



Anti-inflammatory Activity of Essential Oil of *Canarium Strictum* Roxb

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Abstract

Canarium L., used in folk medicine as anti-inflammatory, antibacterial, antifungal, antitumor and hepatoprotective and antioxidant and anti diabetic. The present study investigated the in vivo anti-inflammatory activity of essential oil obtained by hydro distillation of Black dammer resin of *Canarium strictum* Roxb. Anti-inflammatory activity of essential oil of *Canarium strictum* Roxb., EOCS (10-100 mg/kg) has been established by using the carrageenan (acute inflammation model) and formalin (chronic inflammation model) induced paw edema in mice. The dose dependent activity has been observed at the higher dose of EOCS 100 mg /kg ($P < 0.0001$). EOCS exhibited momentous anti-inflammatory activity that was compared with standard drug diclofenac sodium in acute inflammatory animal models. The perceived anti-inflammatory activity might be due to inhibition of histamine, serotonin, kinins, substance P and prostaglandins, and bradykinin at the inflamed area. On the other hand, in the formalin test EOCS 100 mg /kg reduced chronic inflammation most effectively ($P < 0.0001$). Our data supported that EOCS capable to inhibit the paw edema in acute and chronic inflammation in the experimental animal models can be evident to use this oil for the treatment of chronic and acute inflammatory disorders. More elaborate investigation is needed in this aspect and to correlate the possible effect of major terpene which it produced anti-inflammatory activity.

Keywords: Black dammer, Burseraceae, *Canarium strictum*, Essential oil, Inflammation, Oleoresin.

1. Introduction

Canarium L. Comprises of 75 species of an aromatic tree that exist in the rainforest of

tropical Asia, Pacific region and Africa. Among these merely 12% were undergone for biological evaluation. The crude drugs derived from *Canarium* L., possess anti-inflammatory, anti-bacterial, antifungal, antitumor, hepatoprotective, antioxidant, and anti diabetic properties [17]. The resin which was collected

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from the wounded bark of the tree *Canarium strictum* was named as black Dammer resin [9&10]. Indian institute of forest management declared this species endangered in the Nilgiri biosphere [2,15&18]. This plant has enormous medicinal and economic uses in different systems of traditional medicine in India [8]. In the traditional medicine, the decoction or dried powder of resin was given orally for the treatment of skin diseases, hernia, syphilis, epilepsy, asthma, fever, and rheumatism[4]. Also, the resin used as alternate for burgundy pitch in preparing plaster and the externally applied resin cures psoriasis and Pityriasis [20]. The analgesic and anti-inflammatory activity was also reported in resin [21, 22&23]. Black dammer resin contains epi- Ψ -taraxasterol, epi- Ψ -taraxastane diol, 11-keto α -Amyrin, Ψ -taraxasterol, Ψ -epitaraxastane diol, the new ketol, 11- β hydroxyl- α - amyrin acetate, 11-keto α -amyrin, epi- Ψ -taraxasterol, epi- Ψ -taraxastane diol, (+) junenol, canarone, *epi*-khusinol, α -amyrin, β -amyrin, and β -amyrin acetate [13,14].

Many researchers proved that essential oil demonstrated various biological activities like spasmolytic, anti-anxiety, anti-convulsant and anti-nociceptive [7]. The current study is to investigate the possible anti-inflammatory activity of essential oil obtained by hydro distillation of oleoresin of *Canarium strictum* Roxb.

2. Materials and Methods

2.1. Drugs and Chemicals

Carrageenan, Formalin, Diclofenac sodium, Dexamethasone (Sigma Aldrich Chemicals, Bangalore), Diclofenac sodium was dissolved in physiological saline (0.9% NaCl). EOCS dissolved in 2% Tween 80 5 ml/kg was administered to control group along with 5ml of physiologic saline (0.9% NaCl).

