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Investigation of the central and peripheral analgesic activity of ethanolic extract of *Ficus carica* Linn.

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Abstract

Ficus carica Linn is widely used in the preparation of local traditional medicine used for pain relief in Bangladesh. Our present studies make an attempt toward validating this traditional use by investigating anti-nociceptive and anti-inflammatory activities of *F. carica* Linn. Ethanolic extract of whole herb and fruits of *F. carica* Linn showed significant ($p \leq 0.05$) CNS modulated anti-nociceptive activity, in a dose dependent manner, in both Hot Plate and Tail Immersion Tests. Carrageenan induced rat paw edema test gave significant results ($P \leq 0.05$), indicating possible anti-inflammatory action. The extract failed to produce any significant result in Acetic acid induced writhing test. Formalin induced paw-licking test showed that *F. carica* Linn had significant effect in suppressing neurogenic pain ($P \leq 0.05$).

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Introduction

World health Organization (WHO) has stated that up to 80% of the population in many Asian and African countries depend on traditional and complimentary drugs to meet their medical necessities (WHO 2008). It is also an extremely attractive business for many drug vendors which often results in misleading claims being made and confusion in the mind of consumers. Persistent continuation of a regimen with one of these drugs which do not have any pharmacological activity, in reality, would seriously aggravate the morbidity of the patients. For these reasons and others, there has been a demand for ensuring the safety and efficacy of some of these traditional/herbal medicines (Firenzuoli F. and Gor L. 2007).

Ficus carica Linn. ,a member of Moraceae Family, is widely spread in Bangladesh, India, and some other tropical 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 peer shaped, variable in size and colour. The fruit of *F. carica* is a syconium a fleshy hollow receptable with a narrow aperture at the tip. The bark is a cylindrical and pale grey coloured (The Wealth of India). Its fruit, root, bark, and leaves are used to prepare different preparation for diseases like colic, indigestion, loss of appetite, diarrhea, sore throats, coughs, bronchial problems, inflammatory disorders, and cardiovascular disorders in the ayurvedic and other traditional system of medicine (Burkill and I.H., 1935). Antipyretic property of the fruit (Patil VikasV *et al.*, 2010), anti-helmintic property of latex (Alziro de Amorin *et al.*, 1999), and hypoglycemic (Alicia Serraclara *et al.*, 1998), hepatoprotective (Krishna Mohan G *et al.*, 2007), antihelminthic, and antipyretic properties of leaves have been reported (Werbach M., 1993). However, the claim of possessing analgesic and anti-inflammatory activity of this plant has no or very less scientific evidences. The present study was undertaken to evaluate the analgesic and anti-inflammatory activity of ethanolic extract of the whole herb and fruits of *F. carica* to validate the use of this plant in the preparation of medicine traditional system of medicine used for pain management.

Materials and methods

Reagents Used: All reagents and chemicals that were used in the experiments were of analytical grade. Pharmaceutical grade Tramadol, and Diclofenac Sodium were collected from Square Pharmaceuticals Bangladesh Ltd. Normal saline was collected from Beximco Infusion Ltd. All other reagents were procured from Sigma Aldrich (USA) unless mentioned otherwise.

Plant material

For this study, the *F. carica* was collected from Village: Sonpara, Thana: Araihazar; District: Narayangonj, Bangladesh in February 2012 and was identified at the Bangladesh National Herarium, Mirpur, Dhaka where the voucher specimen no: 39875 was for the *F. carica* deposited. The collected plant parts were dried for one week and ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until analysis started.

Preparation of the extract

About 250 gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 1000 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 5 days accompanying occasional 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 pippetting. Then the solution was filtered through Whatman filter paper. The filtered obtained was evaporated using rotary evaporator. It rendered a gummy concentrate of yellowish black colour. The extract was transferred in closed 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 mice. 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 mice 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.

Maintenance and use of test animals

Healthy Swiss Albino mice (5-6 weeks old, of both sex) weighing 20-25g and Spraugue-Dawley rats, weighing 130-160g, of both sex, 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) which gave its consent in absolute accordance with the recommendations of the international Association for the study of Pain (Zimmerman M., 1983).

Grouping and drug administration

The animals were randomly divided into several groups of 8 mice/rats for the planned analgesic and anti-inflammatory 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 Tramadol and Diclofenac Sodium. Treatment groups were treated with three doses (100mg/kg, 200mg/kg, and 300mg/kg) of *F. carica*.

