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in vivo Anti-inflammatory Effect of Purified Aqueous and Methanol Extract of *Trachyspermum ammi* (Linn.) and *Dolichos biflorus* Linn. on Albino Rats

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ABSTRACT

Anti-inflammatory activity of *Trachyspermum ammi* (Linn.) and *Dolichos biflorus* Linn seed extract has been evaluated using an animal model. Anti-inflammatory activity of the extracts was measured by carrageenan-induced rat paw edema method considering diclofenac 1 mg/kg as standard drug (positive control). This study was carried out using a dose of 200, 400 and 600 mg/kg aqueous and methanol extract of both samples orally and edema volume was measured at 1, 2 and 3 h after incorporation of carrageenan by digital plethysmometer. The investigation supports the traditional use of these seeds as an anti-inflammatory agent as well as the presence of biological active compounds.

KEYWORDS

Trachyspermum ammi (Linn.), *Dolichos biflorus* Linn, Anti-inflammatory agent, Carrageenan-induced rat paw edema.

INTRODUCTION

Inflammation is a disorder which involves increase in the number of leukocytes and a variety of complex mediator molecules locally [1] and is a major component of the damage caused by autoimmune diseases [2] as well as the key contributor to pathologies such as cancer, diabetes and cardiovascular diseases [3]. Use of herbal extracts for treatment of inflammatory diseases is well documented in Ayurveda, the ancient medicinal system of India [4]. The biosynthesis of them has also been implicated in the pathophysiology of several cardiovascular diseases, cancer, colonic adenomas and Alzheimer's disease [5]. The usual characteristics of inflammation are pain, swelling, edema, redness and heat. The treatment of inflammatory disease particularly remains a significant research area because the existing therapeutic drugs are not sufficiently capable or their side effects are painful and all the more of long-time therapy [6-8]. Traditional non-steroidal anti-inflammatory drugs (NSAIDs) block the COX pathway that contributes to cell mediated prostaglandin (PGE2) production [9,10]. Several modern drugs are used to treat inflammatory disorders, but their prolonged use may cause severe adverse side effects. Thus there is a common need to develop new anti-inflammatory agents with minimum side effects [11,12].

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Trachyspermum ammi is a polyherbal Ayurvedic medicine, traditionally used as a carminative, antispasmodic and in sciatica. The anti-inflammatory activity of *Trachyspermum ammi* has previously been studied and showed significant effect [13]. The seed of *Dolichos biflorus* Linn. is used in the treatment of pain, constipation, wounds, urinary calculi, cough, edema, asthma, *etc*. [14]. Seed is also beneficial in the treatment of enlarged liver and spleen. The seeds of *Dolichos biflorus* Linn. have been reported to show anti-inflammatory, antioxidant, antihepatotoxic and hypolipidemic activity [15,16]. In India use of *T. ammi* and *D. biflorus* Linn are a part of our diet due to their rich nutritional and medicinal values. Both the samples exhibited potential anti-inflammatory activity both *in vitro* and *in vivo*. However, no scientific data on anti-inflammatory activity of the purified extracts are available. The present study evaluated the potential use of aqueous extracts of *T. ammi* and *D. biflorus* Linn to treat acute inflammatory toxicity. Therefore, this study was designed to screen *in vivo* anti-inflammatory activity of purified aqueous and methanol extract of *Trachyspermum ammi* and *Dolichos biflorus* Linn in animal model of acute inflammation.

EXPERIMENTAL

Carrageenan was purchased from Himedia Labs, India. Throughout the experiments milli Q water was used. All chemicals were commercially purchased and were of AR grade and used without any further purification.

Animals: Adult albino rats (Sprague- Dawley strain) of both sexes weighing between 200-250 g were used for the experiment. The animals were kept in standard conditions with 12 h light and dark cycle. They were allowed to take foods in the form of dry pellet and water.

Sample collection and extraction: The seed of *Trachyspermum ammi* and *Dolichos biflorus* were collected from market, identified and washed with water and dried in air. The samples were kept in both aqueous and methanol solution for 48 h at room temperature (25 °C) at stirring condition. The crude aqueous and methanol extract was collected by centrifugation at 3000 rpm for 15 min. Then the extract was filtered and was lyophilized. Extract was then stored under refrigeration at 4 °C for further studies.

The extracts were purified using gel permeation chromatography. Samples were denoted as TAE: *Trachyspermum ammi* purified aqueous extract, TME: *Trachyspermum ammi* purified methanol extract, DAE: *Dolichos biflorus* purified aqueous extract, DME: *Dolichos biflorus* purified methanol extract.

