

Anti-inflammatory and Antioxidant Activities of *Cordia dichotoma* Forst.

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Cordia dichotoma Forst. has been used in the management of pain and inflammations in traditional medicine. However, the anti-inflammatory activity of the methanolic extract of *C. dichotoma*(MECD) bark has not been reported so far. This work was, therefore, aimed at investigating the anti-inflammatory activity of *C. dichotoma* bark extract. The antioxidant activity was evaluated to justify the anti-inflammatory action of MECD on the basis of its radical scavenging property. The extract of *C. dichotoma* was obtained by Soxhlation of bark powder using methanol as solvent. The anti-inflammatory activity was determined by the carrageenan induced paw edemamodel in ratsat two different dose levels, viz., 250 and 500 mg/kg. The antioxidant activity was evaluated using DPPH radical scavenging assay. The antioxidant activity was performed *in vitro* by DPPH radical scavenging assay using ascorbic acid as the standard drug. In anti-inflammatory activity, maximum inhibition of edema was observed after 4 hours of experimental period. At lower test dose (250 mg/kg b.w.), the percentage inhibition of paw edema was 29.7 %, while 48.6 % inhibition of edema was observed at higher dose (500 mg/kgb.w.). The percentage inhibition of paw edema was significant relative to the control group. The standard indomethacin group also exhibited sufficiently high level of anti-inflammatory effect with 56% inhibition of paw edema at 5 mg/kg dose. In *in vitro* antioxidant activity, the MECD exhibited good DPPH radical scavenging activity with the IC₅₀ vale of 62.46 µg/ml, whereas the standard drug, ascorbic acid showed comparatively more antioxidant activity with IC₅₀ of 27.66 µg/ml. However, our study scientifically validates the folkloric claim as well as traditional uses of *C. dichotoma* as anti-inflammatory medication. It is suggested that the anti-inflammatory activity of *C. dichotoma* may be due to the antioxidant potential of phenolic phyto constituents or plant flavonoids present in the methanolic bark extract.

Keywords: *Cordia dichotoma*, methanolic extract, anti-inflammatory activity, flavonoids, antioxidant property.

Chronic inflammatory diseases remain to be one of the major medical problems around the world. According to the World Health Organization (WHO), chronic inflammatory diseases are

considered as the significant threat to public health. The prevalence of chronic inflammatory disorders is increasing with 17.6 million clinical cases, particularly due to rheumatoid arthritis every

year globally¹. Approximately 3 of 5 people die and 50% of all deaths occur have been attributable to chronic inflammation-related diseases such as cardiovascular diseases (stroke, heart disorders), chronic respiratory diseases (allergic asthma, COPD), rheumatoid arthritis and joint disorders, systemic lupus erythematosus, inflammatory bowel disease (IBD), Crohn's disease, Alzheimer's disease, cancer, obesity, diabetes, chronic kidney disease and many others². Some common FDA approved medications that include Non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs and immuno suppressants are commonly used for the treatment of inflammatory disorders. These drug therapies are required to be administered for a long time and their use often induce some serious adverse effects such as gastrointestinal upset, heart burn, peptic ulcers, headache and so on. Because of this reason, there is an increasing need to develop some new and alternative anti-inflammatory drugs that would not only be efficacious against inflammatory illness and diseases, but also be safe and produce fewer side effects.

Plant-based traditional remedies have been used for the treatment of human diseases for thousands of years. About 80% of population depends on traditional herbal remedies for primary health care across the globe³. Traditional or folklore herbal medicines play a significant role in the management of a variety of human disorders including cancer, neurological disorders, diabetes and pain and inflammatory disorders, just to name a few⁴. WHO has recommended the evaluation of traditional plant-based remedies or herbal preparations for antidiabetic activity because they are more effective and safe as compared to synthetic antidiabetic drugs⁵. In addition, herbal medicines have the property of synergistic action due to the presence of a variety of active constituents in a single drug/ medicinal preparation. Numerous indigenous medicinal plants of India have been found to be useful in the management of pain and inflammations. Ayurveda, Unani and Siddha, are the notable systems of medication documented in ancient practice basically utilizing plants/ plant-based preparations as medicines for curing human ailments/ diseases like diabetes⁶. In view of their traditional and ethnopharmacological importance, herbal medicine may have potential role in the management of pain and inflammations.

Moreover, medicinal plants provide valuable source of new chemical moieties with potential therapeutic properties. Plants that have been found to have anti-inflammatory potential can be screened in search for novel bioactive secondary metabolites or phyto constituents as new and effective, but safe anti-inflammatory agents.