Determination of CNS modulation in analgesic activity

Hot Plate Test: The Hot plate test was performed on the test subjects in a slightly modified version from the one described earlier (Franzotti EM *et al.*, 2000). The animals were placed on hot plate apparatus (Model-35100, manufacturer-UGO Basile of Italy) maintained at a temperature of 54±0.5˚C for a maximum time of 20s per exposure. The control group was administered with 1% tween solution in normal saline (0.9% NaCl) p.o. The treatment groups were treated with *F. carica* (100mg/kg, 200mg/kg, and 300mg/kg p.o.). Naloxone (5mg/kg i.p.) was administered with *F. carica* (100mg/kg, 200mg/kg, and 300mg/kg p.o.) and Tramadol to four different groups, other than the treatment groups.

Tail Immersion test

The tail immersion test was performed according to the procedures used by (Yang XY *et al.*, 2000), with minor modifications. Briefly, the lower two-third of mouse's tail was immersed in a constant temperature water bath at 50±0.2˚C. The reaction time, i.e. the amount of time it takes the animal to withdraw its tail was measured. *F. carica* (100mg/kg, 200mg/kg, and 300mg/kg p.o.), Tramadol (10mg/Kg p.o.), and 1% tween solution in normal saline (0.9% NaCl) (p.o.) were administered to treatment groups. Naloxone (5mg/kg i.p.) was administered with *F. carica* (100mg/kg, 200mg/kg, and 300mg/kg p.o.) and Tramadol to four different groups, other than the treatment groups.

Determination of peripheral analgesia

Acetic-acid induced writhing test: The test was carried out using a modified method from the procedure perviously described (Franzotti EM *et al.*, 2000). *F. carica* at three doses (100mg/kg, 200mg/kg, and 300mg/kg p.o.) were administered to treatment groups. Positive control group was administered with Diclofenac sodium (10mg/kg p.o.) and 1% tween solution in normal saline (0.9% NaCl) p.o. was administered to the control group. 45 minutes after drug treatment, the mice were given 0.7% v/v acetic acid (0.15mL/10mL i.p.) to induce writhing.

Carrageenan induced paw edema test

Carrageenan induced paw edema test was carried out by following the method described previously (Damas J. *et al.*, 1992). Male and female Sprague-Dawley rats were used. The control rats received 1% tween solution in normal saline (0.9% NaCl) p.o.

and the experimental rats received *F. carica* (100mg/kg, 200mg/kg, and 300mg/kg p.o.). Thirty minutes later, the rats were given a subcutaneous injection of 0.05mL of 1% solution of carrageenan in the left hind paw.

Table 1. Effect of Ficus carica on nociceptive responses in the hot plate test.

Group	Dose (mg/Kg)	Latency Period (second)						
		0 min	**30 min**	**1h**	**2h**	**3h**	**4h**	**5h**
Control	-	10.40±0.4	10.72±2.0	10.62±2.3	10.95±2.0	10.96±2.1	8.53±2.1	9.22±1.9
F. carica	100	12.02±0.7	10.22±0.7	8.92±0.8	9.83±0.8	9.98±1.1	9.05±0.7	8.47±0.8
F. carica	200	12.01±0.8	17.85±0.7*	18.02±0.8*	18.37±0.8*	17.53±1.0*	17.05±0.6*	16.77±0.7
F. carica	300	10.02±0.7	19.33±0.7‡	19.67±0.8‡	19.62±0.9‡	19.97±0.9‡	20.00±0.7‡	19.43±0.9‡
Tramadol	10	10.90±0.4	18.67±2.3*	19.83±2.5‡	19.33±2.1‡	19.13±2.1‡	19.67±2.1‡	19.78±1.9‡
Co-treatment with Naloxone								
F. carica + Naloxone	300	10.02±0.7	9.43± 0.6	12.53±0.9	10.32±0.8	9.83±0.9	8.47±0.6	9.40±0.9
Tramadol+ Naloxone	10	8.32±0.5	14.85±1.6	14.48±1.8	14.87±1.6	15.97±1.7*	14.58±1.7*	14.1±1.6*

Values are expressed as Mean ± S.E.M of 8 rats. Differences between groups are determined by One-Way Repeated Measures ANOVA followed by post hoc Dunnett test; and then pair-wise comparison tests were done with Bonferroni correction. *p<0.05 and ‡p<0.01 compared to the control treated group.

