



***In vivo* evaluation of antidiarrhoeal activity of ethanolic extract of leaf and bark of *Ficus carica* Linn.**

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**Abstract**

*Ficus carica* Linn is occasionally used in preparation of local traditional medicines used in the treatment of diarrhoea in Bangladesh. Our present studies make an attempt toward validating this traditional use by investigating antidiarrhoeal activity of *F. carica* Linn. Ethanolic extract of leaf and bark of *F. carica* Linn showed significant ( $p \leq 0.05$ ) decrease in the severity of diarrhoea, in a dose dependent manner, in castor oil induced diarrhoea test. Prostaglandin E2 induced intestinal fluid accumulation test (enteropooling) gave significant results ( $P \leq 0.05$ ), indicating possible antidiarrhoeal action. The extract produced significant ( $P \leq 0.05$ ) reduction of intestinal transit in gastrointestinal motility test with barium sulfate milk in healthy rats. It is evident that *F. carica* Linn have significant antidiarrhoeal activity and may be a potential source of antidiarrhoeal agents.

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## Introduction

Diarrhoea is one of the most prominent reasons (7.1 million incidents per year) of malnutrition and death among the children in the world, especially in the developing countries (Victoria, Bryce, Fontaine, & Monasc, 2000) (Park, 2000). It is manifested by increased gastrointestinal movement, watery or wet stool, and abdominal pain (Aranda-Michel & Gianella, 1999). Bangladesh is very rich in medicinal plants. (Bangladesh National Formulary of Ayurvedic Medicine, 2011) From the ancient ages, many alternative and traditional medicine systems like Ayurveda, Unani, Homeopathy etc has been using different medicinal plants and/or their extracts in the formulation of drugs, however this practice has got very little or no scientific evidences (Tylor, 2000). There is an increasing demand to establish the claimed activity of different medicinal plants and to ensure the safety and efficacy of the plant products (Firenzuoli & Gor, 2007).

*Ficus carica* Linn., a member of Moraceae Family, is widely spread in tropical and subtropical countries. It is a small to moderate sized deciduous tree, 3-10 m high with broad ovate or nearly orbicular leaves, more or less deeply 3-5 lobed, rough above and pubescent below; fruits axillary, normally pear shaped, variable in size and colour. The fruit of *F. carica* is a syconium a fleshy hollow receptacle with a narrow aperture at the tip. The bark is a cylindrical and pale grey coloured (The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products, 1999). The fruit, leaf, bark, and root are used in different traditional medicine (Bangladesh National Formulary of Ayurvedic Medicine, 2011). Different pharmacological properties like antipyretic, anti-helmentic, hypoglycemic etc has been reported (Patil & Patil, 2011). However, the antidiarrhoeal activity of this plant has got no or trivial scientific evidences. The present study was undertaken to evaluate the antidiarrhoeal activity of ethanolic extract of the leaf and bark of *F. carica* to validate the use of this plant in

traditional system of medicine in the treatment of diarrhoea.

## Materials and methods

### Reagents used

All reagents and chemicals that were used in the experiments were of analytical grade. Pharmaceutical grade Loperamide and Indomethacine were collected from Square Pharmaceuticals Bangladesh Ltd. Normal saline was collected from Beximco Infusion Ltd. All other reagents including Atropine sulfate, Prostaglandin E2 were procured from Sigma Aldrich (USA).

### Plant material

For this study, the *F. carica* was collected from Village: Sonpara, Thana: Arai hazar; District: Narayanganj, Bangladesh in February 2012 and was identified at the Bangladesh National Herbarium, Mirpur, Dhaka where the voucher specimen no: 39875 was for the *F. carica* deposited. The collected plant parts were dried for 7 days and ground into a coarse powder by a suitable grinder. The powder was stored in a zipper bag which was then kept in an airtight container and kept in a cool, dark, and dry place for further use.

### Preparation of the extract

About 500 gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 2000 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 5 days with continuous mechanical shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then the filtrate was kept in a beaker for 1 day without any shaking. The next day the supernatant solution was taken by pipetting. Then the solution was filtered through Whatman filter paper. The filtrate obtained was evaporated using rotary evaporator. It became a gummy concentrate of yellowish black colour. The extract was transferred in closed glass container for further use and preservation.

