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A Comparative Evaluation of Anti-inflammatory Activity of the Leaves of *Ficus carica* in Plants of Different Ages

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Abstract: Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. Earliest description of curative properties of medicinal plants is found in Rig-Veda. Charaka Samhita and Sushrusha Samhita give extensive description on various medicinal herbs. The present study was undertaken with an objective to find out comparative evaluation of anti-inflammatory activity of the leaves of *Ficus carica* in plants of different ages. The anti-inflammatory activity was evaluated by rat paw edema model induced by carrageenan for acute inflammation and cotton pellet granuloma model for chronic inflammation. Indomethacin was used as a standard drug. The various extracts were studied for their anti-inflammatory activity in carrageenan-induced hind paw edema in rats and the paw volume was measured plethysmometrically from 0 to 3h after injection. We have determined the anti-inflammatory activity of various extracts of the leaves of *Ficus carica* with oral administration doses of 300 and 600 mg/kg/day of body weight to healthy animals. Positive results for flavonoid, sterols and triterpene, tannin and glycoside compounds were investigated by phytochemical analysis. The ethanolic extract of younger plant showed a greater anti-inflammatory effect compared with the standard drug Indomethacin. Present studies besides confirming anti-inflammatory activity of the ethanolic extract of younger more potent than mature plant help to identify from the comparative study of the leaves of *Ficus carica*.

Key words: *Ficus carica* · leaves · Anti-inflammatory · Ethanolic extract · Moraceae · Indomethacin

INTRODUCTION

Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. Earliest description of curative properties of medicinal plants is found in Rig-Veda. Charaka Samhita and Sushrusha Samhita give extensive description on various medicinal herbs[1].Information on medicinal plants in India has been systematically organized [1-4]. The medicinal properties of certain plants have been known for centuries [5]. More than a quarter of the medicines in use today come from plants, i.e. from traditional medicine. Currently, with the active encouragement of the WHO [6,7].

Ficus carica Linn. (Mar, Hindi & Guj: Anjir) Belongs to family Moraceae. The fig tree (*Ficus carica* L.) is one of the unique *Ficus* species widely spread in tropical and subtropical countries which has edible fruits with high commercial value. Commercial fig production is either located around the Mediterranean Sea or is realized in countries possessing Mediterranean climate as in the case of California, Australia or South America. In Turkey, the major fig producer, around 65% of fig trees are in the western Aegean Region especially in Small and Big Meander valleys [8,9].

Ficus carica Linn. (Syn: *Ficus sycomorous*; family: Moraceae) is commonly referred as "Fig". Its fruit, root and leaves are used in the native system of medicine in different disorders such as gastrointestinal (colic, indigestion, loss of appetite and diarrhea), respiratory (sore throats, coughs and bronchial problems), inflammatory and cardiovascular disorders [10, 11]. Fig has been traditionally used for its medicinal benefits as metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory remedy [12, 13].

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Previous reports concerning the nutrient composition of dried figs have indicated that it has the best nutrient score among the dried fruit, being an important source of minerals and vitamins [14].

Phytochemical studies revealed the presence of numerous bioactive compounds: arabinose, β-amyrins, β-carotines, glycosides, β-setosterols and xanthotoxol [15]. *Ficus carica* has been reported to exhibit antioxidant [16], anti-HSV [17], Haemostatic [18], hypoglycemic [19] and hypo-lipidemic activities [20]. The 6-*O*-acyl- β-d-glucosyl-β-sitosterols along with its palmitoyl, linoleyl, stearyl and oleyl derivatives isolated from the fruit of *Ficus carica* exhibited strong cytotoxic effect [21].

However, the plant has not been studied for anti-inflammatory activity. This study was aimed at providing pharmacologic basis for its folkloric use in inflammation and Other species of *Ficus* viz. *Ficus Racemosa*,[22] *Ficus bengalensis* [23,24] *Ficus inspida*, *religiosa*, *elastica*, *Indica* [25] were found to have anti-inflammatory activity. Based on this, an attempt has been made to evaluate the inflammation potency of *Ficus carica*.

In some places the aerial roots were also used with barks as drugs by the traditional medical practitioners. Although inflammatory and related immune responses are normal defence mechanisms essential to health, they play potentially harmful roles in diseases such as rheumatoid arthritis and asthma [26]. Inflammation is a complex process regulated by many different mediators, including prostaglandins [27, 28]. Nonsteroidal anti-inflammatory compounds can relieve the pain and inflammation associated with elevated levels of prostaglandins in the body [29].

