

## COMPARATIVE EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF MEDICINALLY IMPORTANT PLANTS

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### ABSTRACT

**Objective:** Selected medicinal plants are either singly or in combination have been used traditionally for the management of variety of conditions especially against inflammatory disorders. They include *Aegle marmelos* (L.) Corr., *Desmodium gangeticum* (L.) DC., *Gmelina arborea* Roxb., *Oroxylum indicum* Vent., *Premna optusifolia* R. Br., *Solanum anguivi* Lam., *Solanum virginianum* L., *Stereospermum colais* Mabb. *Tribulus terrestris* L. and *Uraria picta* (Jacq.) Desv. ex DC. They are the constituents of *Ayurvedic Dashamoola* which is a combination of roots of ten plants, owing to a unique combination of ten plants that play specific role in treatment of different conditions. Few of them are known to possess anti-inflammatory activity individually but comparative study of these plants has never been carried out, therefore we have comparatively investigated these plants for their anti-inflammatory activity.

**Method:** Water decoctions of roots were administered orally using *in vivo* Carrageenan induced rat paw edema model.

**Result:** Plants viz. *Aegle marmelos* (28.20%), *Premna optusifolia* (25.78%), *Oroxylum indicum* (24.15%), *Desmodium gangeticum* (26.74%), *Uraria picta* (21.49%) exhibited highest anti-inflammatory activity at a dose of 1.8 ml/kg b.w.

**Conclusion:** plants possessing maximum anti-inflammatory activity can be used more effectively to cure inflammatory ailments. They might be responsible for anti-inflammatory activity of *Dashamoola* formulation.

**Keywords:** Medicinal plants, Comparative efficacy, Anti-inflammatory activity

### INTRODUCTION

Inflammatory process is known to play a major role in most chronic disorders including neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune etc. Various current treatments including steroids and Non Steroidal Anti-inflammatory Drugs (NSAIDs) are clinically important therapeutic agents being used for inflammatory disease treatments. Prolonged use of such drugs causes undesirable and severe side effects. Hence, search for other alternative therapies for both acute and chronic inflammatory diseases is necessary. The use of natural remedies has a long traditional history with minimum or no side effects. Since ages, there is exclusive use of plant drugs in traditional medicines as they represent a large source of natural medicines. They are also known to play a crucial role in management of various inflammatory diseases; therefore, naturally originated agents with such medicinal potential are enviable to surrogate the use of chemical therapeutics [1].

According to the Ayurvedic Pharmacopoeia of India (1990) [2], *Dashamoola* is one of the folk medicine, a polyherbal formulation believed to have the potential for providing relief from inflammation. This formulation is prepared from roots of 10 different plants that are divided into two categories: *Brihatpanchamoola* category includes 5 tree species viz., *Aegle marmelos* (L.) Corr., *Premna optusifolia* R. Br., *Gmelina arborea* Roxb., *Oroxylum indicum* Vent. and *Stereospermum colais* Mabb.; *Laghupanchamoola* category includes 5 shrub species namely, *Desmodium gangeticum* (L.) DC., *Solanum anguivi* Lam., *Solanum virginianum* L., *Tribulus terrestris* L. and *Uraria picta* (Jacq.) Desv. ex DC. Roots of *Dashamoola* plants have always been an integral part of treatment of various ailments in *Ayurveda* as well as in diverse communities across India either singly or in combination. Pawra tribes from Satpura hills of Maharashtra routinely use these individual plants to treat the inflammatory condition [3]. Some of important *Ayurvedic* and *ethnomedicinal* uses of these plants are listed in Table 1 [4-36]. Among the various dosage forms prescribed, water decoction is used widely to treat inflammatory conditions [3].

Despite the wide traditional use in ethnobotany and *Ayurveda*, there are a very few reports in literature on the anti-inflammatory activity

of roots of these plants. Different extracts of roots of plants viz. *A. marmelos* [37], *P. integrifolia* (Syn. *P. optusifolia*) [38], *D. gangeticum* [39, 40], *O. indicum* [41] and *U. picta* [42] have exhibited anti-inflammatory potential in various inflammatory models. Although, there are such modern efforts taken to investigate anti-inflammatory potential of these plants, by far the comparative anti-inflammatory potential of roots of these selected plants along with their combination has not yet been investigated. Therefore, the present study was designed to investigate and compare anti-inflammatory potential of roots of selected ten plants and *Dashamoola* as combination using carrageenan induced rat paw edema as an experimental model of inflammation and to rationalize their therapeutic use.