2.2. Plant Material

Canarium strictum Roxb., (Burseraceae) resin was collected from the wounds trunk of the trees found in Rayriath garden Thrissur, Kerala State, India, authenticated by Prof. P. Jayaraman, Taxonomist, Department of Botany, Plant Anatomy Research Centre, Chennai, India. The voucher specimen (PARC/2010/1475) was deposited in the Herbarium Section of the Department Pharmacognosy, Periyar College of Pharmaceutical Sciences, Periyar Centenary Educational Complex, K. Sathanoor Main Road, Tiruchirappalli-620021, Tamilnadu, India.

Clevenger type of apparatus was used for collection of essential oil from the resin (5 Kg) of *C. strictum* by using hydro distillation method. The resultant yellow coloured oil was collected, and its moisture was removed by using anhydrous sodium sulphate. EOCS was stored in glass vials with Teflon-sealed caps at 2 ± 0.5 °C and stored in a dark place. The percentage yield of the essential oil was 1.6 % (v/w) with a density of 0.91 g/ml.

2.3. Animals

Swiss male and female Balb/C mice (25–30 g) were used for the acute toxicity and anti-inflammatory activity. The animals were kept in plastic wire cages at $25 \pm 1^\circ\text{C}$ and fed with food and water ad libitum. Before the 12 hours experimentation feeding was withdrawn, all mice were acquired after being approved by Institutional Animal Ethics Committee (NCP/IAEC/003/2012).

2.4. Acute Toxicity Studies

An acute toxicity study was carried out according to OECD – 423 guidelines [9]. Swiss Albino mice of both sexes were used. Food was withdrawn before 12 hours. However, the animals were permitted to drink tap water throughout the experiment. The fasted mice were divided into two groups of three animals each. The EOCS was given orally at a dose of 5 mg/kg. Negative control received the same volume of the vehicle (10ml/kg). If death was not observed, the protocol was repeated for higher doses such as 50, 300 and 2000 mg/kg. The animals were kept under observation up to 14 days after EOCS administration to find out delayed mortality or any toxic symptoms observed in 24, 48, and 72 hours [16].

2.5. Anti-inflammatory Experiment

2.5.1. Carrageenan-induced Paw Edema Assay (Acute Inflammatory Model)

The anti-inflammatory activity was assessed by the carrageenan-induced paw oedema method in mice, according to

previously described by Winters CA, 1962 [26] with modifications. The animals were treated by oral route with vehicle (10 ml/kg), Diclofenac sodium (10 mg/kg, p.o) and three doses of (10, 50 and 100 mg/kg) of the EOCS. Half an hour after administration of the various tested samples, oedema induced by injection of carrageenan (0.02 ml of 0.1%, w/v) suspension in normal saline was administered into the sub-plantar region of the right hind paw. Paws thickness was measured with a caliper rule before and 1, 2, 3 and 4h after carrageenan injection. Diclofenac sodium was given as a reference drug whereas the control group received the vehicles that were used to dissolve EOCS. The group treated with vehicle was considered as highest of inflammation and all other treatments were compared to this group. Each of a group comprising 6 mice (n=6).

PEI Percentage inhibition = $(\text{CT}-\text{Co}) \text{ control} - (\text{CT}-\text{Co}) \text{ treated} / (\text{CT}-\text{Co}) \text{ control} \times 100$,
Where Ct is paw size after a specific time interval in hours after carrageenan injection, and Co is paw size before carrageenan injection.

2.5.2. Formalin Test (Chronic Inflammatory Model)

To evaluate the suppression of chronic inflammation induced by formalin, female Balb/C mice were divided into five groups. Group I was kept as control and group II was treated with positive control Dexamethasone 10mg/kg. Group III, IV and V given EOCS 10-100 mg/kg were orally administered for 6

Table 1. Anti-inflammatory activity of (EOCS) essential oil of *Canarium strictum* in Carrageenan induced Paw edema in mice.

