In vivo Antiinflammatory Activity of *Exacum bicolor* Roxb. Leaves

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Ashwini *et al.*: Antiinflammatory Activity of *Exacum bicolor* Roxb

Leaves of *Exacum bicolor* are traditionally used as a remedy for the treatment of inflammatory disorders in the form of a tonic by the tribal community of Western Ghats of Karnataka. The present study was designed to evaluate the antiinflammatory activity of the methanol extract of *Exacum bicolor* leaf using the carrageenan-induced mice paw oedema model. The methanol extract which was shown earlier to possess *in vitro* antiinflammatory activity was further screened for *in vivo* antiinflammatory activity at doses of 250 and 500 mg/kg. Diclofenac sodium 10 mg/kg was used as the reference standard. The plant extract showed significant dose-dependent reduction in paw oedema when compared to the control at all the time intervals, which was also comparable to that produced by diclofenac. Histopathological analysis revealed clear and distinguished cellular damage for all the groups. The results of the current study showed that the methanol extract of the leaf of *Exacum bicolor* possessed significant antiinflammatory potential, which provided supporting evidence to the folklore use of the plant leaves as an antiinflammatory drug.

Key words: Antiinflammatory activity, *Exacum bicolor* Roxb., carrageenan, oedema, histopathology

Exacum bicolor Roxb. (family: Gentianaceae), a perennial herbaceous medicinal plant with attractive flowers is endemic to peninsular India[1,2]. This species has been used traditionally for curing diabetes[1,3], skin disorders[4], inflammation, blood purification to treat malaria[5], as a stomachic[6] and asthma[7]. The plants also exhibited anthelmintic, antioxidant, antiinflammatory and thrombolytic activities[8-10]. Preliminary phytochemical screening of the extracts showed the presence of alkaloids, coumarins, flavonoids, saponin, steroids, terpenoids, glycosides and phenols[8].

Oxidative stress plays a major role in today's modern life style causing diseases such as inflammation, rheumatoid arthritis, cardiovascular, Alzheimer's, diabetes, respiratory, liver, autoimmune, kidney and skin problems[11]. Inflammation is a key factor in all aspects of coronary disease including the initiation and progression of atherosclerotic plaque, plaque rupture and thrombosis where the oxidative stress plays a significant role[12]. Oxidative stress and inflammation are intimately linked with the evolution of cardiovascular disease and acute coronary syndromes[13]. Due to short comings of most synthetic drugs, current research is directed towards the development of herbal medicine, which is considered to be safe and less toxic. Herbal source have been the source of a wide variety of biologically active compounds used as potent therapeutic agents for treating not only inflammation but also related disease conditions where inflammation plays a vital role[14]. Carrageenan is a natural polysaccharide obtained from edible red seaweed is used in experimental medicine, pharmaceutical formulations, cosmetics and industrial applications. A special use of carrageenan is in experimental pharmacology for the testing of antiinflammatory drugs[15], which is followed in the present study. *In vitro* antiinflammatory activity of the methanol extract of *E. bicolor* leaves was reported earlier[10]. According to literature survey, there has been no report on the *in vivo* antiinflammatory activity of *E. bicolor*. The present study was aimed to evaluate the antiinflammatory activity of methanol extract of the leaf in carrageenan-induced mouse paw oedema model.

E. bicolor leaves were collected in November 2012 from *Kumara Parvatha*, at Kukke Subramanya in Sullia taluk, Western Ghats, Karnataka, India. The leaves were identified and authenticated at the National Ayurveda Dietetics Research, Jayanagar, Bengaluru, India (Accession No. SMPU/NADRI/BNG/2010-11/557) and a voucher specimen was deposited in the Herbarium of Biotechnology Department of the Jain University, Bengaluru. The leaves were shade dried and were extracted by continuous hot percolation in a Soxhlet apparatus (50±2°) using methanol (yield: 13 %). The resulting extract was dried using a rotary evaporator and was stored at 4° for further studies.

In the present study Swiss albino mice (20-30 g) were used, which were obtained from the Veterinary College, Hebbal, Bengaluru. Six animals were housed in each polyvinyl cage and were maintained under standard laboratory conditions. The animal house was maintained at 25±2° with 12/12 h dark/light cycle. The mice were provided feed (Vet care; Bangalore) and water (Bisleri, Bangalore) *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Nargund College of Pharmacy, Bengaluru (ethical clearance number IAEC/NCP/94/2015). Extracts and the standard drugs were administered in the form of suspension in water with 1 % dimethyl sulfoxide as suspending agent before 30 min of carrageenan treatment. Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development-423 (OECD-423) guidelines. The methanol extract was found to be safe up to a dose of 2000 mg/kg. Hence, 250 and 500 mg/kg doses were used for the evaluation of *in vivo* antiinflammatory activity. Mouse carrageenan-induced hind paw oedema model was used to study the *in vivo* antiinflammatory activity of the methanol extract[16]. The animals were observed for mortality, signs for gross toxicity and behavioural changes at least once daily for 14 d. Body weights were recorded prior to administration of the plant extract and again on 7th and 14th d. The animals were divided into five groups

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Accepted 11 October 2018
Revised 04 April 2018
Received 01 June 2017
Indian J Pharm Sci 2018;80(6):1155-1159

each composed of six animals. Group I- control group given saline (0.9 %) orally. Group II- carrageenan (1 %) in saline was administered into subplantar region of right hind paw. Group III- standard group was given diclofenac sodium (10 mg/kg) orally. Group IV- test group to which *E. bicolor* methanol leaf extract (250 mg/kg) was administered orally. Group V- test group to which *E. bicolor* methanol leaf extract (500 mg/kg) was administered orally.