MATERIAL AND METHODS

Drugs: Indomethacin (Micro labs, Bangalore), Carrageenan (Sigma Chemicals), Ethanol AR (Th omas Baker Chemical Pvt. Ltd.), Petroleum ether AR (60-80 °C, MCC) were used during the experimental protocol.

Animals: Wistar albino rats (120-200g) of either sex supplied from Yash Farms, Pune India were used. The animals housed under standard laboratory conditions maintained at 25 ± 10 °C and under 12/12 h light/dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India ltd) and water *ad libitum*. The experimental protocol has been approved by the institutional animal ethics committee and by the animal regulatory body of the Indian Government (Registration No.652/02/a/ CPCSEA, dated 25/01/1999).

Plant Material: The younger plant fresh leaves of *Ficus carica* was collected from the foothills of the Satpuda ranges in the district of Jalgaon (MS) in the month of May and June 2008. The plant was identified and authenticated by the Joint Director of Botanical Survey of India, Pune dated 13/08/2008 and letter No.BSI/WC/Tech./2008/355 (VVP-1).

The mature plant fresh leaves of *Ficus carica* was collected from the Nashik district, Maharashtra, India. The plant was identified and authenticated by the Joint Director of Botanical Survey of India, Pune dated 13/08/2008 and letter No.BSI/WC/Tech./2008/355(VVP-2).

Preparation of Extracts: The powdered plant material (450g) was repeatedly extracted in a 5000 ml round bottomed flask with 2000 ml solvents starting with petroleum ether, chloroform and ethanol. The reflux time for each solvent was 40 cycles. The extracts were cooled at room temperature and evaporated to dryness under reduced pressure in rotary evaporator.

Toxicity Studies: The extracts were given at the doses of 300 and 600 mg/kg/day of body weight. All the animals found to be safe at dose of 5000 mg/kg (as per OECD Guidelines).

Evaluation of Anti-Inflammatory Activity

Carrageenan-Induced Paw Edema Method: The albino rats of either gender were divided into eight groups of six animals each. Group I received 0.2 ml of 2% w/v carboxy methyl cellulose suspension orally for 7 days as a control group, Group II received 300 mg/kg body weight of ethanolic extract of leaves of *Ficus carica* (EEFC-I) orally for 7 days, Group III received 600 mg/kg body weight of ethanolic extract of *Ficus carica* (EEFC-II) orally for 7 days, Group IV received 300 mg/kg body weight of chloroform extract of *Ficus carica* (CEFC-I) orally for 7 days, Group V received 600 mg/kg body weight of chloroform extract of *Ficus carica* (CEFC-II) orally for 7 days, Group VI received 300 mg/kg body weight of petroleum ether extract of *Ficus carica* (PEEFC-I) orally for 7 days, Group VII received 600 mg/kg body weight of petroleum ether extract of *Ficus carica* (PEEFC-II) orally for 7 days and Group VIII received 10 mg/kg of body weight of Indomethacin intraperitoneally for 7 days as a standard drug. Acute inflammation was induced in all groups by injecting 0.1 ml of 1% (w/v) carrageenan into the sub-plantar region of the right hind paw of the rats. On the 7th day, paw volume was measured 1h prior to carrageenan injection using a plethysmometer and at form 0 to 3h after the carrageenan injection [30, 31].

Table 1: Effect of leaves of *Ficus carica* (Younger age plant) on carrageenan-induced paw edema in rats[a]

		Mean paw volume in ml						
Treatment	Dose mg/kg	0 min	15min	30min	60min	120 min	180 min	Percent inhibition Vt
Control	2%CMC	0.46±0.008	0.59±0.007	0.78±0.01	0.79±0.01	0.87±0.005	0.95±0.005	Vc 0.74
EEFC-I	300	0.4±0.01*	0.34±0.007*	0.46±0.01*	0.48±0.01*	0.50±0.008*	0.54±0.008*	38.73
EEFC-II	600	0.42±0.008*	0.19±0.01	0.13±0.007*	0.12±0.01	0.11±0.008*	0.10±0.008*	75.90
CEFC-I	300	0.48±0.008*	0.50±0.01	0.55±0.007	0.59±0.01	0.64±0.008*	0.72±0.009*	21.62
CEFC-II	600	0.43±0.01	0.36±0.009*	0.35±0.005*	0.34±0.008*	0.32±0.01	0.36±0.007*	51.35
PEEFC-I	300	0.45±0.009*	0.52±0.01	0.57±0.007*	0.63±0.008	0.68±0.009*	0.75±0.01*	18.91
PEEFC-II	600	0.41±0.009*	0.45±0.007*	0.40±0.01	0.39±0.007	0.38±0.007*	0.37±0.008*	45.94
Indomethacin	10	0.12±0.007	0.13±0.007	0.15±0.01	0.16±0.008	0.17±0.007	0.19±0.009	79.27