Anti-inflammatory activity by Carrageenan induced rat paw edema method: Anti-inflammatory activity was assessed by Carrageenan induced rat paw edema method [17]. Albino rats of either sex weighing 200-250 g were divided in 5 groups (N = 6). Group-I received 0.9 % NaCl solution (control), Group-II, III and IV received extracts (200, 400, 600 mg/kg, P.O. respectively) of sample. Group-V received diclofenac (reference standard 1 mg/kg, P.O) [18]. Animals were treated with drugs by oral route and subsequently 1 h after treatment; 0.1 mL of 1 % suspension of carrageenan in normal saline was injected into the sub planter region of left hind paw to induce edema. The paw volume was measured initially at 0, 1, 2 and 3 h after carrageenan injection using digital plethysmometer. The difference between the initial and subsequent values gave the actual edema volume which was compared with control [17,19].

Measurement instrument: A digital plethysmometer was used for the measurement of edema volume. This method is easy to perform and produce accurate results. In this method the paw must be kept from contact with the wall of the plethysmometer cylinder.

Digital plethysmometer is designed to evaluate the changes in paw volume and is useful to study inflammatory response in lab animals. The volume transducer is formed by to Perspex tubes interconnected and filled with a conductive solution and a platinum electrode in each chamber. Water displacement produced by the immersion of the animal's paw in the measuring tube is reflected in the second tube, inducing a change in the conductance between the two electrodes. The control unit detects the change in conductance and generates an output signal to the display indicating the volume displacement measured in 0.01 mL resolution.

Statistical analysis: Data were tabulated and analyzed using the IBM SPSS version 22 software. Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The effect of TAE and TME on carrageenan induced paw edema in rats which is evident in Table-1 and % of inhibition is shown in Table-2. On the other hand the effect of DAE and DME is depicted in Table-3 and % of inhibition in Table-4. It

TABLE-1
EFFECT OF TAE AND TME ON CARRAGEENAN INDUCED PAW EDEMA IN RATS

Treatment groups (N = 6)	Dose (mg/kg BW)	Edema volume (mL)			
		0 h	1 h	2 h	3 h
Normal saline	10 mL/kg	2.86 ± 0.218	3.50 ± 0.15	3.60 ± 0.21	3.67 ±0.20
Diclofenac	1 mg/kg	2.63 ± 0.21[a]	2.55 ± 0.19[a]	2.40±0.19[a]	2.28±0.18[a]
TAE	200	2.76±0.138[b]	2.71±0.14[a]	2.65 ± 0.14[a]	2.60 ± 0.126[a]
	400	2.66 ± 0.08[b]	2.58 ± 0.06[a]	2.37 ± 0.144[a]	2.29 ± 0.17[a]
	600	2.58 ± 0.284[a]	2.28 ± 0.254[a]	2.03 ± 0.194[a]	1.91 ± 0.07[a]
TME	200	2.78±0.234[a]	2.59±0.16[a]	2.68 ± 0.24[a]	2.62 ± 0.122[a]
	400	2.60 ± 0.13[b]	2.54 ± 0.19[a]	2.41 ± 0.12[a]	2.32 ± 0.19[a]
	600	2.56 ± 0.276[a]	2.31 ± 0.21[a]	2.08 ± 0.26[a]	1.95 ± 0.106[a]

[Each value is in mean ± 2SD (N = 6 rats), [a]P < 0.01, [b]P < 0.05; One way ANOVA followed by Dunnett's multiple comparison tests].

TABLE-3
EFFECT OF DAE AND DME ON CARRAGEENAN INDUCED PAW EDEMA IN RATS

Treatment groups (N = 6)	Dose (mg/kg BW)	Edema volume (mL)			
		0 h	1 h	2 h	3 h
Normal saline	10 mL/kg	2.97 ± 0.194	3.50 ± 0.15	3.60 ± 0.21	3.67 ±0.20
Diclofenac	1 mg/kg	2.65 ± 0.16[a]	2.59 ± 0.2 [a]	2.45±0.14[a]	2.33±0.16[a]
DAE	200	2.82±0.18[b]	2.78±0.13 [a]	2.74 ± 0.13 [a]	2.72 ± 0.12 [a]
	400	2.85 ± 0.08[b]	2.79 ± 0.137 [a]	2.72 ± 0.144 [a]	2.60 ± 0.15 [a]
	600	2.69 ± 0.134[a]	2.39 ± 0.166 [a]	2.09 ± 0.166 [a]	1.85 ± 0.184 [a]
DME	200	2.77±0.208[b]	2.693±0.076 [a]	2.68 ± 0.19 [a]	2.72 ± 0.122 [a]
	400	2.78 ± 0.122[b]	2.69 ± 0.068 [a]	2.65 ± 0.102 [a]	2.60 ± 0.15 [a]
	600	2.64 ± 0.144[a]	2.34 ± 0.214 [a]	2.06 ± 0.226 [a]	1.81 ± 0.106 [a]

[Each value is in mean ± 2SD (N = 6 rats), [a]P < 0.01, [b]P < 0.05; One way ANOVA followed by Dunnett's multiple comparison tests].