Oxidative stress (OS) induced by the reactive oxygen species (ROS) produced from the action of free radicals in the biological matrix may be increased abnormally during diabetes, causing an imbalance between the cellular metabolism and the antioxidant system of the body. The oxidative stress produces several inflammatory cascades that damage the cellular components⁷. Further, oxidative stress is undoubtedly claimed to have significant role in the progression of chronic inflammation mediated diseases. The cellular oxidative stress can be suppressed to an appreciable extent by potentiating the action of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSH) and glutathione peroxidase (GPx) by alternative herbal therapies⁸.

Cordia dichotoma Forst. (also known as Indian cherry) belonging to the family Boraginaceae is an average-sized tree of tropical and subtropical origin. It is widely found in Sri Lanka, India, and other tropical regions of the world. The use of this plant has been on ancient practice for the management of a variety of human disorders. It is also an important plant species found in various traditional Indian systems of medicine including Ayurveda, Unani and Siddha⁹. Seeds of *C. dichotoma* are used for the management of various inflammatory disorders¹⁰. Fruits are used as expectorant, astringent, laxative and anthelmintic⁹. Some common ethnomedical uses of *C. dichotoma* includes antidiabetic, immunomodulator, diuretic, anthelmintic, wound healing, antiulcer, gastro-protective, anti-inflammatory, antileprotic, antidiabetic, and hepatoprotective and antioxidant activities¹⁰. The bark of *C. dichotoma* has been reported to a variety of phyto constituents such as betulin, α -amyrin, octacosanol, β -sitosterol, lupeol-3-rhamnoside, β -sitosterol-3-glucoside, hentricontanol, taxifolin-3,5-dirhmnoside, and hesperitin-7-rhamnoside^{9,11}.

There has been no scientific study on the anti-inflammatory activity of the *C. dichotoma* bark

previously reported in literature. The present study was, therefore, aimed at investigating the anti-inflammatory activity of the methanolic extract of *C. dichotoma* bark (MECD) with a view to justify the traditional use of this particular plant species in the treatment of edema. The antioxidant activity was evaluated to justify the anti-inflammatory action of MECD on the basis of its radical scavenging effect.

MATERIALS AND METHODS

Drugs and chemicals

Carrageenan and indomethacin were purchased from Merck Pvt. Ltd., Mumbai, India. Carboxy methyl cellulose (CMC) was procured from Sigma-Aldrich, Mumbai, India. All other chemicals and solvents used in this study were of analytical grade.

Plant material

The barks of *C. dichotoma* Forst. were collected during the month of April-May, 2012 from the Duhai forest of Ghaziabad, Uttar Pradesh, India. The plant material was identified from CSIR-National Institute of Science Communication and Information Resources (CSIR-NISCAIR), New Delhi. A voucher specimen (NISCAIR/RHMD/Consult/2012-13/2025/33) of the bark of *C. dichotoma* was submitted at the herbarium for future reference.

Extraction methodology

The shade-dried barks of *C. dichotoma* were pulverized to coarse powder, defatted using petroleum ether, and extracted by Soxhlation using methanol as solvent. The methanolic extract was subsequently evaporated to dryness and the concentrated extract so obtained was preserved in a refrigerator at 4 °C for further use. The percentage yield of the methanolic bark extract of *C. dichotoma* (MECD) was found to be 7.11% w/w on dry weight basis.

Phytochemical analysis

The MECD was analysed for the presence of presence of phyto constituents as per the standard procedure previously described in literature¹².

Experimental animals

Adult Wistar female albino rats weighing around 300-330 g were procured from the Institutional Animal House for the experimental study. Acclimatization of animals was done in

accordance with standard laboratory conditions (temperature: 25 ± 2 °C, relative humidity: 50 ± 5 %) with a 12 h light/12 h dark cycle for a week prior to the beginning of experiments, and were provided with free access to the standard pellet diet and drinking water *ad libitum*. The experimental protocol was approved by the IAEC vide approval no. IAEC/DU/58 dated. 24/09/2013.

Acute toxicity study

The oral acute toxicity of MECD was done as per OECD guidelines. The rats were randomly distributed into seven groups of three animals each. The first three groups were administered with MECD at 10, 100, and 1000 mg/kg doses, respectively. The animals were observed for signs of mortality or morbidity or death for 24 hours. Further, doses of 2,000, 3,000, and 5,000 mg/kg were given to rest of the three groups and observed for 48 hours. One group administered with vehicle was treated as normal control⁴.

Evaluation of anti-Inflammatory activity

The anti-inflammatory activity was evaluated by carrageenan induced rat paw edema method according to the previously reported methods¹³⁻¹⁶ with minor modifications. The rats were randomly distributed into five groups containing six rats in each group (one control, one toxic, standard and two test groups). The acute inflammation in rats was induced by the injecting carrageenan (1% w/v solution in 0.9 % w/v sodium chloride, 0.1 ml) in the planter region of rat's paw. The extract was formulated in two different doses (250 and 500 mg/kg body weight) in the form of a suspension using 0.3% CMC. The extract formulations were administered orally into experimental animals one hour prior to injecting carrageenan. Indomethacin (5 mg/kg body weight, oral route) was used as standard drugs.

