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# Assessment of anti-inflammatory potential of *Sesbania bispinosa* Linn. leaf extracts and fractions by acute and chronic models

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**KEYWORDS**

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**Abstract** *Aim and objectives:* Leaf extracts and fractions of *S. bispinosa* were evaluated for anti-inflammatory activity in mice using acute and chronic anti-inflammatory models with aspirin as a reference drug.
*Materials and methods:* Methanol, chloroform and hexane were used to prepare leaf extracts by soxhlet extraction method, while acetone, ethyl acetate and petroleum ether were used to prepare fractions of most active extract. These extract and fractions were evaluated by using carrageenan and formalin-induced inflammation models in albino mice. Extracts and fractions at the dose 250 mg/kg/p.o. and aspirin at 100 mg/kg/p.o. were used for the study.
*Results:* Methanolic extract of *S. bispinosa* (MELSB) revealed significant anti-inflammatory activity which is indicated by 15.22% inhibition of paw edema at 180 min in carrageenan model, while ethyl acetate fraction of methanolic extract showed 16.26% and 15.50% inhibition of paw edema and thickness in carrageenan and formalin models respectively. In chronic model, just like aspirin, time-dependent activity was observed with acetone and ethyl acetate fractions; the greater reduction in paw thickness was observed with ethyl acetate fraction ($^{**}p < 0.01$).
*Conclusion:* The results of the present study suggest that leaves of *S. bispinosa* possess significant level of anti-inflammatory activity and ethyl acetate fraction may be further explored as an anti-inflammatory remedy as it was found to possess higher anti-inflammatory activity among all extracts and fractions as demonstrated in both acute and chronic models.


## 1. Introduction

Inflammation is a normal, protective response of living tissues against stimulus. First of all Aurelius Cornelius explained clinical features and hallmarks of inflammation.[1,2] Apart from the type of stimuli, restoring normal homeostasis is initiated by

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complex physiological process of innate immunity, mediated by pro-inflammatory cells (leukocytes, neutrophils, macrophages & mast cells, etc.) which secrete a number of inflammatory mediators (cyclooxygenase-1, cyclooxygenase-2, histamine etc.), activate enzymes to eliminate the initial cause of cell injury, & remove necrotic cells that causes inflammation.[3–6]

Inhibition of the therapeutic targets (cyclooxygenase-1, cyclooxygenase-2, histamine receptor, etc.) is a potential strategy to control inflammation, and is concerned with the mechanism of action of gluco-corticoids and non-steroidal anti-inflammatory drugs (NSAIDs). Majority of the currently available anti-inflammatory drugs are associated with gastrointestinal, renal system, cardiovascular and other risks.[7–11]

Apart from the synthetic drugs, the number of plants has significant potential to inhibit inflammatory diseases because, it contains a multitude of different molecules that act synergistically on targets of the complex physiological pathways and have found widely used because of several reasons such as awareness about plant-based remedies, the lower cost of phyto-therapy, the fear about the possible side effects of allopathic medicine, phyto-constituents are having low animal and human toxicity and have a higher bioavailability and therefore higher protective efficacy than synthetic drugs.[12–16] So, it is need of time to invent and evaluate more and more herbal drugs with high therapeutic activity, bioavailability and less toxicity.

