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Research Article

Evaluation of anti-inflammatory activity of *Crataeva magna* Lour (DC) root bark in experimental animals

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ABSTRACT

The present study was to evaluate anti-inflammatory activity of ethanolic extract of root bark of *Crataeva magna* Lour (DC) (EECM) at 100, 200 and 400 mg/kg body weight was studied for anti-inflammatory activities in different animal models. Anti-inflammatory activity was carried out by using carrageenan induced paw edema model and cotton pellet induced granuloma model in wistar rats. The anti-inflammatory activity may be due to presence of Phytochemical compounds present in the extract like alkaloids, flavonoid, triterpenoid, phenols, saponins and tannins. The results suggested that EECM possess anti-inflammatory activity in (200 and 400 mg/kg) also showed significant ($p<0.001$) anti-inflammatory activity by reducing the paw edema volume in carrageenan-induced paw edema in rats in the late phase (3 to 5 h) regulated by prostaglandins and leucotrienes and in cotton pellet induced granuloma model EECM decreased dry weight of granuloma. The effect was also comparable to Diclofenac, the standard drug in this study.

Keywords: Anti-inflammatory; Crataeva magna Lour (DC); carrageenan induced paw edema; cotton pellet induced granuloma model.

INTRODUCTION

Inflammation is the concept of living tissues to be injured due to many reasons (Chinaka *et al*., 2014). It is a simplify of patho physiological process mediated through different type of molecules produced by leukocytes, macrophages and mast cells by activation of enzyme and release of the mediator makes the breakdown of tissues and edema formation brings the output of fluid and proteins and the leukocytes accumulate at the inflammatory part (White *et al*., 1999).A suitable response protects against injury and cure the damaged tissue in natural body condition while in condition of clinically inflammation may lead in destroy of tissue and its final to organ dysfunction (Ikeda *et al*., 2008, Sampanda bosle *et al*.,2014).All the steroidal and non-steroidal anti-inflammatory drugs (NSAID's) cause unpleasant side effects during clinical use (Tomar *et al.,*2010). The administration of NSAID for long time may cause gastro-intestinal ulcers, bleeding in human parts and renal failure due to their both constitutive (COX-1) and inducible (COX-2) iso forms of the cyclooxygenases enzymes (Robert, 1976, Peskar, 1977, Tapiero *et al* 2002). Medicinal plants are important source of new organic substances with useful therapeutic effects. The traditional medicinal plants are good sources of potential drugs for treatment of inflammation (Calixlo *et al*., 2003, Shraddha *et al*., 2013). The plants having folkloric use as pain relievers and anti-inflammatory agents. (Dhirender *et al*., 2012, Gupta *et al.,* 2006).

The present research work was initiated to investigate the anti-inflammatory activity of ethanol extract of *root bark of Crataeva magna* in laboratory animals.

Crataeva magna Lour DC (family Capparidaceae) is known as three leaved caper in English, Varuna in Sanskrit and Baruna in Hindi, a small tree with a much branched head, found to be distributed mainly in the warmer (tropical) parts of the world. In folk medicine, its stem pith in the tribal peoples of Kandhamal district of Orissa known as Eastern Ghats of India that the bark is used for lactation after child birth, treat urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation (Sovan patnaik *et al*., 2012, Gangadeep and Kalidhar, 2006) Leaves are deciduous three foliolate; petioles 3.8–7.6 cm long; leaflets 5–15 ovate, lanceolate or obovate, acute or acuminate, attenuate at the base, entire, glabrous on both surfaces, pale beneath, and reticulately veined (Inayathulla *et al*., 2010) . The traditional plant used to treat various ailments in particular to Urolithiasis (Baskar *et al*., 1996), Hepatoprotective (Sunitha *et al.,* 2001), Cardio protective (Sudharshan *et al*., 2006), anti-arthritic and rubifacient (Geetha and Varalakshmi, 2001, Latha *et al.,* 2001). Bark juice of this plant is given orally to prevent child-

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hood diseases among the inhabitants of the Kanyakumari district (Solomon *et al.,* 2011). The literature revealed that wide variety of medicinally important compounds including friedelin, diosgenin, sitosterol, dodecanoic anhydride, saponins, flavonoid, sterols , glucosilinates, cadabicine diacetate, lupeol, betulinic acid, glucocapparin, triacontane, triacontanol, cetyl and ceryl alcohol, octanamide,12-tricosanone ,rutin, quercitin, varunol, methyl pentacosanoate, kaemferol-3-O-α-D-glucoside and quercitin-3-O-α-D-glucoside have been reported from *Crataeva magna* (Mantena *et al.,*2008, Mhd Moniruzzaman and Mohammad Zafar Imam, 2014).

To our best knowledge, there is no report about the anti-inflammatory activity of root bark of *Crataeva magna.* So the ethanolic extract of *Crataeva magna* leaves was investigated for its potential anti-inflammatory activity in different experimental models in mice.