Table 2. Effect of *Ficus carica* on nociceptive responses in the tail immersion test.

Group	Dose (mg/Kg)	Latency Period (second)				
		0 min	**30 min**	**60 min**	**90 min**	**120 min**
Control	-	7.33±1.81	3.25±2.21	4.92±2.91	3.75±2.01	3.83±2.59
F. carica	100	3.47±1.79	13.87±2.32*	13.27±2.19*	13.82±1.78*	13.52±1.78*
F. carica	200	3.72±2.32	21.17±2.29‡	21.48±2.22‡	20.58±1.88‡	18.33±1.89‡
F. carica	300	4.50±1.79	23.50±2.07‡	26.83±2.31‡	28.17±1.83‡	28.00±1.69‡
Tramadol	10	3.83±1.81	21.83±2.21‡	21.90±2.91‡	20.33±2.01‡	17.67±2.59‡
Co-treatment with Naloxone						
F. carica + Naloxone	300	7.15±1.80	7.17±1.89	7.00±2.16	8.00±1.65	5.83±1.79
Tramadol + Naloxone	10	5.00±1.80	7.72±2.07	6.00±2.04	5.33±1.73	7.17±1.66

Values are expressed as Mean±S.E.M of 8 rats. Differences between groups are determined by One-Way Repeated Measures ANOVA followed by post hoc Dunnett test; and then pair-wise comparison tests were done with Bonferroni correction. *p<0.05 and ‡p<0.01 compared to the control group.

Table 3. Effect of *Ficus carica* on nociceptive responses in the Acetic Acid induced writhing test.

Treatment	Dose (mg/Kg)	Number of Writhungs (15-20 min)	Inhibition (%)
Control	-	16.83±0.87	-
F. carica	100	21.83±0.48	Nil
F. carica	200	16.83±0.75	Nil
F. carica	300	18.17±0.60	Nil
Diclofenac Na	10	1.83±0.60	89.74%**

Values are expressed as Mean±S.E.M. of 8 rats. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnett test. *p<0.05 and **p<0.01 compared to the control group.

Dissociation between CNS and peripheral analgesic activity

Formalin induced Paw-licking test: The experimental mice were randomly assigned to six groups; each group had eight mice. The formalin test was conducted based on the method of Tjølsen et al (Tjølsen A. *et al*., 1992). For the formalin test, groups of mice were treated p.o. 1% tween solution in normal saline (0.9% NaCl) (for control), *F. carica* at three doses (100mg/kg, 200mg/kg, and 300mg/kg p.o.) (for treatment group), Tramadol (10mg/Kg p.o.), and Diclofenac Na (10mg/Kg p.o.) (both for positive control). Tramadol was used as the positive control drug for both neurogenic phase and inflammatory phases. Diclofenac Na was used as the positive control drug for the later inflammatory phase.

Naloxone (5mg/kg i.p.) was administered with *F. carica* (100mg/kg, 200mg/kg, and 300mg/kg p.o.) and Tramadol to four different groups, other than the treatment groups. Percentage inhibition was obtained by using this formula (Kumar SVP *et al*., 2010):

$$\frac{T_o - T_t}{T_o} \times 100$$

T_o = mean licking time for the control group

T_t = mean licking time for the test group

Statistical analysis

Results were expressed as mean ± SEM (standard error of mean) of responses. All tests were done using SPSS Software Ver. 20. For Hot Plate test, Tail Immersion test, and Carrageenan induced Rat Paw Edema test, statistical significance was determined by Repeated Measures One-way Analysis of Variance (ANOVA) followed by post hoc Dunnett test. Later, Pair-wise comparison test along with Bonferroni correction were done. For Acetic acid induced writhing test and Formalin test, Statistical significance was determined by One-way Analysis of Variance (ANOVA) followed by post hoc Dunnett test. Then Pair-wise comparison test along with Bonferroni correction were done. The *P* values less than 0.05 were considered to be significant.

Result

Hot-plate test

In the Hot Plate Test, *F. carica* treatment caused significant increase in analgesia in a dose dependent manner. In the presence of Naloxone, an antagonist of opioid receptor, the effect of *F. carica* was reduced profoundly as shown in Table 1.

