Anti-inflammatory and *In-vivo* Antioxidant Activities of *Cressa cretica* Linn., a Halophytic Plant

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Abstract: The methanolic (Fr-Me) and ethyl acetate fraction (Fr-Et) obtained from the aerial parts of *Cressa cretica* L. (Convolvulaceae) exhibited inhibitory effect against acute and chronic models of inflammation, namely, carrageenan-induced paw edema, cotton pellet granuloma, carrageenan air pouch inflammation, vascular permeability, freuds complete adjuvant induced arthritis models. The fractions also inhibited arachidonic acid and other mediator (histamine, serotonin, prostaglandin E2)-induced paw edema in rats in a dose dependent manner. Moreover, Fr-Me and Fr-Et significantly increased plasma superoxide dismutase, catalase, glutathione and glutathione peroxidase activities. On the contrary, the malonaldehyde (as a measure of lipid peroxidation) level was significantly decreased when compared with the control group. Also, it was found that Fr-Et reduced the inflammation and revealed the antioxidant activity more significantly than Fr-Me. Thus study established the anti-inflammatory activity and scavenges the free radicals, which are important mediators that provoke or sustain inflammatory processes.

Key words: *Cressa cretica* Acute · Chronic · Inflammatory mediator- induced anti-inflammatory activities · *In-vivo* antioxidant activity

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesic or antipyretic agents for the clinical treatment of inflammatory diseases such as arthritis, lumbago and rheumatism. These agents exhibit an inhibitory action on the cyclooxygenase that catalyzes the biosynthesis of prostaglandins and thromboxane from arachidonic acid. It has been also reported that reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and peroxynitrite participate in the process of inflammation in various tissues [1]. The inflammatory tissue damage is due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites [2]. The ROS play an important role in the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts and inflammation [3,4]. The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes [5,6]. In addition, ROS propagate inflammation by stimulating release of cytokines such as interleukin-1, tumor necrosis factor-á and interferon-ã, which stimulate recruitment of additional neutrophils and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation [7,8]. ROS may also contribute to several chronic cutaneous inflammatory diseases such as psoriasis, atopic dermatitis and contact dermatitis [9]. In a chronological sequence of reactions, various cytokines, which participate in the pathogenesis of inflammatory reactions are produced. Therefore, compounds that have

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scavenging activities toward these radicals and/or suppressive activities on lipid peroxidation may be expected to have therapeutic potentials for several inflammatory diseases [10]. Although steroidal anti-inflammatory drugs and NSAIDs are currently used to treat acute inflammation, these drugs have not been entirely successful in curing chronic inflammatory disorders while such compounds are accompanied by unexpected side effects. Therefore, there is an urgent need to find safer anti-inflammatory compounds. Traditional medicine has used extracts of different plants for the treatment of a wide variety of disorders including acute and chronic inflammation.

Cressa cretica L. (Convolulaceae), popularly known as 'Rudanti' in Hindi and is a widely grown halophytic plant. Different parts of the plant have been claimed to be valuable in a wide spectrum of diseases [11-13]. In earlier studies *Cressa cretica* Linn flowers exhibited cytotoxic and anti-inflammatory activity *in vitro* [14]. *Cressa cretica* is reported to be antibilous, antituberculosis and expectorant [15]. Shahat *et al.* [16] yielded five flavonoids (quercetin, quercetin-3-O-glucoside, kaempferol-3-O-rhamnoglucoside and rutin) from the aerial parts of *Cressa cretica*. It is also reported the fruits of *Cressa cretica* is a potential source of edible oil. The oil of *C.cretica* was free from any undesirable components and could safely be recommended for human consumption. In addition the antiviral activity from the plant was reported [17]. It is already reported that the areal parts of the plant contains scopoletin umbelliferone, isoflavone glycoside coumaranochrome glycoside [18]. Syringaresinol glucoside and dicaffeoyl quinic acid were also isolated [16]. In the present study, the detailed anti-inflammatory activities of the fractions of *C. cretica* have been performed using different acute, subchronic and chronic inflammatory models. Also, attempts have been made to explore the effect of the extract on the antioxidant defence systems.

MATERIALS AND METHODS

Plant Material: *Cressa cretica* was collected from Nalban island of Chilika lake, Orissa, India and was preliminarily identified at Natural product division, Institute of Mineral and Material Technology, (formerly known as Regional Research laboratory, Bhubaneswar) India and which was later on confirmed from Botanical Survey of India, Howrah, West Bengal, India (CNH/I-I/32/2010/Tech.II/237-3). A voucher specimen has been kept in our laboratory for future reference.

Preparation of Extract: The aerial parts were air-dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve and extracted in a soxhlet extractor with methanol. The extract was decanted, filtered with Whatman No. 1 filter paper and concentrated at reduced pressure below 40?C through rota-vapor (Rotavapor RII, Buchi Labortechnik AG, Switzerland) to obtain dry extract (16.73%w/w). *Cressa cretica* methanolic extract (CME) was adsorbed on to the 250 g of silica gel of 60-120 mesh size and fractionated using solvents of increasing polarity such as hexane (Fr-He), ethylacetate (Fr-Et) and methanol (Fr-Me). The fractions were subjected for preliminary phytochemical screening to show the presence of steroid, alkaloid, glycoside, tannin, triterpenoid, carbohydrates reducing sugar and fatty acids.