### MATERIALS AND METHODS

#### Chemicals

The following drug and chemicals were used: Carrageenan (Himedia, India), Diclofenac sodium (Reactin-100 SR, Cipla, India), 0.9% Saline (Sipra Remedies Pvt. Ltd., India), Carboxy methyl cellulose (CMC) (Merck, India). All other chemicals used in this study were of Analytical grade.

#### Plant material

The fresh roots of all ten plants were collected from their natural habitat. The plant material was taxonomically identified, authenticated and herbaria were deposited in the herbarium of Medicinal Plants Conservation Centre, Pune (MPCC) as mentioned in Table 2.

The roots were shade dried, cut into small pieces and pulverised using mechanical grinder. Powder was passed through 80-mesh sieve and stored in an air-tight container for further use.

#### Plant extraction

Water decoctions of roots were prepared as per the standard procedure mentioned in Ayurvedic Formulary of India (AFI) [4], by taking 1 part of the root powder in 16 parts of water. It was then boiled till 1/8<sup>th</sup> of its volume and filtered through muslin cloth. Same

method was followed for preparation of water decoction of *Dashamoola* (root powder of all ten plants mixed in 1:1 proportion).

### Experimental animals

Adult Wistar albino rats of either sex, weighing 180-200 g, were obtained from National Institute of Biosciences, Pune, India. They were kept under standard husbandary conditions and provided with food and water *ad libitum*. Approval from institutional animal ethical committee was obtained (Ref no. BVDUMC/1960/2011-2012) before initiation of the experiment. All the procedures were followed as per the CPCSEA norms.

### Dose fixation

Dose was calculated by extrapolating the human dose of water decoction (20 ml) as per the posology of water decoction [43] to animal dose (1.8 ml/kg b.w.) based on the body surface area ratio by referring to the table of Paget and Barnes [44, 45].

### Anti-inflammatory study using carrageenan induced rat paw edema model

Carrageenan induced rat paw edema model was used for comparative anti-inflammatory potential [46]. Seventy eight rats were randomly divided into 13 groups (n=6) including Control, Diclofenac sodium and eleven test groups viz. *A. marmelos*, *D. gangeticum*, *G. arborea*, *O. indicum*, *P. optusifolia*, *S. anguivi*, *S. virginianum*, *S. colais*, *T. terrestris*, *U. picta* and *Dashamoola* respectively. Control group received 1% carrageenan in 0.9% saline (0.1 ml). Diclofenac sodium at 10 mg/kg b.w. and test samples at 1.8 ml/kg b.w. dose was orally administered to respective groups 1 hr before the injection with 0.1 ml 1% Carrageenan on the plantar side of right hind paws of the rats. The paw volume was measured after 3<sup>rd</sup> and 6<sup>th</sup> hr interval of carrageenan injection using plethysmometer (Orchid Scientifics, Model PLM 01 PLUS). Results were expressed as the paw volume (ml) and percentage inhibition of edema.

Percentage inhibition of edema was calculated as per the formula:

$$\text{Percentage inhibition of edema} = (V_c - V_t) / V_c * 100$$

Where,

V<sub>c</sub> = Paw volume of control group animal

V<sub>t</sub> = Paw volume of treatment group animal

### Statistical analysis

Results are expressed as mean±SD. Statistical evaluations were made using Two-way ANOVA and values were considered significantly different when P ≤ 0.05 by using Boneferroni post-test. The statistical analysis was done using GraphPad Prism (V5.0).

## RESULTS

Selected plants on Carrageenan induced paw edema in rats showed varied anti-inflammatory activity in comparison with Diclofenac sodium, an anti-inflammatory drug used to reduce acute inflammatory response. As shown in Table 3, Diclofenac sodium (10 mg/kg) reduced the edema volumes about 30.07% and 44.95% in comparison to the control group during the 3<sup>rd</sup> and 6<sup>th</sup> hr of Carrageenan treatment respectively. Compared to the control, oral administration with water decoction plants and their combination as *Dashamoola* at a dose of 1.8 ml/kg b.w. p.o. reduced (P < 0.05) the paw edema. The edema development was observed to be significantly reduced in groups treated with *A. marmelos*, *D. gangeticum*, *O. indicum*, *P. optusifolia*, *U. picta* and *Dashamoola* at both 3<sup>rd</sup> and 6<sup>th</sup> hr after carrageenan injection. The levels of edema volume were reduced by about 24.62%, 15.54%, 10.94%, 9.80%, 9.52% and 9.21% at 3<sup>rd</sup> hr by *Dashamoola*, *A. marmelos*, *P. optusifolia*, *D. gangeticum*, *O. indicum* and *U. picta* treated groups respectively. While at 6<sup>th</sup> hr in *Dashamoola*, *A. marmelos*, *D. gangeticum*, *G. arborea*, *P. optusifolia*, *O. indicum*, *U. picta*, *G. arborea* and *S. colais* treated groups, the edema volume was significantly reduced by about 40.47%, 28.20%, 26.74%, 25.78%, 24.15%, 21.49%, 14.09%, 10.81% and 7.59% respectively. These data imply that all plants (except *S. anguivi* and *S. virginianum*) along with *Dashamoola* exhibit significant anti-inflammatory activity as indicated by significant decrease in paw volume at 3<sup>rd</sup> and 6<sup>th</sup> hr after carrageenan injection.