[a]Figures in parenthesis indicate oedema inhibition percentage, *N*=6 animals per group

*$p<0.001$by Student's t-test $p<0.05$ by Student's t-test

Table 2: Effect of leaves of *Ficus carica* (Younger age plant) on cotton pellet granuloma in rats[a]

Treatments	Dose	Weight of cotton-pellet (mean±S.E.M.)	Inhibition (%)
Control	2%CMC	42.7±0.5	-
EEFC-I	300 mg/kg	23.1±0.4**	45.90
EEFC-II	600 mg/kg	12.1±0.6*	71.66
CEFC-I	300 mg/kg	32.2±0.5	24.59
CEFC-II	600 mg/kg	24.3±0.5	43.09
PEEFC-I	300 mg/kg	33.6±0.6	21.31
PEEFC-II	600 mg/kg	27.5±0.6	35.59
Indomethacin	10 mg/kg	10.4 ±0.5*	75.64

[a]*N*=6 animals per group *$p<0.001$by Student's t-test **$p<0.05$ by Student's t-test

Table 3: Effect of leaves of *Ficus carica* (Mature age plant) on carrageenan-induced paw edema in rats[a]

		Mean paw volume in ml						
Treatment	Dose mg/kg	0 min	15min	30min	60min	120 min	180 min	Percent inhibition Vt
Control	2%CMC	0.45±0.006	0.50±0.009	0.75±0.009	0.80±0.008	0.87±0.008	0.96±0.007	Vc 0.72
EEFC-I	300	0.41±0.01	0.48±0.008	0.52±0.01	0.53±0.01	0.54±0.007	0.59±0.005	28.93
EEFC-II	600	0.28±0.007	0.30±0.008	0.30±0.007	0.31±0.01	0.37±0.007	0.38±0.006	55.09
CEFC-I	300	0.51±0.007	0.52±0.01	0.54±0.008	0.57±0.008	0.58±0.007	0.59±0.01	23.37
CEFC-II	600	0.30±0.006	0.35±0.007	0.37±0.006	0.40±0.009	0.41±0.009	0.44±0.009	47.45
PEEFC-I	300	0.53±0.008	0.56±0.008	0.57±0.007	0.59±0.007	0.60±0.009	0.62±0.005	19.67
PEEFC-II	600	0.41±0.009	0.42±0.005	0.42±0.008	0.43±0.01	0.50±0.01	0.51±0.004	37.5
Indomethacin	10	0.12±0.007	0.13±0.007	0.15±0.01	0.16±0.008	0.17±0.007	0.19±0.009	79.27

[a]Figures in parenthesis indicate oedema inhibition percentage, *N*=6 animals per group

*$p<0.001$by Student's t-test $p<0.05$ by Student's t-test

Table 4: Effect of leaves of *Ficus carica* (Mature age plant) on cotton pellet granuloma in rats[a]

Treatments	Dose	Weight of cotton-pellet (mean±S.E.M.)	Inhibition (%)
Control	2%CMC	45.3±1.2	-
EEFC-I	300 mg/kg	35.5±1.3**	45.90
EEFC-II	600 mg/kg	26.4±1.6*	71.66
CEFC-I	300 mg/kg	36.3±1.4	24.59
CEFC-II	600 mg/kg	29.5±1.5	43.09
PEEFC-I	300 mg/kg	37.4±1.1	21.31
PEEFC-II	600 mg/kg	32.6±1.2	35.59
Indomethacin	10 mg/kg	10.9 ±0.5*	75.93

[a]*N*=6 animals per group *$p<0.001$by Student's t-test **$p<0.05$ by Student's t-test

This method is for the same with the younger and mature age plant of the *Ficus carica*.

Mean increase in the paw volume was measured and percentage inhibition was calculated.

Percentage of inhibition =100 (1-Vt/Vc)

where, Vc = edema volume in control and Vt = edema Volume in test/standard compound. [Table 1 and 3]

Cotton Pellet Granuloma Method: This study was carried out by cotton pellet implantation method in rats [32]. This method used here was with light ether anesthesia, sterile cotton pellets (10mg) were implanted subcutaneously in the axilla and groin regions of the rats [33]. The animals were treated orally with various extracts at different doses (300 and 600 mg/kg) daily for 7 consecutive days. Animals in the control group received either normal saline with CMC. Indomethacin (10mg/kg, orally) was given to animals in the reference groups. They were sacrificed on the 8th day, the cotton pellet removed, freed from extraneous tissue and dried overnight at 60°C and weighed. The inhibition percent of the dry weight of the granuloma were calculated and compared [Table 2 and 4].