Animals: All the experiments were carried out using Wistar albino rats (150-220g) of either sex obtained from animal house, Birla Institute of Technology, Mesra, Ranchi, India and kept in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of $24 \pm 2°C$ and relative humidity of 60-70 %. A 10:14 light: dark cycle was followed. All animals were allowed to free access to water and fed with standard commercial rat chaw pallets (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Reg. No: 621/02/ac/CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India.

Carrageenan-induced Paw Oedema: Pedal inflammation was produced in rats according to the method described by Winter *et al.* [19]. The rats were treated orally with 100 and 200 mg/Kg of Fr-Et and Fr-Me of *C. cretica,* while the control and standard groups received saline (p.o.) and indomethacin (10 mg/kg, p.o.) respectively. One hour after the administration of extracts, indomethacin or saline, 0.1 ml of 1 % carrageenan was injected into the left hind paw of each animal under the sub plantar aponeurosis. Measurement of paw volume was carried out by recording the volume using a plethysmometer. Paw volumes were measured immediately before and 1-6 hrs after carrageenan injection.

Cotton Pellets-induced Granuloma: The cotton pellets-induced granuloma in rats was studied according to the method Ismail *et al.* [20]. The rats were anaesthetized and

sterile cotton pellets weighing 10 ± 1 mg were implanted subcutaneously into both sides of the groin region of each rat. The fractions (Fr-Et and Fr-Me) at the concentration of 100 and 200 mg/kg was administered orally for seven consecutive days from the day of cotton pellet implantation. The control and standard groups received saline (p.o.) and indomethacin (10 mg/kg, p.o.) respectively for the same period, on 8th day the animals were anaesthetized and the pellets together with granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60°C for 24 h to constant weight, after that the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation. The antiproliferative effects of fractions were compared with control.

Carrageenan-induced Air Pouch Formation: According to the modification of the procedure of Ghosh *et al.* [21], carrageenan induced air pouch was performed. Six days to drug treatment, air pouch formation in the intrascapular region of rats by initial subacutaneous injection of 20ml sterile air and successive injection of 10ml sterile air every 3 days to sustain its patency. On day 0, vehicle (saline), dexamethasone 0.1 mg/kg, Fr-Et 100mg/kg, Fr-Et 200mg/kg, Fr-Me 100mg/kg, Fr-Me 200mg/kg were orally administered 1h prior to carrageenan injection (0.1 ml of 1.0% solution) into the pouch. After 15h, the pouch cavity was opened and exudates were collected. The volumes of the exudates were measured using a graduate tube. Aliquotes were diluted Turk solution and polymorphonuclear leukocytes were counted in a standard hemocytometer chamber. Experiments were performed in triplicate.

Vascular Permeability: The effect of Fr-Et and Fr-Me on vascular permeability was studied by the method described by Turner [22]. Rats were divided into six groups of 6 animals each. Group1: vehicle control; Group II: 100 mg/kg Phenyl butazone; Group III: Fr-Et 100mg/kg, Group IV: Fr-Et 200mg/kg, Group V: Fr-Me 100mg/kg, Group VI: Fr-Me 200mg/kg. One hour after drug treatment, 1% Evans Blue in normal saline was injected intravenously into the tail vein at a dose of 0.3 ml/100 g body weight. Half-an-hour later, each paw was injected with 0.1 ml of 1% carrageenan in normal saline. Blueness of the paw was graded from 1 to 9 based on the intensity of the colour after 1, 4 and 20 hours.

Freund's Complete Adjuvant (FCA) Induced Arthritis: The methodology used was that of Zhaoliang *et al.* [23]. Rats were divided into six groups of 6 animals each. Group I: model control; Group II: prednisone acetate 10mg/kg (positive control); Group III: Fr-Et 100mg/kg, Group IV: Fr-Et 200mg/kg, Group V: Fr-Me 100mg/kg, Group VI: Fr-Me 200mg/kg respectively. Both right and left hind paw cubages of all rats were measured before treatment. On day 1 of the experiments the animals were treated with standard and the test substances. Three days later, all the rats were treated with FCA (0.1 ml) by intradermal injection on the pad of the right hind paw. On the 1st through the 5th and on the 7th, 11th, 13th 16th, 19th day after treatment, the cubage of both right (site of primary infection) and left (site of secondary infection) hind paws were measured and assessed for any anti-inflammatory effects which were measured as the degree of swelling.

Arachidonic Acid-induced Inflammation: To further explore the mechanism of anti-inflammatory action, the arachidonic acid-induced inflammation in rats was studied as described by Di-Martino *et al.* [24]. Paw edema was induced by a single subplanter injection of 0.1 ml of arachidonic acid in 0.2 M. carbonate buffer (pH 8.43-8.56) into the right hind paw of rats 30 rain after treatment with different fractions, or aspirin (cyclooxygenase inhibitor) (100 mg/kg) or indomethacin (cyclooxygenase inhibitor) or chlorpheniramine maleate (antihistaminic) (25 mg/kg) or cyproheptadine (antihistaminic and antiserotonin) (25 mg/kg) or control vehicle intraperitoneally. The edema volume was measured after 30 min using a plethysmometer.