MATERIALS AND METHODS

Root bark of *Crataeva magna* Lour DC were collected in and around local forest area of Kanyakumari, Tamilnadu and authenticated by the Botanist Prof.Chelladurai, Department of Botany, Govt. Siddha Medical College, Tirunelveli. A voucher herbarium specimen number KMCP/CM/01/2015 was also preserved in the K.M.College of Pharmacy, Madurai.

Preparation and Extraction of Plant material

The root bark is collected were subjected to dried in shade and then coarsely powdered. The 500 gms of powdered root bark of *Crataeva magna* Lour DC were defatted with petroleum ether and extracted successively with chloroform and ethanol using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extract was filtered through a cotton plug, followed by whattman filter paper (no.1). The extract was evaporated under reduced pressure using rotovac evaporator.

ANTI INFLAMMATORY ACTIVITY

Animals

Albino rats of either sex weighting 180-200g were used. The animals were fed with standard animal feed (Hindustan Lever Ltd) and water *ad libitum*. All the animals were acclimatized to the laboratory conditions prior to experimentation.

Acute toxicity studies

Acute toxicity study was performed for the extracts to as certain safe dose by acute oral toxic class method as per 423 guidelines (OECD)

Carrageenan induced rat-paw edema method

Carrageenan induced rat paw edema method was followed for acute anti-inflammatory study (Winter *et al.*, 1962, Goldenberg and Ilse, 1977). The wistar rats were divided into five groups (n = 6)

Group 1- Carrageenan control,

Group 2- Diclofenac (10 mg/kg, p.o.),

Group 3- EECM (100 mg/kg, p.o.),

Group 4- EECM (200 mg/kg, p.o.),

Group 5- EECM (400 mg/kg, p.o.).

Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% lambda Carrageenan (Sigma Chemical Co., USA) suspension in sterile normal saline in the left hind paw of each rat. Rats were pretreated orally with EECM and Diclofenac (10mg/kg p.o.) 1 h before carrageenan injection.

The rat paw volume up to the ankle joint was measured using Plethysmometer (Ugo Basile, Italy) from 0-6 h at an interval of 1 h. The mean changes in injected paw volume with respect to initial paw volume were calculated. Percentage inhibition of paw volume between treated and control group was calculated using following formula

$$\% \text{ Inhibition} = (1 - Vt/Vc \times 100)$$

Where, Vc and Vt represent mean increase in paw volume in control and treated groups, respectively.

Cotton-pellet granuloma method (Winter and Porter, 1957, Meir *et al.,* 1950, Pelzer *et al.,* 1998, Shenoy *et al.,* 2010)

The rats were divided into five groups (n = 6):

Group 1- Vehicle control

Group 2- Diclofenac (10 mg/kg, p.o.),

Group 3- EECM (100 mg/kg, p.o.),

Group 4- EECM (200 mg/kg, p.o.),

Group 5- EECM (400 mg/kg, p.o.).

Autoclaved cotton pellets weighing 35 ± 1 mg each were implanted subcutaneously through small incision made along the axilla or flank region of the rats anesthetized with anesthetic ether. EECM and Diclofenac (10 mg/kg p.o.) were administered once daily for seven consecutive days from the day of cotton pellet insertion. On the eighth day all rats were sacrificed and the cotton pellets covered by the granulomatous tissue were excised from animal body and dried in hot air oven at 600C for 24 h and weighed.

Statistical Analysis

Values were expressed as mean ± SEM and statistically analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying One Way ANOVA with Dunnett's test and Two Way ANOVA with Bonferroni test, $p < 0.05$ was considered to be significant.

Table 1: Effect of EECM in Carrageenan induced paw edema in rats

Treatment	Dose (Mg/kg, p.o.)	Change in paw volume (ml)		
		1h	3h	5 h
Vehicle control	-	0.71 ± 0.07	2.41 ± 0.18	2.45± 0.09
Diclofenac	10	0.35 ± 0.09* (49.18)	0.43 ± 0.14*** (73.84)	0.17 ± 0.04*** (91.57)
EECM	100	0.64 ± 0.15 (10.69)	1.65 ± 0.05** (20.16)	2.35 ± 0.19*** (21.34)
EECM	200	0.67 ± 0.05 (24.10)	1.54 ± 0.18*** (33.50)	1.15 ± 0.09*** (59.87)
EECM	400	0.52 ± 0.06 (42.27)	0.87 ± 0.21*** (53.17)	0.65 ± 0.05*** (84.36)

Table 2: Effect of EECM on cotton pellet-induced granuloma in rats

Treatment	Dose (mg/kg)	Dry weight of granuloma (mg)	Percent Inhibition (%)
Vehicle control	-	129 ± 5.1	-
Diclofenac	10	60 ± 2.3***	49.43
EECM	100	115 ± 4.9	11.51
EECM	200	101 ± 4.4***	20.31
EECM	400	83 ± 3.2***	33.74

RESULTS

Acute oral toxicity

The EECM did not exhibit any toxic symptoms and mortality when given orally at dose of 2000 mg/kg b.w. Hence, the extract was found to be safe at the dose of 2000 mg/kg b.w. Therefore three doses 100, 200 and 400 mg/kg b.w. were selected for pharmacological studies.