Statistical Analysis: Statistical analysis was carried out using Graph Pad Prism 4.0 (Graph Pad software San Diego, CA). Results were expressed as Mean ± SEM. Statistical significance was calculated by Student's t-test <0.001 was considered as significant.

RESULTS AND DISCUSSION

Younger Age Plant

Carrageenan-induced Rat Paw Edema Method-Acute Inflammatory Study: The carrageenan-induced paw edema results of younger age plant were showed in Table 1. When ethanolic extract of *Ficus carica* at 300 mg/kg body weight per day (EEFC-I) given orally as a suspension the paw volume was reduced by 38.73%, whereas the case of the ethanolic extract of *Ficus carica* at 600 mg/kg body weight per day (EEFC-II) shows a 75.90% inhibition after 3 h, which indicated that the effect of ethanolic extract of *Ficus carica* is reflected in a dose-dependent manner. Both EEFC-I and EEFC-II showed an inhibitory effect on carrageenan-induced paw edema thus exhibiting anti-inflammatory effect against acute inflammation. Chloroform extract of *Ficus carica* at 300 mg/kg body weight per day (CEFC-I) reduced the paw volume by 21.62% and chloroform extract of *Ficus carica* at 600 mg/kg body weight per day (CEFC-II) exhibited a 51.35% reduction in paw volume after 3 h. in contrast petroleum ether extract of *Ficus carica* at 300 mg/kg body weight per day (PEEFC-I) reduced the paw volume by 18.91% and petroleum ether extract of *Ficus carica* at 600 mg/kg body weight per day (PEEFC-II) exhibited a 45.94% reduction in paw volume after 3 h. Therefore, petroleum ether extract of *Ficus carica* did not possess significant anti-inflammatory activity when compared with control and Indomethacin treated animals (Table 1). It may be due to the absence of flavonoids in the petroleum ether extract.

Cotton Pellet Granuloma Method

Chronic Inflammation Study: The results of cotton pellet granuloma were given in Table 2. The paw volume were reduced by 45.90% with ethanolic extract of *Ficus carica* at 300 mg/kg body weight per day (EEFC-I) when given orally as a suspension whereas in case of ethanolic extract of *Ficus carica* at 600mg/kg body weight per day (EEFC-II) showed 71.66% inhibition after 3 h. This indicated that the effect of ethanolic extract of *Ficus carica* is reflected in dose dependent manner. Both EEFC-I and EEFC-II showed inhibitory effect on cotton pellet granuloma thus, exhibiting anti-inflammatory effect against chronic inflammation. In case of chloroform (CEFC-I) and Petroleum ether extract (PEEFC-I) of *Ficus carica* at 300mg/kg body weight per day reduced the paw volume 24.59% and 21.31%. Chloroform (CEFC-II) and petroleum ether extract (PEEFC-II) of *Ficus carica* at 600mg/kg body weight per day exhibited 43.09% and 35.59% reduction in paw volume after 3 h. As a result, chloroform and petroleum ether extract of *Ficus carica* did not possess significant anti-inflammatory activity when compared with control and indomethacin treated animals (Table 2). It may be due to absence of flavonoids in the petroleum ether extract.

Mature Age Plant

Carrageenan-Induced Rat Paw Edema Method

Acute inflammation study: In this study, the results of carrageenan-induced paw edema were given in Table III. the paw volume was reduced by 28.93% with ethanolic extract of *Ficus carica* at 300 mg/kg body weight per day (EEFC-I) when given orally as a suspension, whereas the case of the ethanolic extract of *Ficus carica* at 600 mg/kg body weight per day (EEFC-II) showed a 55.09% inhibition after 3 h. which indicated that the effect of ethanolic extract of *Ficus carica* was reflected in a

dose-dependent manner. Both EEFC-I and EEFC-II showed an inhibitory effect on carrageenan-induced paw edema thus, exhibiting anti-inflammatory effect against acute inflammation. Chloroform extract of *Ficus carica* at 300 mg/kg body weight per day (CEFC-I) reduced the paw volume by 23.37% and chloroform extract of *Ficus carica* at 600 mg/kg body weight per day (CEFC-II) exhibited a 47.45% reduction in paw volume after 3 h. Petroleum ether extract of *Ficus carica* at 300 mg/kg body weight per day (PEEFC-I) reduced the paw volume by 19.67% and petroleum ether extract of *Ficus carica* at 600 mg/kg body weight per day (PEEFC-II) exhibited a 37.5% reduction in paw volume after 3 h. Therefore, petroleum ether extract of *Ficus carica* did not possess significant anti-inflammatory activity when compared with control and indomethacin-treated animals (Table 3). It may be due to the absence of flavonoids in the petroleum ether extract.