## DISCUSSION

Carrageenan induced paw edema is the most suitable and well established model used to evaluate the anti-inflammatory effect of natural products on acute inflammation. The carrageenan induced rat paw edema is a biphasic process where the first phase is attributed to release of histamine and serotonin retaining upto 1<sup>st</sup> hr after carrageenan injection, while the second phase is due to prostaglandins which proceeds through 4<sup>th</sup> hr. The plateau phase between first and second phase is maintained by kinin like substances upto 3<sup>rd</sup> hr [38, 47, 48].

In the present investigation, Diclofenac sodium, an anti-inflammatory drug was used as a standard to reduce acute inflammatory response in terms of swelling. In comparison with control group, treatment with *G. arborea*, *S. colais* and *T. terrestris* reduced swelling significantly at 6<sup>th</sup> hr after carrageenan injection. This suggests their effect on inflammation in the second phase of carrageenan induced inflammation. In addition to this, *A. marmelos*, *D. gangeticum*, *O. indicum*, *P. optusifolia*, *U. picta* and *Dashamoola* significantly reduced paw swelling at both 3<sup>rd</sup> and 6<sup>th</sup> hr (Figure 1) after carrageenan injection. However, *S. anguivi* and *S. virginianum* had no effects on inflammation (Table 3).

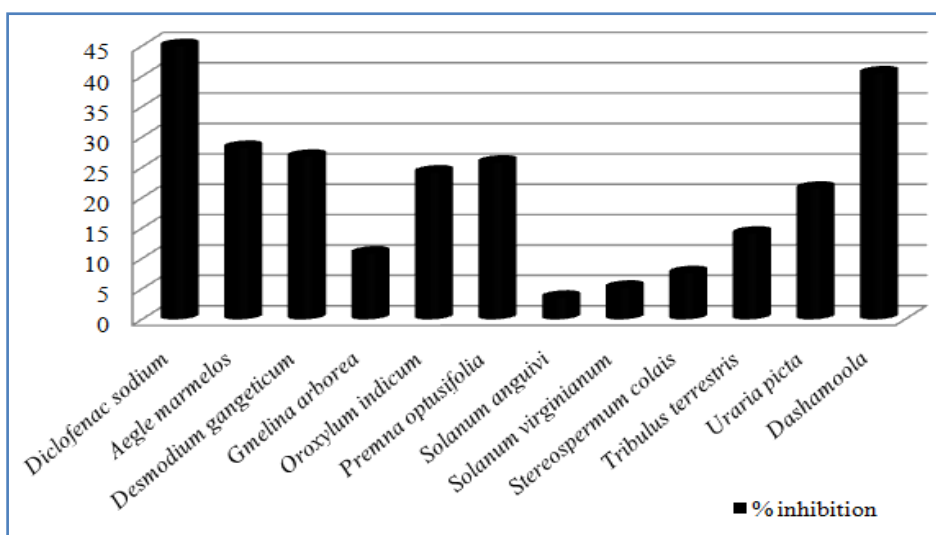


Fig. 1: It shows anti-inflammatory activity in percentage inhibition shown by selected plants and formulation at 6<sup>th</sup> hr.