Tail Immersion test

Table 2 shows that the analgesic effect of *F. carica* was also significant, at two of the three doses (200 and 300 mg/Kg) in Tail immersion test.

Acetic acid induced writhing test

Intraperitoneal injection of 0.7% acetic acid given to the control group caused 16.83±0.87 writhes in a 5 minute interval. The treatment with *F. carica* failed to induce any decrease in the number of writhing (Table 3).

Carrageenan induced Paw edema test: The injection of carrageenan at rat paw created an edema that increased gradually (Table 4). *F. carica* 200mg/kg showed significant anti-inflammatory activity starting from 2h after the injection of carrageenan to throughout the experiment time with a highest reduction of 34.38% (5h after the carrageenan injection). Whereas, *F. carica* 300mg/Kg showed significant anti-inflammatory activity starting from 1h after the injection of carrageenan to throughout the experiment time with a highest reduction of 38.28% (5h after the carrageenan injection). *F. carica* 100mg/Kg did not show significant activity.

Formalin induced paw-licking test

In the Formalin induced paw-licking test, *F. carica* treated mice groups except 100mg/kg group showed significant activities in the early phase pain responses (200mg/kg 81.07% and 300mg/kg 88.37%) compared to that of the control group. These two doses also showed significant analgesic activity at later phase of the experiment (200mg/kg 62.40% and 300mg/kg 76.21%). In combination studies using Naloxone, an antagonist opioid

receptor, the analgesic activity of the Tramadol was diminished in both phases. The analgesic activity of Diclofenac Na was not diminished by the co-treatment with Naloxone. Co-treatment with naloxone also affects the analgesic activity of *F. carica* (300mg/kg) in both the phases of the experiment, suggesting that there might be involvement of opioid receptor in the analgesic activity of *F. carica* (Table 5).

Table 4. Effect of *Ficus carica* on anti-inflammatory responses in Carrageenan induced rat paw edema rest.

Group	Dose (mg/Kg)	Volume of Paw (ml)						
		0 min	30 min	1 h	2h	3h	4h	5h
Control	-	0.77±0.01	0.95±0.08	1.01±0.07	1.16±0.11	1.17±0.08	1.26±0.12	1.28±0.13
F. carica	100	0.73±0.04	0.99±0.03	0.95±0.02	1.07±0.14	1.07±0.09	1.11±0.06	1.17±0.05
F. carica	200	0.79±0.04	0.98±0.06	0.89±0.03	0.90±0.2*	0.91±0.4*	0.89±0.1*	0.84±0.1*
F. carica	300	0.75±0.03	0.90±0.02	0.82±0.1*	0.86±0.3*	0.88±0.1*	0.81±0.1*	0.79±0.1*
Diclofenac Na	10	0.76±0.05	0.94±0.03	0.95±0.21	0.90±0.4*	0.89±0.1*	0.85±0.1*	0.78±0.1*

Values are expressed as Mean ± S.E.M. of 8 rats. Differences between groups are determined by One-Way Repeated Measures ANOVA followed by post hoc Dunnett test; and then pair-wise comparison tests were done with Bonferroni correction. *p<0.05 compared to the control treated group.

Table 5, Effect of *Ficus carica* on nociceptive response in the formalin test.

Group	Dose (mg/Kg)	Early Phase		Later Phase	
		Licking time (s)	Inhibition (%)	Licking time (s)	Inhibition (%)
Control	-	84.50±12.79	-	43.42±13.50	-
F. carica	100	59.83±3.59	29.20	31.83±1.96	26.70
F. carica	200	16.00±0.89*	81.07*	16.33±1.78*	62.40*
F. carica	300	9.83±0.87‡	88.37‡	10.33±0.49‡	76.21‡
Tramadol	10	4.17±1.49‡	95.07‡	4.50±0.764‡	89.64‡
Diclofenac Na	10	62.50±8.65	26.04	5.50±1.88‡	87.33‡
Co-treatment with naloxone					
F. carica + Naloxone	300	81.33±2.85	3.75	41.33±3.12	6.67
Tramadol + Naloxone	10	44.50±2.79	47.34	23.17±1.38	46.64
Diclofenac Na + Naloxone	10	71.83±5.87	15.00	4.83±1.08‡	88.88‡

Values are expressed as Mean±S.E.M. of 8 rats. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnett test. *p<0.05 and ‡p<0.01 compared to the control group.