*Acute toxicity study*

Doses of 50, 100, 250, 500, 1000, 2500 and 5000 mg/Kg of extracts were administered orally to rats. The extracts were given at the doses of 250 and 500 mg/Kg of body weight/day. All the animals were found to be safe at highest dose (5000mg/Kg). Then the rats were observed for incidence of mortality or any sign of toxicity up to 24 h. OECD Guideline (OECD Guideline 425) were followed in maintaining dosing schedule (Werbach, 1993).

*Maintenance and use of test animals*

Healthy Sprague-Dawley rats, weighing 130-160g, of both sexes, were procured from Jahangir Nagar University Animal House. The test subjects were provided with standard rat pellet diet and filtered drinking water ad libitum. This study was approved by an ethics committee of North South University (LSEC-15G-2012).

*Grouping and Drug administration*

The animals were randomly divided into several groups of 10 rats for the planned antidiarrhoeal tests. Control groups were treated p.o. with 1% tween solution in normal saline (0.9% NaCl) at a volume that would not cause any additional psychological or physiological stress to the animals. Positive controls were treated with Loperamide, Atropine sulfate, and Indomethacine, where applicable. Treatment groups were treated with three doses (100mg/kg, 200mg/kg, and 300mg/kg) of *F. carica* extract.

*Determination of antidiarrhoeal activity*

The method described by Chatterjee (1993) was followed in conducting the experiment (Ecobichon, 1997), but with minor modifications. 18 hour fasted rats were randomly assigned to five groups, ten rats per group, designated as Group A (0.5% v/v Tween 80 in normal saline, 10ml/Kg p.o.), Group B (*F. carica* 100mg/Kg p.o.), Group C (*F. carica* 200mg/Kg p.o.), Group D (*F. carica* 300mg/Kg p.o.), and Group E (Atropine sulfate 0.1mg/Kg i.p.). 1 hour after treating

the groups with assigned treatments, the barium sulfate milk (10% w/v in 0.5% Na CMC) was administered orally. Then the rats were sacrificed after 30 minutes. The length of GI traversed by the barium sulfate was measured and expressed as a percentage of the total length of small intestine. The percentage inhibition of barium sulfate traverse of groups other than A was calculated by taking the percentage inhibition value of Group A equal to zero.

*Castor oil induced diarrhoea*

The method described by Moly Thomas and F. Gricilda Shoba (2001) was followed in conducting the experiment (Shoba & Thomas, 2001), but with minor modifications. 18 hour fasted rats were randomly assigned to five groups, ten rats per group, designated as Group A (0.5% v/v Tween 80 in normal saline, 10ml/Kg p.o.), Group B (*F. carica* 100mg/Kg p.o.), Group C (*F. carica* 200mg/Kg p.o.), Group D (*F. carica* 300mg/Kg p.o.), and Group E (loperamide 3mg/Kg p.o.). The rats were kept in specially designed cages which had facilities to collect and observe the stool. 1 hour after treating the groups with assigned treatments, the castor oil (0.5ml/Kg) was administered orally. After six hours long observation, the stools were collected, counted, and weighed. The percentage inhibition of the total number of feces, total number of diarrhoeal feces, and weight of the feces for the groups other than the Group A was calculated by taking the percentage inhibition value of Group A equal to zero.

*PGE<sub>2</sub> induced enteropooling*

18 hour fasted rats were randomly assigned to six groups, ten rats per group, designated as Group A (0.5% v/v Tween 80 in normal saline, 10ml/Kg p.o.), Group B (0.5% v/v Tween 80 in normal saline, 10ml/Kg p.o.) Group C (*F. carica* 100mg/Kg p.o.), Group D (*F. carica* 200mg/Kg p.o.), Group E (*F. carica* 300mg/Kg p.o.), and Group F (Indomethacine 10mg/Kg p.o.). Just after treating the groups with the assigned treatments, 100mcg/Kg PGE<sub>2</sub> was orally administered to every groups except Group A. Each

and every rats were sacrificed 30 minutes after the administration of PGE<sub>2</sub> and the total intestine was isolated, dissected longitudinally, and cleared to collect the intestinal contents in different properly labeled test tubes. The weight and volume of the intestinal contents were measured (Awouters, Nimegeers, Lenaerts, & Janssen, 1978).

#### Phytochemical screening

The extract was screened for the presence of alkaloids, flavonoids, tannins, glycosides, resins, phenols, carbohydrate, sterols, volatile oils and saponins using standard test procedures (Sofowor, 1993).