Inflammatory Mediators-induced Paw Edema: In another set of experiment, different inflammatory mediators/phlogistic agents were used as oedemogens [25]. The respective strength of the oedemogens, the volume injected and the time for determination of edema volumes are indicated in parentheses; histamine (10 3 g/ml, 0.1 ml, 60 rain) (Sigma); serotonin (10 ~ g/ml, 0.1 ml, 30 rain) (Sigma); prostaglandin E2 (10 ~ g/ml, 0.I ml, 30 rain) (Sigma); were injected into the hind paw of the rat after 30 rain of administration of different fractions and control vehicle (dist. water) intraperitoneally to groups of rats. The edema volume was determined as mentioned previously [19].

Plasma Antioxidant System and Lipid Levels in Rats

Experimental Design: Five groups of six rats each were used. Animals were treated with Fr-Et and Fr-Me once a day, with the doses of 100, 200 mg/kg, while the control group was treated with Sodium CMC (10 ml/kg). After 21 days, blood was collected into heparinized tubes from rats fasted during overnight. Samples were immediately centrifuged at 1500 rpm for 5 min and the plasma was separated. Superoxide dismutase, catalase, glutathione peroxidases and lipid peroxidation (malonadialdehyde level) were determined as follows.

Measurement of Superoxide Dismutase Activity: The activity of superoxide dismutase (SOD) was determined by monitoring the inhibition of the autoxidation of pyrogallol [26]. At 25°C and 320 nm, the rate of pyrogallol oxidation was recorded with a Shimadzu UV 1201 spectrophotometer (Shimadzu). Activity was expressed as the amount of enzyme that inhibits the oxidation of pyrogallol by 50%, which is equal to 1 unit. The result for SOD activity was expressed as U/mg protein.

Measurement of Catalase Activity: Catalase activity was determined according to Beutler [27]. Briefly, 1 mol/l Tris-HCl, 5 mol/l EDTA (pH 8) and 10 mol/l H_2O_2 were added to plasma and the mixture was incubated at 37.8 °C. The change in absorbance of the system at 230 nm was followed for 10 min. The result for catalase activity was expressed as the mol of H_2O_2 degraded per min/mg/proteins. Proteins were determined using bovine serum albumin as a standard [28].

Measurement of Glutathione Activity: Glutathione was estimated using Ellman's reagent (5,5¢dithiobis-(2-nitrobenzoic acid) [DTNB]). The sulphydryl groups present in glutathione forms a colored complex with DTNB, which was measured colorimetrically at 412 nm [29]. The amount of glutathione was determined using its molar extinction coefficient of 13600/m/cm and expressed in terms of ìmol/mg of protein.

Measurement of Peroxidase Activity: Peroxidase estimation is based on periodide formation. Periodide can be spectrophotometrically determined at 353 nm and this is directly proportional to the peroxidase concentration in the reaction mixture containing approximate amounts of H_2O_2 and enzyme [30]. One unit of peroxidase activity is defined as the change in absorbance per minute and expressed in terms of units per milligram of protein.

Lipid Peroxidation Intermediates: Lipid peroxidation was assayed by the measurement of malondialdehyde (MDA) levels on the basis of reaction with thiobarbituric acid [31]. Briefly, 0.2 ml of serum was mixed with thiobarbituric acid reagent (0.375% thiobarbituric acid and 15% trichloroacetic acid in 0.25 N HCl). The reaction mixture of serum and thiobarbituric acid reagent was placed in boiling water for 15 min, cooled, centrifuged and then the optical density of the supernatant was recorded at 532 nm. A standard curve was obtained with a known amount of 1.1.3.3.-tetraethoxypropane, using the same assay procedure.

Statistical Analysis: Pharmacological data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's *t* test with equal sample size. The difference was considered significant when *p* value < 0.05. All the values were expressed as mean ± standard error mean (S.E.M.).

RESULTS

The preliminary phytochemical analyses of the fractions revealed fatty acids and fixed oils are present in hexane fraction, Steroids, terpenes, coumarin glycosides and reducing sugars and phenols are present in the ethylacetate fraction, flavonoids, reducing sugars and phenols are present in methanol fraction.