Group 1 (Control): 0.3 % CMC (10 ml/kg body weight), orally

Group 2 (Toxic): 1 % Carrageenan (0.1 ml)

Group 3 (Standard): Indomethacin (2 ml, 5 mg/kg body weight), orally + 1 % Carrageenan (0.1 ml), orally

Group 4 (Low dose test): MECD (250 mg/kg body weight), orally + 1 % Carrageenan (0.1 ml), by injection

Group 5 (High dose test): MECD (500 mg/kg body weight), orally + 1 % Carrageenan (0.1 ml), by injection

The paw volume was measured using a Plethysmometer at 0, 1, 3 and 4 hours after the administration of carrageenan. The percentage inhibition of paw edema was estimated using the following formula.

% Inhibition of edema = $(V_t - V_c / V_t) \times 100$
 where, V_t = Paw volume in test group, V_c = Paw volume in control group

Antioxidant activity

The *in vitro* antioxidant activity was evaluated by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay method^{5, 8}. A solution of 0.1 mM DPPH in methanol was prepared and 2.4 ml of this solution was mixed with 1.6 ml of the MEXD in methanol at different test concentrations (10-200 μ g/ml). The reaction mixture was vortexed thoroughly and

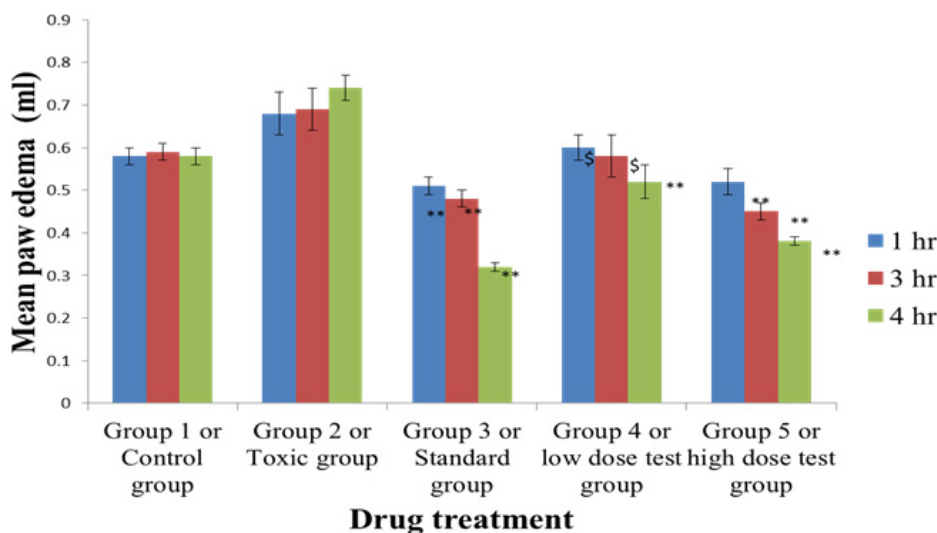


Fig. 1. Effect of MECD on carrageenan-induced paw edema

Values are expressed as mean \pm SEM ($n=6$); statistically significant at $**p < 0.01$, compared to normal control.

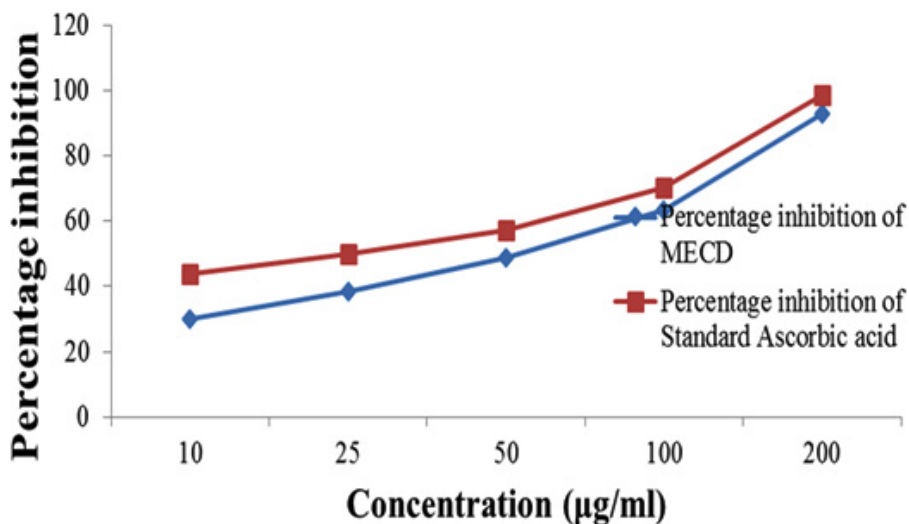


Fig. 2. DPPH radical scavenging activity
 Values are expressed as mean \pm SEM ($n=3$)

kept at room temperature in the dark for 30 min. The absorbance of the mixture was determined spectrophotometrically at 517 nm. Ascorbic acid was used as standard drug. The experiment was carried out in triplicate observations. The percentage of radical scavenging activity was calculated using the following formula. Then % of inhibition of scavenging activity was plotted against concentration and from the graph IC_{50} was calculated.