#### Statistical analysis

Results were expressed as mean  $\pm$  SEM (standard error of mean) of responses. All tests were done using SPSS Software Ver. 20. Statistical significance was determined by One-way Analysis of Variance (ANOVA) followed by post hoc Dunnett test. The *P* values less than 0.05 were considered to be significant.

### Result

#### Gastrointestinal motility test

*F. carica*, at all doses except 100mg/Kg, significantly decreased the traverse of the barium sulfate through the gastrointestinal tract (100mg/Kg, 200mg/Kg, and 300 mg/Kg cause reduction of 22.88%, 29.81%, and 42.50% respectively as compared with control group, group A). A similar reduction of 48.56% was observed with the positive control group, group E (TABLE 1).

#### Castor oil induced diarrhoea

The *F. carica* extract, in a dose dependent manner, significantly reduced the diarrhoeal condition (TABLE 2) with highest result at the dose of 300mg/Kg. The percentage of inhibition of number of watery feces as and stool weight was found 59.52% and 74.29% respectively at the dose of 300 mg/kg. This result was similar to the result of the standard drug loperamide (69.40% and 85.82%).

Table 1. Effect of ethanol extract of *F. carica* on gastrointestinal motility (by barium sulfate traverse).

Group	Barium sulfate traverse (%)	Inhibition (%)
A= Vehicle	78.21 $\pm$ 6.4	-
B= <i>F. carica</i> 100mg/Kg	60.31 $\pm$ 3.1	22.88%
C= <i>F. carica</i> 200mg/Kg	54.89 $\pm$ 5.3*	29.81%*
D= <i>F. carica</i> 300mg/Kg	44.98 $\pm$ 4.6*	42.50%*
E= Artopine 0.1mg/Kg	40.23 $\pm$ 5.2*	48.56%*

Values are expressed as Mean  $\pm$  S.E.M of 10 rats. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnett test \**p*<0.05 compared to the vehicle group.

#### PGE<sub>2</sub>-induced enteropooling

PGE<sub>2</sub> caused a significant increase in the fluid volume of the rat intestine when compared with vehicle control group, group A. *F. carica* extracts significantly inhibited, in a dose dependent manner, PGE<sub>2</sub> induced enteropooling in rats in terms of volume and weight of intestinal content (TABLE 3). The effect of 300 mg/Kg (inhibition of volume 73.34% and inhibition of weight 73.93%) was comparable to the effect of standard drug Indomethacine (82.49% inhibition of volume and 81.55% inhibition of weight).

#### Phytochemical screening

Phytochemical screening of *F. carica* extract proved the presence of alkaloids, flavonoids, glycosides, saponins, sterols, carbohydrate, tannins, and volatile oil (TABLE 4).

### Discussion

Castor oil induced diarrhoea test was employed to evaluate the antidiarrhoeal activity of *F. carica*. Our findings demonstrated significant activity of *F. carica* (100mg/Kg, 200mg/kg, and 300mg/kg) in this model. Ricinoleic acid, an active component of castor oil, induces permeability changes in mucosal fluid and electrolyte transport. This causes a hypersecretory

response and diarrhoea (Trease & Evans, 1996). Ricinoleic acid significantly increases the PGE<sub>2</sub> conc. in the intestinal lumen and also causes an increase of the secretion of the water and electrolytes into the gastrointestinal tract (Gaginella, Stewart, Olson, & Bass, 1975). It is reported that prostaglandin

biosynthesis inhibitors delayed castor oil induced diarrhoea (Doherty, 1981). Hence, the test extract, *F. carica*, might reduce the incidence of diarrhoea by inhibiting prostaglandin biosynthesis.

**Table 2.** Effect of different doses of *F. carica* extract on castor oil induced diarrhoea.

Group	Total number of feces	Inhibition (%)	Total number of diarrhoeal feces	Inhibition (%)	Total weight of feces (g)	Inhibition (%)
A= Vehicle	21.40±3.9	-	17.34±1.2	-	9.10±0.23	-
B= <i>F. carica</i> 100mg/Kg	17.86±2.8	16.54%	13.22±1.1	23.70%	6.20±0.89*	31.87%*
C= <i>F. carica</i> 200mg/Kg	14.23±1.1*	33.51%*	10.01±0.89*	42.72%*	4.11±0.23*	54.84%*
D= <i>F. carica</i> 300mg/Kg	13.11±0.90*	38.74%*	7.02±0.54*	59.52%*	2.34±0.45*	74.29%*
E= Loperamide 3mg/Kg	9.10±0.67*	57.48%*	5.29±0.32*	69.49%*	1.29±0.32*	85.82%*

Values are expressed as Mean ± S.E.M of 10 rats. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnett test \*p<0.05 compared to the vehicle group.