In vivo anti-inflammatory activity of *C. cretica* fractions in carrageenan induced paw edema model is shown in Fig. 1. The Fr-Et and Fr-Me significantly inhibited carageenan induced edema in a dose depended manner. The inhibitory effect of Fr-Et was more pronounced than that of Fr-Me at a dose level of 200mg/kg b.w. The concentration required to inhibit the paw edema for both the fractions were statistically comparable to the standard reference drug indomethacin 10mg/kg. Further, the effect of Fr-Et and Fr-Me were evaluated on the proliferative phase of inflammation in cotton-pellet granuloma model. Fr-Et and Fr-Me, when administered orally, produced reduction in the weight of granuloma induced by cotton-pellet. Both the wet and dry weights of granuloma were less in fraction treated groups as compare to control group in a dose dependent manner. The effect of Fr-Et 200mg/kg treated group (wet weight 109.45 ± 1.86mg and 32.6± 2.172mg) was comparable with the standard reference drug, indomethacin (104.45 ± 2.078mg and 29.04 ± 1.166mg).
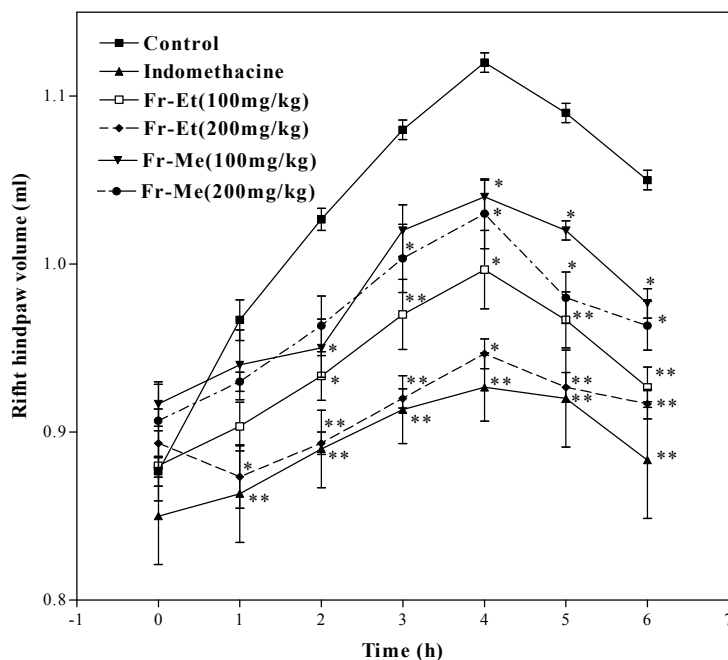
Fig. 1: Effect of Ft-Et and Fr-Me on Carrageenan-induced edema. n=6; points represents arithmetic mean, bars, S.E.M.; Significantly $^*p < 0.05$; $^{**}p < 0.01$.

Table 1: Effect of Fractions of *Cressa cretica* on Cotton pellet-induced granuloma

Treatment	Dose	Granuloma Weight (mg)	
Control	10ml/kg	Wet	176.0±1.538
		Dry	59.333±1.437
Indomethacine	10mg/kg	Wet	104.45±2.078***
		Dry	29.04±1.166***
Fr-Et	100mg/kg	Wet	139.19±1.865**
		Dry	44.843±1.398***
	200mg/kg	Wet	109.45±1.860***
		Dry	31.6±2.172***
Fr-Me	100mg/kg	Wet	145.67±1.891**
		Dry	49.68±1.021*
	200mg/kg	Wet	128.33±2.603**
		Dry	40.67±1.453***

Values are expressed as mean ± S.E.M. for six animals; Comparisons-All groups with control group; Significantly $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

In the carrageenan induced air pouch model, dexamethasone (a positive control) significantly (p<0.001) reduced the average volume of the exudates (Figure 2A). Treatment with Fr-Et and Fr-Me significantly decreased the carrageenan induced formation of exudates in a dose depended manner. Counts of polymorphonuclear leukocytes in the carrageenan induced air pouch significantly (p<0.05) decreased in the experimental groups treated with Fr-Et and Fr-Me fractions (Figure 2B). Counts of polymorphonuclear leukocytes at the treatment of 200mg/kg Fr-Et were comparable to that in the treatment of dexamethasone as a positive control. These findings obtained in the carragennan-induced air-pouch model also proposed anti-inflammatory activity of *C. cretica*.