TABLE-2
PERCENTAGE INHIBITION OF PAW EDEMA EXHIBITED BY TAE AND TME

Treatment groups	Dose (mg/kg BW)	Inhibition (%)			
		0 h	1 h	2 h	3 h
TAE	200	3.5	22	26	29
	400	6.9	26	34	37
	600	9.7	34	43	47
TME	200	2.7	26	25	28
	400	9	27	33	36
	600	10	34	42	46

TABLE-4
PERCENTAGE INHIBITION OF PAW EDEMA EXHIBITED BY DAE AND DME

Treatment groups	Dose (mg/kg	Inhibition (%)			
		0 h	1 h	2 h	3 h
DAE	200	5	20	23	25
	400	4	20	24	29
	600	9	31	43	49
DME	200	6.7	23	25	26
	400	6.3	23	26	29
	600	11	33	42	50

showed significant anti-inflammatory activity at doses 200, 400 and 600 mg/kg compared to the control group (Fig. 1).

The most commonly used primary experiment to screen herbal products for their anti-inflammatory activities to measure the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent. Carrageenan induced rat paw edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the inflamed tissue surroundings. The significant anti-inflammatory activity shown by the extracts (200, 400 and 600 mg/kg) over a period of 3 h in the animal model was quite similar to the result exhibited by the group treated with the standard drug *i.e.* diclofenac sodium. The highest percentage inhibition activity was found in the dose of 600 mg/kg with the mean percentage inhibition of 47 and 46 % for TAE and TME respectively, 50 and 49 % for DAE and DME after 3 h of administration. The anti-inflammatory activity of methanol extract was less than that of the aqueous extract. This was obtained in both cases. These results indicated that the extract acts in later phases in dose dependent manner, probably involving arachidonic acid metabolites, which

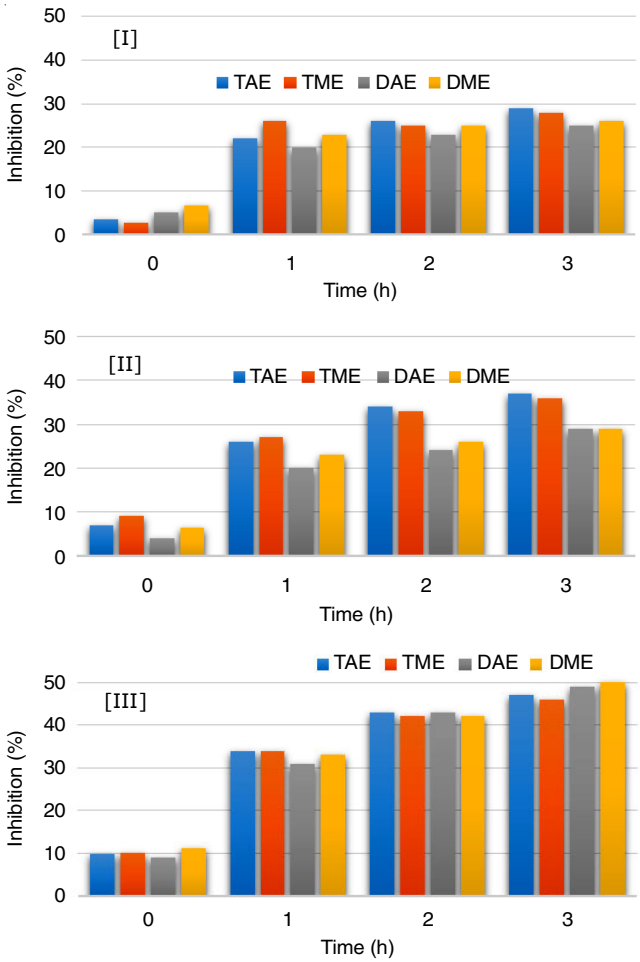

Fig. 1. Percentage inhibition of paw edema exhibited at different doses [I] 200 mg/kg [II] 400 mg/kg and [III] 600 mg/kg

produced an edema dependent on neutrophils mobilization. Phenolic compounds present in this extracts probably have played the key role in its anti-inflammatory activity observed [20,21].

Percentage inhibition of paw edema was measured by the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Mean increase in paw volume of control} - \text{Mean increase in paw volume of test group}}{\text{Mean increase in paw volume of control}} \times 100$$

Conclusion

It may be concluded from the results observed in the experiment that the purified aqueous and methanol extracts of *Trachyspermum ammi* and *Dolichos biflorus*have good anti-inflammatory activity in dose dependent manner. The results support the conventional use of these seeds in inflammatory conditions and suggest the presence of biologically active compounds. Further investigations should be carried out to explore the biologically active compounds present in it.

Ethical approval: All experiments were conducted as per the guidelines of the Animal Ethics Committee CPCSEA [Ethics committee approval memo no. PR-HC/6-119/2895(8)] according to Government of India accepted principles for laboratory animal use.

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