Group	Paw edema volume								
	0HR	1 HR		2HR		3HR		4HR	
Dose mg/kg	Mean± SEM	Mean± SEM	%PEI						
Control	0.0448 ± 0.0019	0.1950 ± 0.0156		0.4167 ± 0.0170		0.5083 ± 0.0193		0.4783 ± 0.0207	
Diclofenac Na 10mg/kg	0.0443 ± 0.0020	0.1433** ± 0.0133	34.08%	0.1367** ± 0.0157	74.88%	0.1133** ± 0.0130	85.11%	0.1067** ± 0.0130	85.60%
EOCS 10mg/kg	0.0437 ± 0.0019	0.1833* ± 0.0181	7.05%	0.1650** ± 0.0143	67.38%	0.1411** ± 0.0151	78.98%	0.1334** ± 0.0164	79.30%
EOCS 50mg/kg	0.0448 ± 0.0018	0.1634** ± 0.0158	21.0%	0.1567** ± 0.0130	69.91%	0.1405** ± 0.0178	79.35%	0.1269** ± 0.0156	81.06%
EOCS 100mg/kg	0.0451 ± 0.0025	0.1617** ± 0.0183	22.37%	0.1421** ± 0.0108	73.91%	0.1230** ± 0.0105	83.19%	0.1193** ± 0.0127	82.88%

Results expressed as mean ± S.E.M (N=6) *** P<0.0001; ** P<0.001; * P<0.05 compared to the vehicle treated group (ANOVA followed by Dunnett's test).

successive days. Chronic inflammation was induced by subplanter injection of freshly prepared 0.02 ml of 2% formalin on the right hind paw of all the animals 1 hour after the tested drugs was administered orally.

Dexamethasone 10mg / kg was given through i.p., 15 minutes before the formalin injection. By using the meter rule the paw thickness was measured for 6 consecutive days after injection of formalin [4&24].

The percentage of inhibition was calculated by using the given formula

Percentage inhibition of inflammation = $\frac{1-T}{CX100}$

T- Paw volume difference in test, C- Paw volume difference in control

3. Results and Discussion

Mortality was observed in 2 animals out of 3 at 2000 mg/kg of EOCS, and then the similar dose was repeated over again to affirm the toxic dose. The dose for anti-nociceptive activity was fixed at 10-100 mg/kg of EOCS for dose dependent study. The treated mice did not show any behavioural modifications during the assessment period (72 h). There were no changes in body weight or food and water intake between the control and the treated groups.

3.1. Carrageenan-induced Paw Edema Test

Figure 1 showed dose dependent anti-inflammatory effect in the carrageenan-induced paw oedema model of inflammation. Higher dose (EOCS100 mg/kg) inhibited the edema by 22.37% during the first hour compared to 34.08% of positive control Diclofenac sodium 10mg/kg, (P<0.0001). In the second hour EOCS significantly

suppressed the inflammation by 73.91% compared to 74.88% of Diclofenac sodium (Table 1). In the third and fourth hours, the percentage of edema inhibition by EOCS 100mg/kg was 83.19% and 82.88% respectively in unison the positive control also inhibited the inflammation by 85.11% and 85.60% ($P < 0.0001$) respectively.

3.2. Formalin Test

The results of anti-inflammatory activity of the essential oil isolated from *Canarium strictum* against formalin induced inflammation model was given in Table-2. The dose dependent chronic anti-inflammatory action has been demonstrated by EOCS100

mg/kg produced the highest suppression of inflammation in all the 6 days observation. The activity of EOCS began from the first hour after formalin administration and persevered right through experiment in a dose-dependent manner and likewise, positive control dexamethasone also reduced edema significantly compared to the vehicle control. The oral administration of EOCS 100mg/kg emulsion diminished the inflammation most effectively ($P < 0.0001$). EOCS 10 mg/kg and 50 mg/kg also reduced paw edema significantly compared with the vehicle group (Table-2). Generally, the observed activity was more or less similar to the positive control Dexamethazone.