Since inflammation is characterised by increase in vascular permeability, we further studied the effect of Fr-Et and Fr-Me on capillary permeability using Evans Blue dye. Fr-Et and Fr-Me reduced a significant (p<0.05) decrease in vascular permeability as indicated by the blueness of the paw. The median score of vascular permeability with Fr-Et was comparable to that of the standard drug, phenylbutazone (Fig. 3). However the permeability score for Fr-Et is more than that of Fr-Me.
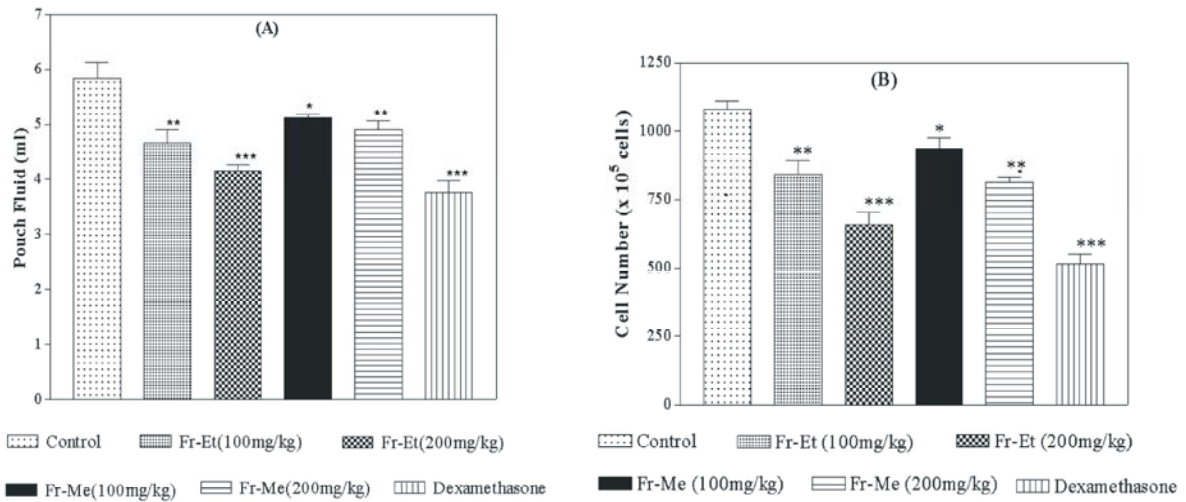
Fig. 2: Inhibitory effect of Fr-Et and Fr-Me on the volumes of exudates (A) and polymorphonuclear leukocytes (B); Values are expressed as mean ± S.E.M. for six animals; Comparisons-All groups with control group; Significantly $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$
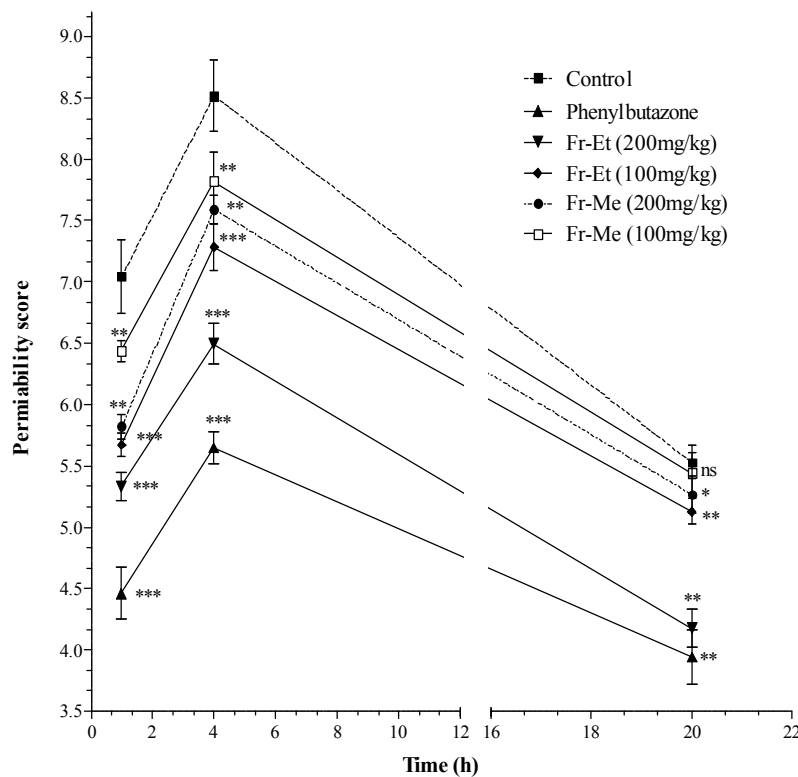

Fig. 3: Effect of Ft-Et and Fr-Me on Carrageenan-induced vascular permeability. n=6; Points represents arithmetic mean, bars, S.E.M.; Significantly $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; ns: not significant vs. control.

We have further tested the fractions of *C.cretica* for its ability to inhibit chronic inflammation induced by FCA. The results of Fr-Et and Fr-Me on paw swelling of the primary (right paw) and secondary (left paw) sites in FCA induced arthritic rats are given in tables 2 and 3 respectively. The results of the statistical measures showed a significant difference in mean FCA induced paw swelling of the primary infection of the treated groups in different days as compared to model control group. Fr-Me inhibited swelling in primary infection, but the decrease in

Table 2: Effect of Fr-Et and Fr-Me on swelling of the right hind paw in FCA-induced arthritic rats (the primary affection)

Group	Model Control	Prednisone acetate (10 mg/kg)	Fr-Et (100 mg/kg)	Fr-Et (200 mg/kg)	Fr-Me (100 mg/kg)	Fr-Me (200 mg/kg)
Cubage before inflammation (ml)	0.986±0.012	1.01±0.057	0.98±0.006	1.013±0.003	1.00±0.005	0.986±0.01
Cubage of right hind paw after inflammation (ml)						
1 day	1.383±0.015	1.38±0.021	1.373±0.007	1.393±0.01	1.40±0.004	1.386±0.015
2 day	1.853±0.014	1.77±0.005**	1.82±0.013	1.8±0.006*	1.83±0.012	1.833±0.014
3 day	1.923±0.013	1.706±0.015**	1.81±0.017**	1.776±0.02**	1.896±0.026*	1.84±0.023*
5 day	1.8±0.006	1.64±0.011**	1.753±0.009	1.69±0.011**	1.804±0.024	1.76±0.035
7 day	1.85±0.011	1.52±0.012***	1.69±0.005***	1.68±0.016***	1.78±0.018**	1.73±0.038**
9 day	1.773±0.005	1.453±0.008**	1.603±0.023**	1.526±0.031**	1.76±0.021	1.67±0.022**
11 day	1.603±0.009	1.417±0.003**	1.54±0.005**	1.507±0.013**	1.6±0.013	1.58±0.03*
13day	1.55±0.007	1.427±0.004**	1.54±0.003	1.453±0.009**	1.56±0.014	1.57±0.026
16day	1.576±0.01	1.453±0.014***	1.49±0.006*	1.447±0.009***	1.52±0.02*	1.55±0.017*
19 day	1.64±0.004	1.423±0.01***	1.487±0.019***	1.44±0.021***	1.56±0.019**	1.51±0.003***