*Sesbania bispinosa* Linn., (Fabaceae) is wild shrub and also cultivated throughout India as its leaves are used as fodder especially for sheep & goats in summer seasons[17] and leaves are good source of protein supplementation with high quality nutrients. The various parts of *S. bispinosa* showed the presence of phyto-constituents such as; flavonols, tannins, saponins, phytosterols, anthocyanins, alpha-ketoglutaric acid, oxaloacetic acid, and pyruvic acid [18–20] It is traditionally used in Indian folk medicines for various ailments. Roots are used as contraceptive, hepato-protective, anti-helminthic & carminative. It is also used for the cure of tuberculous glands in dysuria, in retention of urine, leucoderma & as an anti-dote in scorpion stings, for the relief of fever, ulcer, diabetes etc. Root and bark are used as bitter tonic, nervous disorders, as central nervous system stimulant.[18,19] Barks are also used to treat the ulcer, leucorrhea, vitiated conditions of pitta, anemia, bronchitis, tumor, dysentery, inflammations, cirrhosis of liver and hypertension. Seeds are emmenagogue, stimulant, astringent and also used in treatment of diarrhea, excessive menstrual flow, to reduce enlargement of spleen and skin disease. Its leaves are useful in diabetes, colic, skin diseases, as anti-helmintic and also in inflammatory rheumatic conditions.[18,21–23] However, anti-inflammatory effects are not yet experimentally proved. Hence the present study aimed to evaluate anti-inflammatory activity of leaves of *S. bispinosa* by acute and chronic models.

## 2. Material and methods

### 2.1. Collection and identification of plant

The leaves of *S. bispinosa* were collected in the month of December, 2014, from local area of Kandhar Dist. Nanded, Maharashtra, India. The plant was identified as *S. bispinosa* Linn., belonging to a family of Fabaceae, by P.G Department of Botany, N.E.S. Science College, Nanded, Maharashtra, India, with a voucher specimen no. Auth: S-1/26/08/13.

### 2.2. Chemicals

All chemicals used in the study were of laboratory grade: methanol, chloroform, hexane, petroleum ether, ethyl acetate, acetone, acetic anhydride, carrageenan, formalin, Tween 80, carboxy methyl cellulose and aspirin and were obtained from S.D. Fine chemicals, Mumbai, India.

### 2.3. Preparation of extracts and fractions

The leaves were washed with distilled water and shed dried to prepare the coarse powder. The powder of *S. bispinosa* was extracted employing solvents such as methanol, chloroform and hexane by using Soxhlet apparatus.[24] Most effective extract (methanol extract) obtained in carrageenan-induced paw edema test, was suspended in distilled water (1:3, v/v) and successively fractioned with petroleum ether, ethyl acetate and acetone. All the fractions obtained were dried with rotary evaporator and kept at 2–8 °C until the time of use.[25]

### 2.4. Experimental animals

Swiss albino mice of either sex weighing 25–30 g were used. They were housed under standard laboratory conditions at 23 °C (± 3 °C) temperature and 12 h light and dark cycle maintained, free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol (IAEC resolution number: 3-X dated 22/06/2014).

### 2.5. Anti-inflammatory activity

#### 2.5.1. Carrageenan-induced paw edema test

All extracts and fractions were administered with the gastric gavage and 50 µl of 1% v/v of carrageenan was injected in the left hind paw subplantar region after 30 min of oral administration of test extracts and aspirin.[26,27] The paw volume was measured by Plethysmometer (ORCHID Scientific & Innovative India, Pvt. Ltd. India). Before injecting carrageenan, the average volume of the right and left hind paw of each mouse was measured at 30 min, 60 min, 120 min, and 180 min after injection.[1] Animals were categorized into control and test groups; control group represented negative control (without treatment of standard/extracts/fractions) and positive control (standard i.e. aspirin).

*2.5.1.1. Anti-inflammatory activity of extracts.* Five groups of six animals each of either sex were used: Group I (negative control) received suspension of 1% CMC (Carboxymethyl Cellulose) at the dose of 10 ml/kg.p.o.; Group II (positive control) received suspension of aspirin at the dose of 100 mg/kg.p.o.; Groups III–V served as treatment (250 mg/kg.p.o.); Group III received suspension of methanolic extract of leaves of *S. bispinosa* (MELSB); Group IV received suspension of chloroform extract of leaves of *S. bispinosa* (CELSB); and Group V received suspension of hexane extract of leaves of *S. bispinosa* (HELSB).