To reinforce the above findings, we employed PGE<sub>2</sub> induced intestinal fluid accumulation (enteropooling) test. The test doses of *F. carica* significantly inhibit the fluid accumulation in the similar extent as the standard drug loperamide did. It has been reported that PGE<sub>2</sub> cause diarrhoea and the mechanism has been suggested that it alters gastrointestinal movement and water and electrolyte transport (Pierce, Carpenter, Elliott, & Greenough, 1971) (Awouters, Nimegeers, Lenaerts, & Janssen, 1978). Combining the results of present study with these reports it can be suggested that *F. carica* exert antidiarrhoeal activity by inhibition of action of PGE<sub>2</sub> on gastrointestinal tract.

To further ascertain its antidiarrhoeal activity, gastrointestinal motility test using barium sulfate milk marker was done. The *F. carica* extract showed dose dependent reduction of gastrointestinal movement. This reduction in peristaltic movement might increase

the GI emptying time which eventually might increase the re-absorption of water and electrolytes.

The phytochemical analysis of the *F. carica* showed the presence of alkaloids, saponins, flavonoids, sterols and/or terpenes and sugars. Flavonoids and saponins have been reported to possess antimotility and antisecretory activity on gastrointestinal tract (Agbor, Leopold, & Jeanne, 1999) (Galvez, Crespo, Jimerez, Suarez, & Zarzuelo, 1991) (Oben, Assi, Agbor, & Musor, 2006). Steroids are useful for the ability to increase intestinal absorption of Na<sup>+</sup> and water (Su, Leung, Bi, Huang, & Chen, 2000). Protein tannates, formed from proteins in the presence of tannin, make the intestinal mucosa more resistant and hence, reduce secretion (Oben, Assi, Agbor, & Musor, 2006).

**Table 3.** Effect of different doses of *F. carica* extract on castor oil induced intestinal fluid accumulation (enteropooling).

Group	Volume of intestinal content (ml)	Inhibition (%)	Weight of intestinal content (g)	Inhibition (%)
A= Vehicle	1.08±0.81	-	1.39±0.73	-
B= PGE <sub>2</sub> control	6.34±0.23	-	7.21±0.88	-
C= <i>F. carica</i> 100mg/Kg	3.22±0.21*	49.22%*	3.89±0.13*	46.04%*
D= <i>F. carica</i> 200mg/Kg	2.10±0.31*	66.88%*	2.56±0.09*	64.49%*
E= <i>F. carica</i> 300mg/Kg	1.69±0.22*	73.34%*	1.88±0.15*	73.93%*
F= Indomethacine 10mg/Kg	1.11±0.41*	82.49%*	1.33±0.72*	81.55%*

Values are expressed as Mean ± S.E.M of 10 rats. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnett test \*p<0.05 compared to the PGE<sub>2</sub> control group.

**Table 4.** Phytochemical screening of *F. carica* extract.

Test	Inference
Alkaloids	+
Flavonoids	+
Glycosides	+
Phenols	-
Resins	-
Saponins	+
Tannins	+
Sterol	+
Carbohydrate	+
Volatile oil	+

### Conclusion

The results suggest that the ethanolic extract of *F. carica* contains pharmacologically active substances that have potent inhibitory action on gastrointestinal movement and secretion. This might be due to the inhibition of biosynthesis of PGE<sub>2</sub> and/or the inhibition of the action of PGE<sub>2</sub> on the gastrointestinal tract. This might also be due to the presence of various types of chemical components like flavonoids, saponins, sterols etc which might reduce the occurrence and severity of diarrhoea. Presence of several types of

mechanism of antidiarrhoeal activity of the crude extract is also an option. Hence, its traditional use in preparations used in the treatment of diarrhoea held the test of time, not by its mere placebo effect but by some potent molecules hidden in this ethanolic extract of *F. carica*. But our study did not go further to elucidate the mechanism behind the activity but the effects of the extract on physiological and pathophysiological conditions. We believe that further studies are required to completely understand the mechanism of antidiarrhoeal action of *Ficus carica* Linn and thus its better usage can be ensured.

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### Conflict of Interest

The authors have no conflict of interest to declare.

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