Values are expressed as mean ± S.E.M. for six animals; Comparisons-all Groups with control group. Significantly $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 3: Effect of Fr-Et and Fr-Me on swelling of the left hind paw in FCA-induced arthritic rats (the secondary affection)

Group	Model Control	Prednisone acetate (10 mg/kg)	Fr-Et (100 mg/kg)	Fr-Et (200 mg/kg)	Fr-Me (100 mg/kg)	Fr-Me (200 mg/kg)
Cubage before inflammation (ml)	1.00±0.004	0.986±0.011	0.977±0.014	1.013±0.010	1.007±0.008	1.016±0.014
Cubage of right hind paw after inflammation (ml)						
1 day	1.060±0.006	0.990±0.015**	1.043±0.010	1.020±0.005*	1.060±0.016	1.046±0.003
2 day	1.050±0.013	1.044±0.012	1.037±0.003	1.020±0.017	1.047±0.003	1.040±0.006
3 day	1.140±0.016	1.083±0.010**	1.130±0.004	1.090±0.011*	1.123±0.014	1.103±0.009
5 day	1.106±0.009	1.067±0.017	1.117±0.009	1.074±0.012	1.107±0.017	1.083±0.009
7 day	1.173±0.012	1.104±0.019**	1.143±0.009	1.094±0.008**	1.150±0.005	1.137±0.016*
9 day	1.207±0.014	1.133±0.009	1.180±0.007	1.150±0.015	1.173±0.019	1.176±0.020
11 day	1.160±0.005	1.140±0.013	1.145±0.018	1.127±0.007	1.166±0.021	1.145±0.024
13day	1.174±0.01	1.103±0.005**	1.134±0.012	1.107±0.014**	1.155±0.018	1.150±0.026*
16day	1.127±0.018	1.074±0.018	1.103±0.019	1.080±0.013	1.130±0.011	1.136±0.012
19 day	1.236±0.008	1.170±0.006**	1.201±0.013*	1.166±0.018**	1.210±0.022	1.188±0.019**

Values are expressed as mean ± S.E.M. for six animals; Comparisons-all Groups with control group. Significantly $^*p < 0.05$; $^{**}p < 0.01$
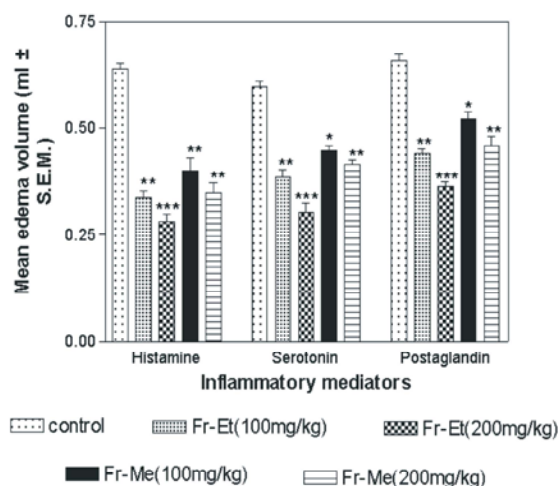

Fig. 4: Effect of Fr-ET and Fr-Me on histamine, serotonin and prostaglandin E_2-induced paw edema; each value represents the mean ± S.E.M. from six animals in each group. Significantly $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ vs. control.

swelling was not significant for all the different days (Table 2). The decrease was significant on all the days for Fr-Et (200mg/kg). The results of the decrease in FCA induced paw swelling of the secondary infection was significant on day 7, 13 and 19 for Fr-Et (200mg/kg) and Fr-Me (200mg/kg) and day 3 for Fr-Et (200mg/kg) when compared to model control group. The fractions of *C. cretica* significantly (p< 0.05) reduced the paw edema formation induced by various phlogistic agents, viz histamine, serotonin, prostaglandin E_2 (Figure 4). Arachidonicacid-induced edema in rat paw was the model used to distinguish between lipoxygenase inhibitors and selective cyclooxiginase inhibitors. Neither aspirin nor indomethacin blocked the edema formation (Table 4). Fr-Et and Fr-Me inhibited the edema formation at the tested dose levels. Chlorpheniramine maleate and cyproheptadine also significantly inhibited the edema formation induced by arachidonic acid.