**Table 1: It shows Ayurvedic and ethnomedicinal uses of roots of ten plants of Dashamoola**

Plant name	Ayurvedic uses	Ethnomedicinal uses
<i>Aegle marmelos</i> (L.) Corr.	Colic, Diseases of nervous system, Dyspepsia, Edema, Emesis, Rheumatism and Vomiting [4]	Diarrhoea, Gastric troubles, Intermittent fever, Snakebite and Vomiting [5-8]
<i>Desmodium gangeticum</i> (L.) DC.	Amenorrhoea, Diseases of eye, Fever, Piles, Pthysis, Tuberculosis, Vomitin and, Worm infestation [4]	As antidote in snake bite, Asthma bronchitis, Cough, Diarrhea, Dysentery, Fever, Mouth ulcer, Rheumatism, Sedative agent and medicine for abortion, To cure premature ejaculation, Toothache, Typhoid and Vomiting [9-15]
<i>Gmelina arborea</i> Roxb.	Burning sensation, Edema, Excessive thirst, Fever, Piles and Polydipsia [4]	As antidote in scorpion sting, For urinary discharges and strangury, Piles, Washing and healing of septic wounds and Wound healing [9, 16-19]
<i>Oroxylum indicum</i> Vent.	Cough, Diseases of Abdomen, Diseases of nervous system, Ear diseases, Edema, Loss of movement of leg, Rheumatism and Stillness [4]	As antidote and abortifacient, Burning maturation, Diarrhoea, Dysentery, Jaundice, Rheumatism and Stomach trouble [9, 20-22]
<i>Premna optusifolia</i> R. Br.	Bronchitis, Constipation, Chyluria, Diabetes, Disorder, Dyspepsia, Fever, Gonorrhoea, Headach, Inflammation, Liver, Piles and Swelling [23]	Bodypain, Cough and Snakebite [24, 25]
<i>Solanum anguivi</i> Lam.	Colic, Diseases of Heart, Dyspepsia and Fever [4]	Anthrax, Asthma, Blackleg, Bronchitis, Cough, Haematuria, Jaundice, Leprosy and Toothache [26-31]
<i>Solanum virginianum</i> L.	Cough, Fever, Hoarseness, Ozaena, Pleurodyria and Intercostals neuralgia [4]	Asthma, Chest pain, Cough, Gum pain, Small pox and Tooth-ache [11, 28, 32, 33]
<i>Stereospermum chelonooides</i> (L.f.) DC.	Burns, Edema, Hiccough, Hyperacidity, Piles and Vomiting [4]	For curing nervous disorders and to regularize menstrual disorders [9, 20]
<i>Tribulus terrestris</i> L.	Cough, Diseases of Heart and Dysuria [4]	For male weakness, expelling of kidney stones, prevention of white discharge in women, in strangury and to treat urinary troubles [14, 17, 31, 34, 35]
<i>Uraria picta</i> (Jacq.) Desv. ex DC.	Acute diarrhea, Blepharophimosis, Blood dysentery, Bone fracture, Burning sensation, Cough, Fever, Gout, Insanity, Ulcer Vomiting [4]	As abortifacient, Dysentery, Snake bite and Sore mouth [10, 33, 36]

**Table 2: It shows Dashamoola ingredients**

Scientific name	Sanskrit name	Family	Herbarium number
<i>Aegle marmelos</i> (L.) Corr.	<i>Bilva</i>	Rutaceae	MPCC 3591
<i>Desmodium gangeticum</i> (L.) DC.	<i>Shalparni</i>	Fabaceae	MPCC 146
<i>Gmelina arborea</i> Roxb.	<i>Gambhari</i>	Verbenaceae	MPCC 783
<i>Oroxylum indicum</i> Vent.	<i>Shyonak</i>	Bignoniaceae	MPCC 3592
<i>Premna optusifolia</i> R. Br.	<i>Agnimantha</i>	Verbenaceae	MPCC 2593
<i>Solanum anguivi</i> Lam.	<i>Bruhati</i>	Solanaceae	MPCC 2627
<i>Solanum virginianum</i> L.	<i>Laghukantakari</i>	Solanaceae	MPCC 2762
<i>Stereospermum colais</i> Mabb.	<i>Patala</i>	Bignoniaceae	MPCC 90
<i>Tribulus terrestris</i> L.	<i>Gokshur</i>	Zygophyllaceae	MPCC 1097
<i>Uraria picta</i> (Jacq.) Desv. ex DC.	<i>Prishniparni</i>	Fabaceae	MPCC 2645

**Table 3: It shows effect of water decoction of Dashamoola plants and Dashamoola formulation on inflammation in carrageenan induced rat paw edema**