Cotton Pellet Granuloma Method

Chronic Inflammation Study: The results of cotton pellet granuloma were given in Table IV. The paw volume were reduced by 45.90% with ethanolic extract of *Ficus carica* at 300mg/kg body weight per day (EEFC-I) when given orally as a suspension whereas in case of ethanolic extract of *Ficus carica* at 600 mg/kg body weight per day (EEFC-II) showed 71.66% inhibition after 3 h. which indicated that the effect of ethanolic extract of *Ficus carica* was reflected in dose dependent manner. Both EEFC-I and EEFC-II showed inhibitory effect on cotton pellet granuloma, thus exhibiting anti-inflammatory effect against chronic inflammation. In case of chloroform (CEFC-I) and petroleum ether extract (PEEFC-I) of *Ficus carica* at 300 mg/kg body weight per day reduced the paw volume 24.59% and 21.31%. chloroform (CEFC-II) and petroleum ether extract (PEEFC-II) of *Ficus carica* at 600 mg/kg body weight per day exhibited 43.09% and 35.59% reduction in paw volume after 3 h. As a result chloroform and petroleum ether extract of *Ficus carica* did not possess significant anti-inflammatory activity when compared with control and indomethacin treated animals (Table 4). It may be due to absence of flavonoids in the petroleum ether extract.

DISCUSSION

Among several traditional claims, the usefulness of *Ficus carica* in fever, inflammation and pain had been emphasized more in literature. Hence, it was considered that investigations for these medicinal properties might give scientific authentication to the traditional claims, still this plant has not been subjected to the systemic pharmacological screening mentioned above so far. In the present study, the anti-inflammatory activity of various extracts of the leaves of *Ficus carica s* has been established. The test extracts with two different doses of 300 and 600 mg/kg/day were found to inhibit the carrageenan-induced rat paw edema, significantly, which has significant predictive value for anti-inflammatory agents by inhibiting the mediators of acute inflammation. Inflammation has different phases. The first phase was caused by an increase in vascular permeability, the second one by infiltrate of leucocytes and the third one by granuloma formation. Anti-inflammatory activity was determined by inhibition of carrageenan induced inflammation, which was one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan induced edema was bi-phasic. The first phase was attributed to the release of histamine, serotonin and kinins and the second phase was related to the release of prostaglandins and bradykinins [34-38].We observed that EEFC-I and EEFC-II showed significant inhibition against carrageenan-induced paw edema in the dose dependent manner. But chloroform and pet ether extract failed to possess the anti-inflammatory effect may be due to absence of flavonoid [39]. This response tendency of the extract in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the ethanolic extract. This anti-inflammatory effect of EEFC-I and EEFC-II may be due to the presence of flavonoids. It has been reported that a number of flavonoids possessed anti-inflammatory activity [40].The presence of flavonoids might be responsible for the anti-inflammatory activity in ethanolic extract. Thus, it is concluded that the ethanolic extract of leaves of *Ficus carica* produced significant anti-inflammatory activity in dose dependent manner. Besides the carrageenan-induced rat paw edema model, the production of protanoids has been through the serum expression of COX-2 by a positive feedback mechanism. Therefore, it is suggested that the action mechanism of test extracts may be related to the prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of Nonsteroidal anti-inflammatory drugs in the inhibition of inflammatory process induced by carrageenan. The cotton Pellet granuloma, which has certain advantages for natural product testing first, the response is local and involves the skin. Thus, the topical application avoids drug metabolism and excretion. Secondly, this model uses very small amounts of drugs. Like-wise, the granulomatous tissue formation is related to the chronic inflammatory process, which is characterized by several phases [41].

From these investigations, it may be concluded that the various extracts of *Ficus carica* showed anti-inflammatory effects similar to those observed for non-steroidal drugs such as, phenylbutazone and indomethacin. The activity of the *Ficus carica* extracts and young leaves extracts was significantly higher than that of the mature leaves extracts. It is important to point out that the presence of flavonoids may be responsible for the anti-inflammatory activity. Further investigations are under process in our laboratory to isolate and characterize the specific active components of the plant extract which is responsible for observed pharmacological actions.

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