*2.5.1.2. Anti-inflammatory activity of fractions*. Group I (negative control): This group received suspension of 1% CMC at the dose of 10 ml/kg.p.o. Group II (positive control): This group received suspension of aspirin at the dose of 100 mg/kg.p.o. Group III: This group received suspension of acetone fraction (AF) of MELSB. Group IV: This group received suspension of ethyl acetate fraction (EAF) of MELSB. Group V: This group received suspension of petroleum ether fraction (PEF) of MELSB.

### 2.5.2. Formalin-induced arthritis

Five groups of six animals each of either sex were used. Group I (negative control): This group received suspension of CMC at the dose of 10 ml/kg.p.o. Group II (positive control): This group received suspension of aspirin at the dose of 100 mg/kg.p.o. Group III–V served as treatment (250 mg/kg.p.o). Group III: This group received suspension of acetone fraction (AF) of MELSB. Group IV: This group received suspension of ethyl acetate fraction (EAF) of MELSB. Group V: This group received suspension of petroleum ether fraction (PEF) of MELSB. All the groups were fed with the gastric gavage and injected with 0.02 ml of 2.5% v/v of formalin solution in the left hind paw in subplantar region after 30 min of oral administration of standard drug/test/vehicle on day 1.[28,29] The mice were then treated with different fractions/drug/vehicle for 7 days. The paw thickness of each mouse was measured by using Vernier caliper daily before treatment.[1]

## 3. Statistical test

All the data were statistically analyzed using ANOVA followed by Dunnett test. Values are expressed as Mean ± SD ($n = 6$). Level of significance set as $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with control.

## 4. Results

### 4.1. Carrageenan-induced paw edema test

The average left hind paw volumes are presented in Table 1. For the control group, the injection of carrageenan caused localized edema, after 30 min. The swelling increased progressively from 30 min onwards. A maximum volume of paw was observed in animals of negative control at 180 min (0.289 ± 0.008 ml) after carrageenan injection. MELSB and HELSB caused significant decrease in the paw volume ($^{**}p < 0.01$) (0.289 ± 0.008 to 0.245 ± 0.011 and 0.259 ± 0.008) with 15.22% and 12.11% inhibition of inflammation respectively. The standard drug, aspirin significantly reduced ($^{**}p < 0.01$) the paw edema (0.204 ± 0.013 i.e. 29.41% inhibition of inflammation). The results reveal that the chloroform extract lacked anti-inflammatory activity while methanolic extract (MELSB) significantly inhibited inflammation.

As seen from Table 2, ethyl acetate fraction (EAF) of MELSB demonstrated significant reduction ($^{**}p < 0.01$) in the paw volume of mice induced by carrageenan; decreasing the paw volume from 0.289 ± 0.008 (in the negative control) to 0.242 ± 0.015** ($^{**}p < 0.01$) at the dose of 250 mg/kg/p.o. amounting 16.26% inhibition of paw volume which was comparable to aspirin, which reduced paw volume to 0.204 ± 0.013** ($^{**}p < 0.01$) at 100 mg/kg/p.o. AF of MELSB also demonstrated significant anti-inflammatory activity, whereas, PEF exerted significant but lesser activity as compared to other fractions.

### 4.2. Formalin-induced arthritis

Fig. 1 depicts the formalin-induced left hind paw thickness results. Administration of formalin caused increase in paw thickness in all groups. In case of negative control, the highest increase in paw thickness was observed on day 3 and slowly reduced, but not restored to its original paw thickness which was on day 1.

Formalin-induced paw thickness was reduced significantly ($^{**}p < 0.01$) with administration of AF, EAF and PEF which was found to be time-dependent and highest reduction in paw thickness was observed on day 8. EAF showed highest activity among all fractions and its anti-inflammatory effects were comparable to aspirin.