Group (n=6)	3 <sup>rd</sup> hr		6 <sup>th</sup> hr	
	Paw volume (ml) (Mean ± SD)	% inhibition	Paw volume (ml) (Mean ± SD)	% inhibition
Control	1.63±0.02	-	1.82±0.02	-
Diclofenac sodium	1.14±0.04***	30.07	1.00±0.02***	44.95
<i>Aegle marmelos</i>	1.38±0.05***	15.54	1.31±0.03***	28.20
<i>Desmodium gangeticum</i>	1.47±0.04***	9.80	1.33±0.05***	26.74
<i>Gmelina arborea</i>	1.66±0.02 <sup>ns</sup>	-1.55	1.62±0.04***	10.81
<i>Oroxylum indicum</i>	1.48±0.11***	9.52	1.38±0.07***	24.15
<i>Premna optusifolia</i>	1.45±0.04***	10.94	1.35±0.13***	25.78
<i>Solanum anguivi</i>	1.60±0.09 <sup>ns</sup>	1.85	1.76±0.07 <sup>ns</sup>	3.54
<i>Solanum virginianum</i>	1.62±0.03 <sup>ns</sup>	0.69	1.73±0.07 <sup>ns</sup>	5.19
<i>Stereospermum colais</i>	1.63±0.05 <sup>ns</sup>	0.21	1.68±0.06*	7.59
<i>Tribulus terrestris</i>	1.59±0.05 <sup>ns</sup>	2.26	1.56±0.16***	14.09
<i>Uraria picta</i>	1.48±0.04***	9.21	1.43±0.07***	21.49
<i>Dashamoola</i>	1.23±0.04***	24.62	1.08±0.02***	40.47

Values are Mean± SD (n=6); Means were compared by using Bonferroni post-test; \*P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*P ≤ 0.001, \*\*\*\*P ≤ 0.0001.

Among all selected test drugs, anti-inflammatory effect of *Dashamoola* combination (40.47%) was close to that of Diclofenac sodium (44.95%) at 6<sup>th</sup> hr (Table 3, Figure 1). This indicates that, although individual plants of *Dashamoola* possess anti-inflammatory

activities, *Dashamoola* combination may prove to be the best anti-inflammatory agent in acute inflammatory processes which may be attributed to the synergistic effects of the components of the formulation.

Few of the plants have already proven their anti-inflammatory potential. The extracts of roots of *A. marmelos* has been shown to have anti-diarrhoeal potential in castor oil induced diarrhoea model [49] and a significant anti-inflammatory potential in Carrageenan induced paw edema and Cotton pellet induced granuloma model [37]. Methanol extract of roots of *P. integrifolia* (Syn. *P. optusifolia*) have shown to possess anti-inflammatory potential in carrageenan-induced rat paw edema and acetic acid-induced mouse vascular permeation [38].

Ethanol extract of roots of *D. gangeticum* have shown anti-ulcer activity in gastric ulcer induced by cold restraint, aspirin, pyloric ligation and alcohol models [39]. Water decoctions of its roots and aerial parts have shown anti-inflammatory potential in carrageenan induced paw edema and cotton pellet granuloma models [39, 40]. *Gangetin* from its root has been shown to possess anti-inflammatory potential in carrageenan-induced rat paw edema, cotton pellet granuloma and granuloma pouch models [50]. Flavonoid and alkaloid fractions of *D. gangeticum* have proven anti-inflammatory potential in carrageenan induced paw edema [51].

According to Zaveri and Jain, petroleum ether, chloroform, ethyl acetate and n-butanol extracts of roots of *O. indicum* exhibit anti-inflammatory action in carrageenan-induced rat paw edema model, cotton pellet-induced granuloma formation in rats and tail flick method in rats [41]. Its 50% alcoholic extract has anti-ulcer activity in ethanol-induced gastric ulcer model [52]. Ethanol extract of whole plant of *U. picta* was studied using bradykinin and histamine induced rat paw edema and carrageenan induced inflammation for anti-inflammatory potential [42]. Water extract of root bark of *S. suaveolens* (Syn. *S. colais*) have shown to possess anti-inflammatory potential in carrageenan-induced rat paw edema [53].

These investigations are in accordance with the present results. Although, basic components of the formulation possess varying anti-inflammatory potential, the combination of the plants indicates enhanced anti-inflammatory potential. These observed effects might be due effects of different phytochemical constituents of ingredient plants and specific combinations and synergistic/additive in formulation. Results of present investigation support the rationale for use of individual plants as well as for having diverse formulations with combination of different plants [54] which will give a unique advantage of enhanced desired activity.

## CONCLUSIONS

In conclusion, our data suggest that *Aegle marmelos*, *Premna optusifolia*, *Oroxylum indicum*, *Desmodium gangeticum* and *Uraria picta* significantly reduced inflammation. Hence, it can be assumed that they possess significant anti-inflammatory activity and might be responsible for anti-inflammatory activity of *Dashamoola* formulation. This study rationalizes the folk use of roots of investigated plants either singly or in formulation against inflammation. Further studies to develop novel formulation with significant anti-inflammatory potential can be undertaken.

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