After 3 weeks of administration of Fr-Et and Fr-Me fractions of *C.cretica* to rats, SOD, catalase, glutathione,

Table 4: Comparison of the effect of Fr-Et and Fr-Me and various standard drugs on arachidonic acid-induced paw edema in rats

Group	Dose (p.o)	Edema volume (ml ± S.E.M)	% of inhibition
Control	3.0 ml/kg	0.691±0.005	-
Fr-Et	100mg/kg	0.412±0.014**	40.38
Fr-Et	200mg/kg	0.300±0.006***	56.58
Fr-Me	100mg/kg	0.541±0.010*	21.70
Fr-Me	200mg/kg	0.478±0.007**	30.82
Aspirin	100mg/kg	0.656±0.018ns	5.06
Indomethacin	10mg/kg	0.662±0.014ns	4.19
Chlorpheniramine maleate	25mg/kg	0.357±0.011***	48.34
cyproheptadine	25mg/kg	0.388±0.015**	43.85

Values are expressed as mean ± S.E.M. for six animals; Comparisons-all groups with control group. Significantly $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; ns: not significant.

Table 5: Effect of *C.cretica* on antioxidant enzymes

Treatment	SOD (units/min/mg protein)	CAT (μ mol H_2O_2 consumed/min/mg protein)	Glutathione (μ g/mg protein)	GPx (μ mol glutathione oxidised/ min/mg protein)	Lipid peroxidation (nM MDA/g of protein
Control	13.35±0.583	55.29±1.320	11.38±0.159	8.348±0.151	2.67±0.025
Fr-Et (100mg/kg)	16.67±0.262*	69.39±0.795**	12.39±0.064*	10.67±0.043***	1.33±0.007***
Fr-Et (200mg/kg)	22.04±0.381***	84.76±1.231***	15.59±0.041***	11.58±0.086***	0.97±0.009***
Fr-Me (100mg/kg)	14.43±0.343*	61.57±0.764**	11.65±0.072	8.97±0.025*	2.31±0.013
Fr-Me (200mg/kg)	17.86±0.049**	74.69±0.098***	14.37±0.009***	9.87±0.008**	1.88±0.027**

Values are expressed as mean ± S.E.M. for six animals; Comparisons-all groups with control group. Significantly $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

glutathione peroxidases were increased significantly while lipid peroxidation (measured as MDA content) was decreased significantly. An increase in antioxidant enzyme activity and reduction in lipid peroxidation by *C. cretica* fractions may result in reducing a numbers of deleterious effects due to accumulation of oxygen free radicals which could results a beneficial action against pathological alteration specially in inflammatory diseases.

DISCUSSION

The preliminary phytochemicals tests showed the presence of flavonoids, sterols and coumarin glycosides etc. in extracts of *C. cretica*. The plant constituents are sterols, particularly the β-sitosterol, stigmasterol type, Coumarins glycosides like umbelliferone, scopoletin, dicaffeoyl quinic acid were previously isolated from the plant [32]. Also the plant contains flavonol glycosides like kaempferol, quercetin, rutin, [16] are known to have anti inflammatory, antinociceptive and antirheumatic activities. In general, kaempferol glycosides were found to show higher activity than quercetin. Polyphenols particularly tannins, flavonoids etc are well known natural antioxidants. Moreover, Yoshida and Niki, [33]showed the antioxidant effects of the phytosterols β-sitosterol, stigmasterol, against lipid peroxidation. The coumarins are typical phenolic compounds and, therefore, act as potent metal chelators and free radical scavengers. They are

powerful chain-breaking antioxidants. Therefore the anti-infalmmatory activity and antioxidant activity observed in this study may be due to the effect of a combination of some of the identified constituents [34]. Phytochemical screening showed the content of polyphenols in ethyl acetate extract is higher than in the methanol extract.

The experimental data of present study indicates that *C. cretica* significantly reduced carrageenan induced inflammation which involves three distinct phases of mediator release including histamine and serotonin in first phase, kinins in second phase and prostaglandin in third phase [35]. But, lipoxygenase inhibitors also possess significant anti-inflammatory effect against carrageenan-induced paw edema [36]. So inhibition of carrageenan-induced paw edema by Fr-Et and Fr-Me could also be due to its inhibitory effect on lipoxygenase (the enzyme involved in lipoxygenase pathway of inflammation).

Cotton pellet granuloma the most suitable methods for studying the efficacy of drugs against proliferative phase of inflammation. The dry weight of the pellets correlates well with the amount of granulomatous tissue [37]. The result indicates that the extract can inhibit sub chronic inflammation in which various types of cellular migration are (e. g. fibroblast) involved [38].

The carrageenan-induced air-pouch model in rats was used to examine anti-inflammatory activity of fractions (Fr-Et, Fr-Me) of *C. cretica* on fluid extra vacation, leukocyte accumulation in exudates. Treatment with the fractions of

C. cretica decreased significantly the carrageenan induced the exudates and counts of polymorphonuclear leukocytes indicating the sub chronic anti-inflammatory activity. As an *in vivo* model, vascular permeability assay is a model typical of the first stage inflammatory reactions. In vascular permeability assay mediators of inflammation, released following stimulations, leads to dilation of arterioles and venules and increased vascular permeability [39].

Our findings suggest that the anti-inflammatory effect of Fr-Et and Fr-Me in various models, like carrageenan induced paw oedema, carrageenan air pouch granuloma and adjuvant induced arthritis is due to its ability to decrease capillary permeability which results in decrease in fluid exudation. It is possible that the fractions inhibits the release of mediators like histamine, serotonin, prostaglandins which play a significant role in increasing vascular permeability [40-44].