$$\text{Percentage inhibition} = (A_o - A_t / A_o) \times 100$$

where, A_o and A_t are absorbance values of the control and the test compound (standard/ test), respectively

Statistical Analysis

Results are presented as mean \pm standard error of mean (SEM). The one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used to analyse data. Statistical analysis was performed using the IBM SPSS 19.0 statistical software package, for Windows. Statistical differences at 1% ($p < 0.01$) level of probability between the groups were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis

Qualitative phytochemical analysis showed the presence of alkaloids, steroids, cardiac glycosides, flavonoids, tannins, terpenoids, saponins and carbohydrate in the MECD.

Acute oral toxicity

Results of oral acute toxicity study reveals that the LD_{50} of the MECD was above 5,000 mg/kg body weight. No gross behavioural changes, other symptoms of toxicity and mortality were observed up to 48 hours of experimentation. The body weight and food consumption of treated rats were normal as compared to vehicle control.

Anti-inflammatory activity

The MECD showed anti-inflammatory activity against carrageenan-induced paw edema model in rats. The extract exhibited dose-dependent anti-inflammatory activity. Maximum inhibition of edema was observed after 4 hours of experimental period. At lower test dose (250 mg/kg b.w.), the percentage inhibition of paw edema was 29.7 %, while 48.6 % inhibition of edema was observed

at higher dose (500 mg/kg b.w.). Results of anti-inflammatory activity are presented in Figure 1. Both lower and higher test doses of the MECD demonstrated significant ($p < 0.01$) inhibition of paw edema as compared to control group. The standard indomethacin group also exhibited sufficiently high level of anti-inflammatory effect with 56% inhibition of paw edema at 5 mg/kg dose, relative to the control group. The anti-inflammatory effect of MECD is comparable to some extent with that of the standard drug, indomethacin.

Antioxidant activity

In the *in vitro* radical scavenging activity, the significant reduction in the DPPH radicals was seen due to the free radical scavenging property of MECD. The antioxidant activity rose with increasing the concentration of the test extract, reaching maximum level with the IC_{50} value of 62.46 μ g/ml. On the other hand, the standard drug, ascorbic acid exhibited comparatively more antioxidant activity with IC_{50} of 27.66 μ g/ml (Figure 2). However, the MECD extract of *C. dichotoma* bark possesses antioxidant activity.

Results reveal the anti-inflammatory potential of the MECD of *C. dichotoma* bark along with antioxidant property. It is attributed that the anti-inflammatory effect might be due to the phytochemical content such as phenolic compounds, flavonoids, saponins or alkaloids of the extract. Many studies have investigated the antioxidant potential of plant polyphenols and flavonoids. Phenolic phytoconstituents and flavonoids have been attributed to exhibit pharmacological effects against heart diseases, cancer, neurological disorders, diabetes, inflammatory disorders and so on owing to their radical scavenging actions¹⁷⁻¹⁹. Literature reports suggest that the flavonoids content of plant extract may be responsible for the anti-inflammatory effect which might be because of their radical scavenging actions²⁰. The antioxidant activity of MECD can help reduce the oxidative stress induced generation of inflammatory mediators and associated tissue damages. Restoring the levels of antioxidant enzymes, herbal drugs could act as free-radical scavengers and eventually prevent generation of ROS and thereby OS induced cellular damages^{21,22}. In this study, the antioxidant activity of MECD may be the underlying reason behind

its anti-inflammatory action. Phenolic compounds and flavonoids of MECD could reduce cellular oxidative stress induced inflammatory damages.

CONCLUSION

It is concluded that the methanolic extract of *C. dichotoma*(MECD) bark possesses anti-inflammatory activity in carrageenan-induced paw edema model in rats. Our study scientifically validates the folkloric claim as well as traditional uses of *C. dichotoma* as anti-inflammatory medication. It is suggested that the anti-inflammatory activity of *C. dichotoma* may be due to the presence of phenolic phytoconstituents or plant flavonoids in the methanolic bark extract. Further studies can be carried out in order to identify the specific phytochemical(s) responsible for the anti-inflammatory potential of *C. dichotoma*.

Conflict of interest

The authors declare that there are no conflicts of interest.

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