Paw oedema was induced by injecting 50 µl[17] of 1 % carrageenan (Sigma-Aldrich) in physiological saline into subplantar tissues of hind paw to produce acute inflammation[18-21]. The volume of oedema was measured at intervals of 0, 2, 4, 8 and 24 h by the mercury displacement method using a Plethysmograph (Letica, Comella, Spain). Percent inhibition (% IE) of oedema was calculated using the Eqn., % IE = (Vc− Vt)/Vc×100, where Vc is the inflammatory increase in paw volume in control group of animals and Vt is the inflammatory increase in paw volume in drug-treated animals. Inhibition of paw volume in drug-treated group was compared with carrageenan control group (group 1).

Histopathological examination of mice paws were carried out to assess the effects of inflammation. After 24 h of carrageenan injection, mice were sacrificed and paws were fixed in 10 % formaldehyde. The tissues were cut into 4 µm thick slices with microtome and placed on adhesive glass. Standard haematoxylin and eosin (H and E) staining was performed for morphological observation.

In the present study it was observed that there were no signs of mortality and none of the mice showed clinical toxic signs such as anorexia, depression, lethargy, dermatitis throughout the examination with a dose level up to 2000 mg/kg of *E. bicolor* leaf methanol extract when administered to mice. A carrageenan-induced mouse paw oedema model was used to study *in vivo* antiinflammatory activity and the volume of paw oedema was measured using a Plethysmometer. Carrageenan showed increase in paw oedema volume (0.42 ml) at 6th h (figs. 1 and 2) whereas saline group showed an increase in volume of 0.32 ml (fig. 2). The development of oedema in the rat hind paw following the injection of carrageenan has been described as a biphasic, age-weight-dependent event in which various mediators operate in sequence to produce the inflammatory response[15]. The present study with *E. bicolor* the leaf extracts at the doses of 250 and 500 mg/kg showed 0.3 and 0.33 ml of increase in volume of paw oedema, respectively. *E. bicolor* extracts at different doses were significant and comparable to control group (saline). Diclofenac and *E. bicolor* extracts (250 and 500 mg/kg) showed 96, 90 and 91 % inhibition, respectively at 6 h. According to previous reports, the methanol extract of *Enicostemma axillare* was assessed for antiinflammatory activity by *in vitro* methods using albumin denaturation assay, proteinase inhibitory activity, membrane stabilization and antilipoxygenase activities, which showed a significant inhibitory activity[22]. *E. bicolor* leaf methanol extract exhibited significant *in vitro* membrane stabilization on human red blood cell with IC_{50} value of 37.4 µg/ml and there was also a significant correlation (R^2>0.98) between total phenolic content and antiinflammatory activity[10]. In the present study, *E. bicolor* leaf methanol extract exhibited significant and *in vivo* antiinflammatory activity at 250 and 500 mg/kg with percent inhibition of 90 and 91 %, respectively. Diclofenac (10 mg/kg) inhibited inflammation by 96 %. In the histopathological sections of mice paw (fig. 3) there was no cellular infiltration (fig. 3a), which represented that saline did not cause any damage to the cells. In contrast, there was swelling followed by cellular damage due to the carrageenan injection (fig. 3b). After treatment with diclofenac, the cellular damage was not much prominent as the cells were intact. *E. bicolor* at the doses of 250 and 500 mg/kg significantly reduced the oedema and also the cellular damages when compared to carrageenan (fig. 3d and e). In contrast to the present study, *E. littorale* extract at 100 mg/100 g exhibited 54 % antiinflammatory activity in carrageenan-induced inflammation[20]. Alcohol and petroleum ether extracts of *Gentiana lutea* rhizomes at 500 and 1000 mg/kg showed dose-dependent antiinflammatory activities in carrageenan-induced rat paw oedema. Both extracts showed significant dose-dependent antiinflammatory activities[21]. Antiinflammatory activity was assessed using carrageenan-induced rat paw oedema model in ethanol extract of *Swertia chirata* root. The extract was found to reduce the formation of oedema significantly ($p<0.001$) at the 400 mg/kg dose level and showed 57.81 % ($p<0.001$) inhibition of oedema volume at the end of 3 h[22].