Anti-inflammatory activity

Carrageenan induced paw edema in rats

Treatment with EECM at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg exhibited a significant decrease in paw volume. EECM at 100 mg/kg showed significant ($p< 0.05$) and at 200 & 400 mg/kg also showed significant ($p< 0.001$) decrease in paw volume at 3rd and 5th h. Diclofenac (10 mg/kg) exhibited a significant ($p< 0.001$) reduction in paw volume at 3rd and 5th h as compared to vehicle control. The percentage inhibition of change in paw volume of EECM at 100 mg/kg, 200 mg/kg and 400 mg/kg was found to be 20.16%, 33.50 % and 53.17 % respectively at 3h. However the maximum percentage inhibition was found to be at 5th h 20.16 %, 59.87 % and 84.36 % for 100 mg/kg, 200 mg/kg and 400 mg/kg of EECM respectively. The percentage inhibition of Diclofenac (10 mg/kg) was found to be 73.84 % and 91.57 % at 3rd & 5th h respectively when compared with carrageenan control animals (Table 1).

Values are expressed as mean ± SEM for six animals and analyzed by Two way ANOVA followed by Bonferroni test, *$p< 0.05$, ** $p< 0.01$, ***$p< 0.001$ when compared to carrageenan control. The figures in parenthesis indicate the percent inhibition.

Cotton pellet-induced granuloma in rats

In cotton pellet granuloma, the EECM (200 and 400 mg/kg) significantly ($p< 0.001$) inhibited the granuloma formation when compared to vehicle control group. The degree of inhibition was dose dependent. The EECM at 100, 200, and 400 mg/kg inhibited the granuloma formation by 11.51%, 20.31% and 33.74% respectively. Diclofenac (10 mg/kg) significantly ($p< 0.001$) inhibited the granuloma formation by 49.43% (Table 2).

Values are expressed as mean ± SEM for six animals and analyzed by One way ANOVA followed by Dunnett's test, ***$p< 0.001$ when compared to vehicle control.

DISCUSSION

The traditional medicine is widespread and plants having a large source of structurally novel compounds that might serve as development of novel drugs (Delaheras *et al.,* 1998). The present investigation was carried out to scientifically evaluate the traditional claim of root bark of *Crataeva magna* Lour DC as anti-inflammatory. It was already reported that, the compound lupeol isolated from *C. nurvala* stem bark and its ester lupeol linoleate have shown anti-inflammatory activity in arthritic rats (Geetha and Varalakshmi, 2001).On acute oral toxicity the extract was found to be safe up to 2000 mg/kg. Phytochemical screening showed the presence of saponins, phenols, flavonoid, alkaloids, triterpenoid and tannins.

Carrageenan induced paw edema which is a classical model of acute inflammation used for steroid and non steroid anti-inflammatory drugs (Wu *et al.,* 2006). Carrageenan induced inflammation has a significant value for anti-inflammatory agents acting by inhibiting the exposure of acute inflammation (Badole *et al.,* 2011). Carrageenan is used in animal models of inflammation

to test anti-inflammatory activity because dilute carrageenan solutions (1-2%) injection causes swelling and pain (Lee *et al.,* 2009). The edema produced by sub plantar injection of carrageenan in rat hind paw is biphasic over 4 or more hours. The early phase is attributed due to release of serotonin and histamine while later phase is sustained by prostaglandins and leucotrienes (Vinegar *et al.,*1987) and continuity between two phases is provided by kinins (Okai,1991) The EECM was found to significantly inhibit carrageenan induced rat paw edema by prostaglandins and leucotrienes.

Cotton Pellet induced granuloma in rats is a severe model of inflammation which has been widely used to assess activity of anti-inflammatory drugs on proliferative phase of inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed (Babu *et al*., 2009) In the present study significant activity of EECM was seen against cotton pellet induced granuloma in rats indicating ability of EECM in reducing number of fibroblasts and synthesis of collagen and mucopolysaccharide, natural proliferative events of granulation tissue formation. The presence of phenolic compounds and triterpenoid (lupeol) in the root bark of *Crataeva magna* ethanolic extract may be responsible for the anti-inflammatory activities in both the models (Shanmuga priya *et al.,* 2005).Therefore anti-inflammatory activity of EECM can be attributed to its Phytochemical compounds present in the extract.

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