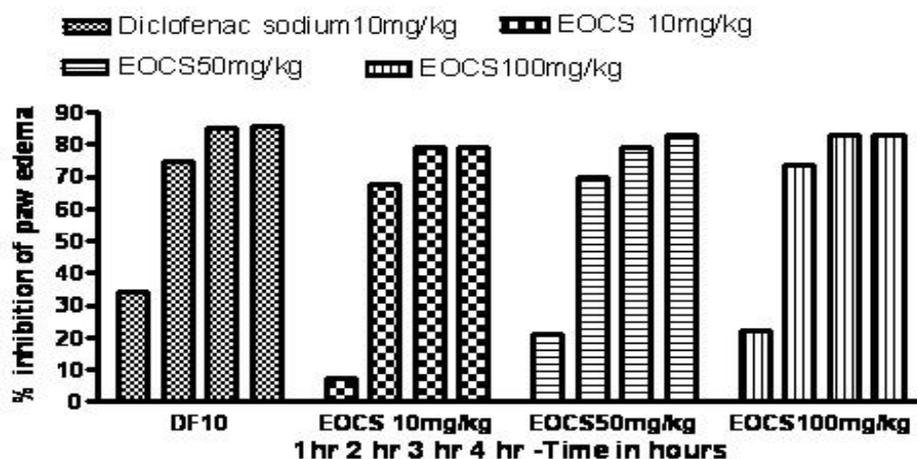


Figure 1. Inhibition of carrageenan induced paw edema.

Table 2. Anti-inflammatory activity of (EOCS) essential oil of *Canarium strictum* in formalin induced paw edema in mice.

Groups	0 Day	1 day	2 day	3 day	4 day	5 day	6 day	DPS 6 – 0 day	%PEI
Control	0.2500 ± 0.0428	0.3450 ± 0.0160	0.3533± 0.0105	0.3550± 0.0158	0.3450 ±0.0095	0.3400± 0.0096	0.3417± 0.0140	0.0917	
DMZ 10mg/kg	0.2333 ±0.042 1	0.2483 **±0.0 124	0.2433* *±0.011 7	0.2533* *±0.006 1	0.2367* * ±0.0120	0.2417** ±0.0153	0.2400* *±0.012 1	0.0067	92.69 %
EOCS 10mg/kg	0.2500 ±0.050 0	0.2933 ± 0.0168	0.2800* *±0.010 5	0.2717* *±0.010 1	0.2850* ±0.0180	0.2800* ±0.0216	0.2867* ±0.0178	0.0367	59.97 %
EOCS 50mg/kg	0.2167 ±0.030 7	0.2333* *±0.012 8	0.2367* *±0.018 8	0.2317* * ±0.0113	0.2383* * ±0.0119	0.2300** ±0.0093	0.2417* *±0.014 9	0.025	72.73 %
EOCS10 0mg/kg	0.2167 ±0.047 7	0.2450* *±0.011 4	0.2267* *± 0.0105	0.2383* *±0.015 7	0.2350* * ±0.0123	0.2433** ±0.0080	0.2350* *±0.011 1	0.0183	80.04 %

PEI percentage of inhibition, % = $(1-T/C) \times 100$, T = Variation of paw size of the mice after administration of formalin at different time intervals C= Control group DPS-Differences in paw size. Results expressed as mean ± S.E.M (N=6) *** P<0.0001; ** P<0.001; * P<0.05 compared to the vehicle treated group (ANOVA followed by Dunnett's test).

Most of the steroidal and non-steroidal anti-inflammatory drugs are presently used to treat acute inflammation, these drugs has not been fully successful in terms of treating chronic inflammatory disorders as such drugs are associated by unforeseen side effects [6 & 25]. This study designed to confirm the folkloric medicinal property of the black Dammer resin which is given for the treatment of rheumatism [5] when it is taken orally and applied topically. Moreover, the presence of terpenoidal compounds in black dammer resin is also responsible for imparting curative

effect of chronic pain. Therefore, we distilled the essential oil from oily resin that is collected from stem bark of *Canarium strictum*.

