenmesi, ve saflaştırılması için yeni çalışmalar gereklidir.

Anahtar Kelimeler: Anti-diyareik aktivite, Ficus microcarpa, Hintyağı, Metanolik ekstre.

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