Discussion

Two well known models of thermal nociception, hot-plate test and tail immersion test were employed to double check on possible involvement of spinal, supra-spinal pathways, and μ-opiate receptor agonism in regulation (CNS modulation) of pain response by *F. carica*. Our findings demonstrated significant activity of *F. carica* (200mg/kg and 300mg/kg) in either model. Hence, involvement of the central nervous system in the regulation of pain is probable here.

To reinforce the above findings, we employed the formalin induced paw-licking test. This test is capable of discerning between nurogenic pain (early phase, acute, non-inflammatory and CNS modulated) and inflammatory (chronic and peripheral pain) (Chau TT. *et al.*, 1989). The neurogenic pain (first phase) is caused by direct chemical stimulation of nociceptive afferent fibers (predominantly C fibers) which can be suppressed by opiate like morphine (Amarlal JF *et al.*, 2007). The inflammatory pain (second phase) is caused by the release of inflammatory mediators like histamine, prostaglandins, bradykinin, serotonin in the peripheral tissues (Dalal A. *et al.*, 1999), and from functional changes in the spinal dorsal horn (Brito ARMS. *et al.*, 1986). Our results showed that *F. carica* had effect on neurogenic pain suppression (first phase) along with effective anti-nociceptive effect in the peripheral inflammatory (second phase) pain. Co-treatment with naloxone partially blocked the activity of both *F. carica* and Tramadol in both the phases while that of Diclofenac Sodium remained unaffected. Hence, we have definitive evidence to conclude that *F. carica* has potent CNS modulated pain suppression activity along with significant anti-inflammatory effect.

To further ascertain its anti-inflammatory activity, we performed the Acetic Acid induced writhing test and carrageenan induced paw edema test. Carrageenan induced edema is commonly used as an experimental model for acute inflammation, and is proven to be biphasic (Dirig DM *et al.*, 1998). The early phase (1-2 hours) of the carrageenan model is chiefly mediated by serotonin and histamine release and increased synthesis of prostaglandins in the damaged paw tissues. These induce inflammation and paw swelling. The later phase is sustained by prostaglandin release and is also mediated by bradykinin, leukotrienes, poly-morphonuclear cells, and prostaglandins produced by tissue macrophages (Derardt R. *et al.*, 1980). *F. carica* showed, in a dose dependent manner, significant anti inflammatory activity from the early phase throughout the later phase indicating its possible ability to hinder endogenous synthesis or release of inflammatory mediators such as prostaglandins, histamine, serotonin, bradykinin and leukotrienes.

The acetic acid induced writhing test was carried out to confirm the peripheral analgesic activity of *F. carica*. The acetic acid used in this test increased the prostaglandin level (mainly PGE_2) in the peritoneal fluid of the mice (Bley KR. *et al.*, 1998). Prostaglandins induce abdominal constriction by activating and sensitizing the peripheral chemo-sensitive nociceptors which are mostly responsible for causing inflammatory pain (Bley KR. *et al.*, 1998). In our study, *F. carica* failed to significantly attenuate the writhing in mice in response to i.p. acetic acid administration. Hence, probable peripheral analgesic mechanism of *F. carica* solely by inhibition of prostaglandin release, in this case, could be ruled out.

Conclusion

In summary, our present study has successfully elucidated the likely mechanism of anti-nociceptive and anti-inflammatory effect of *F. carica*. We have drawn a sound conclusion that *F. carica* has CNS modulated effect in pain inhibition, based on three different in-vivo models. Its anti inflammatory activity has been also confirmed by one in-vivo model. Through this study, it is apparent that the mechanism of action of *F. carica* is similar to that of the commonly used centrally active opioid analgesics. Hence, its traditional use in preparations to relief pain held the test of time, not by its mere placebo effect but by some potent

analgesic and anti-inflammatory molecules hidden in this ethanolic extract of *F. carica*. We believe *F. carica* calls for further studies to elucidate its active component/components responsible for analgesic and anti-inflammatory activity to discover its true potential as therapeutic agent.

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Conflict of Interest

The authors have no conflict of interest to declare.

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