In FCA induced chronic inflammation model a number of inflammatory mediators are released, namely prostaglandin [45], free radicals [46], cytokinins [47] and substance P [48]. It is possible that Fr-Et and Fr-Me might be inhibiting some of these mediators. Further, the fractions of *C. cretica* also inhibited edema formation induced by histamine, serotonin, prostaglandin E_2 which suggests that Fr-Et and Fr-Me also possess potential antihistaminic, antiserotonin and antiprostaglandin activity.

The metabolites of arachidonic acid formed via cyclooxygenase and lipoxygenase pathways represent two important classes of inflammatory mediators. Prostaglandins (products formed via cyclooxygenase pathway) PGE_2 in particular, is known to cause or enhance the cardinal signs of inflammation. Similarly leukotriene (LTB_4) is a mediator of leukocytes activation in inflammation and inhibitors of lipoxygenase may have potential therapeutic value. The recent description of the phlogistic activities of leukotrienes [49] have stimulated search for agents that inhibit both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism [50]. Arachidonic acid-induced paw edema in rats is an *in vivo* model to distinguish between cyclooxygenase and lipoxygenase inhibitors [24]. Our present findings showed that neither indomethacin nor aspirin blocked the arachidonic acid-induced edema formation while *C. cretica* fractions inhibited edema formation in dose-dependent manner (Table 5). Chlorpheniramine maleate (antihistaminic) and cyproheptadine (antihistaminic/antiserotonin agent) also had inhibitory effect on edema formation which appears to be due to inhibition of mast cell mediator release, suggesting that mast cell mediator release may contribute at least in part to arachidonic acid-induced paw edema. It has been observed that fractions (Fr-Et, Fr-Me) at tested dose levels of *C. cretica* inhibited histamine and serotonin-induced edema and also carrageenan-induced edema which involves release of histamine and serotonin. Hence, the inhibitory effect of these fractions of *C. cretica* on arachidonic acid-induced edema could be partly due to inhibition of mast cell mediator release. Since the percentage inhibition of edema with Fr-Et 200mg/kg (56.58%) was more than that produced by either aspirin (cyclooxygenase inhibitor) (5.06%) or indomethacin (cyclooxygenase inhibitor) (4.19%) or chlorpheniramine maleate (antihistaminics) (48.34%) or cyproheptadine (antihistaminic/ antiserotonin agent) (43.85%), there appears to be a likely involvement of lipoxygenase inhibitory effect of the ethylacetate fraction towards inhibition of edema formation (Table 5).

Superoxide is known to participate in the formation of chemotatic factors and recruitment of polymorphonuclear cells (PMNs). Hence, our studies indicated the fractions, which could scavenge the superoxide anion, might inhibit the recruitment of PMNs and thereby reduce inflammation [51]. Reactive oxygen species such as superoxide anion (O_2-), hydroxyl radical (OH.) and hydrogen peroxide (H_2O_2) play an important role in the inflammatory process induced by ethanol, carrageenan or carbon tetrachloride [52-54].

The mechanisms explaining the anti-inflammatory activity of flavonoids or phenolics are (a) antioxidative and radical scavenging activities, (b) regulation of cellular activities of inflammation-related cells, (c) modulation of the activities of arachidonic acid metabolism enzymes (phospholipase A2, cyclooxygenase, lipoxygenase) and nitric oxide synthase, (d) modulation of the production of other proinflammatory molecules, (e) modulation of proinflammatory gene expression [55]. Some flavonoids display a remarkable array of biochemical and pharmacological actions that affect the function of immune and inflammatory cells such as T cells, B cells, macrophages, neutrophils, mast cells, or basophils [56]. Several flavonoids specifically affect enzyme systems critically involved in the generation of inflammatory processes, especially tyrosine and serine-threonine protein kinases. Quercetin also inhibited human neutrophil degranulation as well as catalytic activity of the released elastase [57]. Oral administration of rutin reduced in a dose-dependent manner the polymorphonuclear neutrophils chemotaxis to FMLP in a

model of rat paw oedema. Several flavonoids such as kaempferol, quercetin have been reported as potent inhibitors of b-glucuronidase and lysozyme release from neutrophils. These flavonoids significantly inhibited arachidonic acid release from membranes, an effect that was correlated with degranulation [58].

Coumarins (scopoletin, dicaffeoyl quinic acid) have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities. The hydroxycoumarins are typical phenolic compounds and, therefore, act as potent metal chelators and free radical scavengers. They are powerful chain-breaking antioxidants. The methods used for the identification of phytochemical constituents are preliminary in nature therefore further studies are required to identify the presence of other constituents and the relative concentration of each constituents in the plant.

From this investigation it can be concluded that on preliminary screening the extract of *C. cretica* produced a significant anti-inflammatory effect and antioxidant activity. Further work relating to isolation and characterization of the active constituents present in the plant and studies on various pharmacological evaluations, as well as resolve the exact phytoconstituents accountable for the activity, is under way by our research team in our laboratory.

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