## 5. Discussion

The use of medicinal plants has been an important alternative as therapeutic source of treatment of various diseases and disorders. Its rising acceptance in the medical community has been due to the fact that several plants with novel biologically active compound have been scientifically investigated and their efficacy and safety have been verified.[30] The continuous research in the field of synthetic drugs in recent years is accompanied by numerous unwanted side effects, such as NSAIDs that have gastric ulcer and glucocorticoids are associated with adrenal suppression, as major side effects but plants have their unique place with least side effects.[10,11,15]

**Table 1** Influence of extracts of leaves of *S. bispinosa* on carrageenan-induced paw edema.

| Groups | Paw Volume (ml) | | | | | % inhibition at 180 min |
|---|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 120 min | 180 min | |
| Negative control | 0.117 ± 0.016 | 0.17 ± 0.015 | 0.22 ± 0.018 | 0.265 ± 0.016 | 0.289 ± 0.008 | |
| Aspirin | 0.11 ± 0.018 | 0.13 ± 0.015** | 0.157 ± 0.014** | 0.182 ± 0.014** | 0.204 ± 0.013** | 29.41% |
| MELSB | 0.107 ± 0.017 | 0.14 ± 0.009** | 0.172 ± 0.008** | 0.209 ± 0.008** | 0.245 ± 0.011** | 15.22% |
| CELSB | 0.117 ± 0.016 | 0.157 ± 0.006 | 0.22 ± 0.016 | 0.26 ± 0.009 | 0.287 ± 0.009 | 0.69% |
| HELSB | 0.12 ± 0.018 | 0.154 ± 0.006** | 0.19 ± 0.011** | 0.23 ± 0.009** | 0.254 ± 0.008** | 12.11% |

Values are expressed as Mean ± SD. ($n = 6$), ANOVA followed by Dunnett test.
$^{*}p < 0.05$, when compared with negative control.
$^{**}p < 0.01$, when compared with negative control.

**Table 2** Influence of fractions of methanolic extract of leaves of *S. bispinosa* on carrageenan-induced rat paw edema.

| Groups | Paw volume (ml) | | | | | % inhibition at 180 min |
|---|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 120 min | 180 min | |
| Negative control | 0.117 ± 0.016 | 0.17 ± 0.015 | 0.22 ± 0.018 | 0.265 ± 0.016 | 0.289 ± 0.008 | |
| Aspirin | 0.11 ± 0.018 | 0.13 ± 0.015** | 0.157 ± 0.014** | 0.182 ± 0.014** | 0.204 ± 0.013** | 29.41% |
| AF | 0.109 ± 0.015 | 0.144 ± 0.013** | 0.182 ± 0.012** | 0.219 ± 0.015** | 0.255 ± 0.011** | 11.76% |
| EAF | 0.105 ± 0.014 | 0.142 ± 0.010** | 0.175 ± 0.014** | 0.205 ± 0.006** | 0.242 ± 0.015** | 16.26% |
| PEF | 0.117 ± 0.019 | 0.155 ± 0.009 | 0.189 ± 0.014** | 0.229 ± 0.012** | 0.264 ± 0.011** | 8.65% |

Values are expressed as Mean ± SD. ($n$ = 6), ANOVA followed by Dunnett test.
*$p < 0.05$, when compared with negative control.
** $p < 0.01$, when compared with negative control.

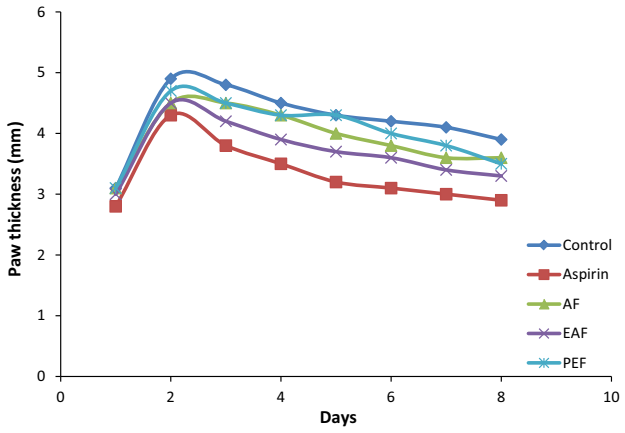


**Figure 1** The paw thickness of various fractions of MELSB. EAF exhibits time-dependent anti-inflammatory effects in a similar fashion to that of aspirin.