Carrageenan and formalin induced paw edema was effectively controlled in all the phases by the EOCS against in vivo animal models. After carrageenan administration, the resultant inflammation episode was categorized into first and second phase. During its first phase (0-2.5 hour) of the inflammation episode, there would be release of inflammatory mediators such as; histamine,

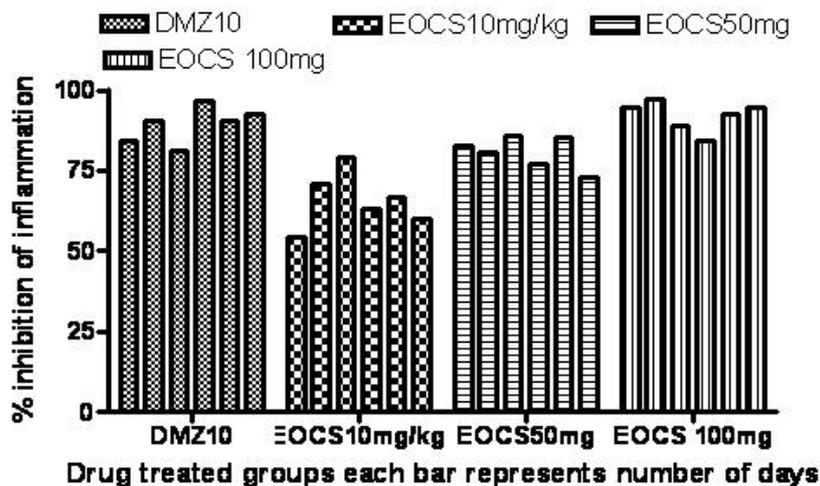


Figure 2. Inhibition of formalin induced paw edema.

serotonin and kinins up to 2.5 hours. The second phase is mediated by the release of leukotrienes, prostaglandins, proteases, and superoxide radicals from 2.5 hours to 6 hours [4&21]. In the same manner, there are two inflammation episode progressed during the formalin test. In the neurogenic first phase, there is a direct chemical stimulus occurring in the nociceptive afferent fibres, chiefly C fibres, and the release of substance P [1], During the inflammatory second phase, there would be release of inflammatory mediators such as prostaglandins, histamine, bradykinin, and serotonin, [12&19] at the inflamed affected area. The paw oedema was diminished by the EOCS in both tests indicating the inhibition of various inflammatory mediators.

In the chronic inflammatory model, inhibition of formalin-induced paw edema in animal models is considered as one of the most suitable test procedures to screen anti-inflammatory agents as it closely resembles

human arthritis [11]. Elevated level of TNF- α , IL-1, and IL-6 are mostly destructive and are also concerned with some of the pathologic responses that occur in chronic inflammatory diseases such as rheumatoid arthritis [3]. Inflammation has been well-known as a major risk factor for different human diseases. The chronic inflammatory episodes influence on the pathological progression of chronic illnesses, exemplified by infiltration of inflammatory cells. Chronic illnesses rheumatoid arthritis is considered as the most common inflammatory disease and is a major cause of disability [10]. Decrease of the chronic inflammatory response is a helpful approaches towards fight several human diseases.

4. Conclusion

This study undoubtedly confirmed that the essential oil of *Canarium strictum* Roxb., exhibited a potent dose dependent anti-inflammatory activity by inhibiting the

mediators of inflammation. The probable reason might be the presence of bioactive terpenoids in the essential oil. This is the first time we are reporting the anti-inflammatory activity of essential oil of black dammer resin. Hence, the current study had extents with a demonstration of antinociceptive activity. It was done to isolate and identify major terpenes presented in EOCS by GCMS and other spectral means.

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