In a previously reported study of *E. bicolor* leaf methanol extract, Fourier-transform infrared spectroscopy spectral analysis revealed the identity of the functional groups such as alcohols, phenols, alkanes, amines, aromatic compound, aldehyde and ethers. Gas

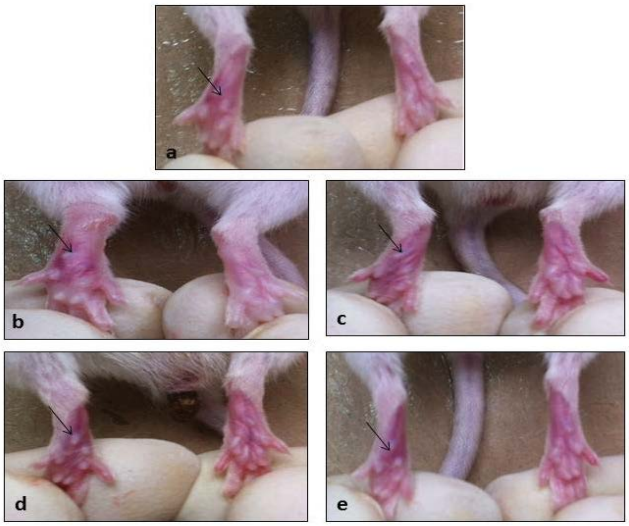

Fig. 1: Carrageenan-induced mice paw oedema
(a) Saline-treated; (b) carrageenan-induced; (c) diclofenac (10 mg/kg); (d) *E. bicolor* 250 mg/kg and (e) *E. bicolor* 500 mg/kg

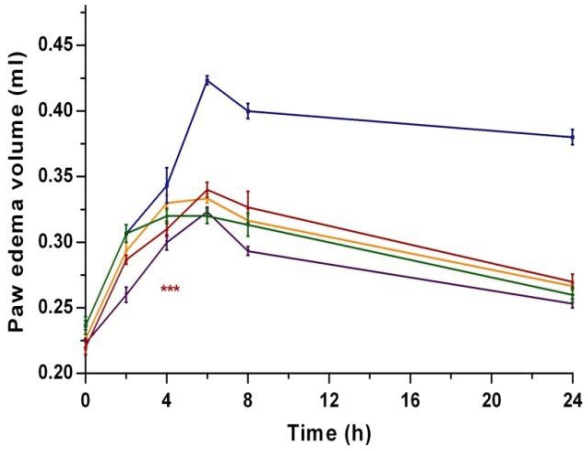

Fig. 2: Antiinflammatory effect of *Exacum bicolor* extract on carrageenan-induced mice paw oedema model
Values are man±SEM for group of five animals. ***P<0.001 vs. control group (saline) analyzed by two way ANOVA followed by Dunnett's multiple comparisons test. (—●—) Saline; (—■—) carrageenan (1 %); (—♦—) diclofenac (10 mg/kg); (—▲—) *E. bicolor* (250 mg/kg); (—▼—) *E. bicolor* (500 mg/kg)

chromatography mass spectrometry analysis revealed the presence of phytoconstituents such as erythrocentaurin, neophytadiene, hexadecanoic acid, 6-octadeccenoic acid, (+–)-inophylum D, 4,6,8(14)-cholestatriene and methyl 3,4-diphenylpyrrolo[2,1,5-cd]indolizine-1-carboxylate. These phytocompounds might be responsible for the cause of antiinflammatory response in *E. bicolor* leaf methanol extracts[10].

The present investigation has provided the scientific basis for the traditional uses of *E. bicolor* Roxb. leaves in inflammation having significant antiinflammatory activity. This report on *in vivo* antiinflammatory study on carrageenan-induced mice paw oedema was for the first time. Further pharmacological investigations are underway to find out the exact active constituents responsible for antiinflammatory activity and to elucidate its mechanism of action.

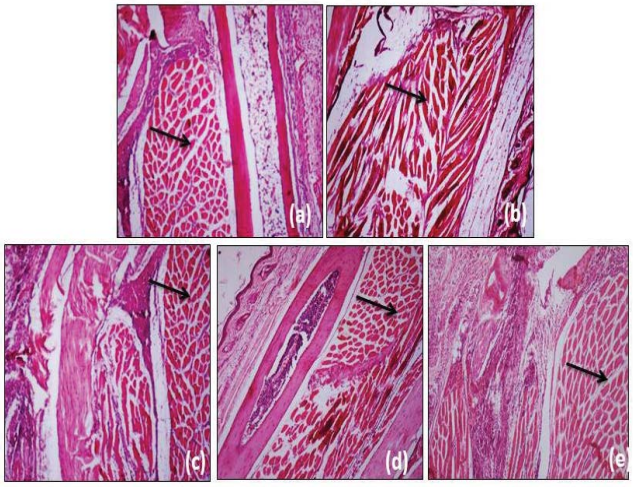

Fig. 3: Histological changes in edematous paws after carrageenan injection
(a) Saline control group; (b) carrageenan model group; (c) diclofenac (10 mg/kg); (d) 250 mg/kg of *E. bicolor* group; (e) 500 mg/kg of *E. bicolor* group; magnification ×10

Acknowledgements

The authors are grateful to the Center for Post Graduate Studies, Jain University, Bangalore, India, for providing financial assistance to the first author to carry out the research work, lab facilities and also infrastructural facilities. We also thank Nargund College of Pharmacy, Bangalore for ethical clearance and providing animal house facility to carry out *in vivo* studies.

Conflict of interest:

Nil.

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