The present study was carried out to evaluate anti-inflammatory efficacy of *S. bispinosa* in carrageenan and formalin-induced mice paw edema and thickness models.

Carrageenan-induced paw edema is a popular and widely accepted acute model for the study of anti-inflammatory activity of compounds such as both steroidal anti-inflammatory drugs and NSAIDs.[1,31] The local injection of carrageenan induces inflammatory reaction in two different phases. The initial phase (0–1 h) has been accredited to the action of inflammatory mediators such as histamine, 5-HT, bradykinin and serotonin on vascular permeability. The late phase (1–6 h) is a result of overproduction of prostaglandins.

The inflammatory reaction involves three phases through orderly release of several mediators. The early phase (the first 90 min) has been attributed to the release of histamine and serotonin; the second phase (1.5–2.5 h) is the result of kinin and prostaglandins, while in the third phase (after 3 h) mainly involves action of prostaglandins with development of hyperalgesia due to increase of cyclooxygenase-2 level, and its peak reaches in within 4 h after carrageenan injection.[32,33]

In the present investigation, animals treated with MELSB and HELSB demonstrated significant reduction in paw volume. More active fraction, MELSB was selected for further fractionation using various solvents such as ethyl acetate, acetone and petroleum ether and these fractions were evaluated for anti-inflammatory activity by both acute and chronic models i.e. carrageenan and formalin induced paw edema. Results obtained from the carrageenan-induced paw swelling test revealed that EAF and AF of MELSB possess time-dependent significant anti-inflammatory activity while PEF of MELSB showed little late anti-inflammatory effect. The findings of this study suggest that the methanolic extract and fractions thereof may possibly act by inhibiting the release or action of histamine, serotonin and kinin and prostaglandin.

Formalin-induced arthritis model is most suitable for screening of anti-inflammatory and anti-arthritic compounds. This model predicts anti-proliferative effects of agents.[34] Injection of formalin into hind paw produces localized pain and inflammation, which is biphasic response, an early neurogenic component followed by a later tissue-mediated response.[1] AF, EAF and PEF of MELSB have shown marked inhibition of inflammation. Time-dependent activity was observed with AF and EAF, and the greater reduction in paw thickness was observed with ethyl acetate fraction (**$p < 0.01$) just like aspirin. This indicates that EAF and AF of MELSB actions confirm its anti-proliferative effect and EAF may further be explored for its use in arthritis.

In both the models, ethyl acetate fraction has shown greater anti-inflammatory effects as compared to acetone and petroleum ether fractions. Interestingly, acetone fraction showed more inhibition of inflammation in acute model and less activity in chronic model than petroleum ether fraction. It indicates that the PEF is having components which inhibit late phase inflammatory mediators and AF possesses the phytoconstituents responsible for inhibition of early phase inflammatory mediators. The observed anti-inflammatory effects of extracts and fractions may be attributed to the presence of steroids, triterpenoids, alkaloids, saponins, flavonoids and phenols which may be responsible for inhibition of inflammatory mediators such as histamine, 5-hydroxytryptamine, bradykinin, serotonin and prostaglandins.[35] Further the role of sterols for the observed anti-edema effect cannot be ruled out as these are present in *S. bispinosa* and several studies have demonstrated anti-inflammatory effects of phytosterols.[18–20,36]

## 6. Conclusion

Findings of the present study suggest that ethyl acetate fraction of *S. bispinosa* leaves possesses significant anti-inflammatory activity, as demonstrated in both acute and chronic models and may be further explored as an anti-inflammatory remedy. However, isolation, separation and identification specific component responsible for anti-inflammatory activity of ethyl acetate fraction may be required.

## Conflict of interest

We have no conflict of interest to declare.

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