March 2010 ISSN : 0974 - 7508 Volume 6 Issue 1

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 6(1), 2010 [77-81]

# *In vivo* assay of various extracts of *Leucas aspera* spreng for anti-inflammatory activity

**G.P.Kalaskar[1], M.Girisha[1], M.G.Purohit[1]*, B.S.Thippeswamy[2], B.M.Patil[2]**

[1]Pharmaceutcial Chemistry Division, Department of Chemistry, Gulbarga University, Gulbarga - 585 106, Karnataka, (INDIA)

[2]Department of Pharmacology, K.L.E.S's College of Pharmacy, J.N.M.C.Campus, Belgaum - 590 010, Karnataka, (INDIA)

E-mail : mgpurohit_glb@rediffmail.com

*Received: 1st February, 2010 ; Accepted: 11th February, 2010*

## ABSTRACT

Plant *Leucas aspera* Spreng is a member of Labiatae family. It is used in ayurvedic (herbal) medication in India for relieving of inflammation. In this study, dried whole plant was subjected for soxhlet extraction successively using Pet ether, Chloroform and Ethanol. The anti-inflammatory activity of the extracts against Carrageenan induced Paw edema (acute model), cotton pellet induced granuloma (Chronic model) and ulcerogenic studies (Sub acute model) in rats were carried out. The COX-II enzyme inhibition study was also carried out as an *in vitro* method. Amongst these three extracts, ethanolic extract showed good activity in all the models.



## KEYWORDS

*Leucas aspera*;
Anti-inflammatory;
COX-II enzyme.

## INTRODUCTION

Since thousands of years, people living in rural and semi urban areas in India are dependent on herbal preparations for treatment of their illness. Herbal preparations are found to be effective. Plant synthesizes novel structures by biosynthetic pathway. The isolation and biological studies of medicinal plants leads to the identification of herbal medicine for specific illness.

One of the approaches to discover newer anti-inflammatory agents is to search for their presence in natural sources. *Leucas aspera* Spreng (Family-Labiatae) is an annual herb found throughout India as a weed in cultivated fields, wastelands and roadsides. Traditionally, juice of the leaves is applied in psoriasis, chronic skin eruptions, insecticidal and its paste is applied topically to inflamed areas[1,2]. Some reports have been published on the chemical constituents such as long-chain compounds, aliphatic ketols, phenols, β-sitosterol, triterpenes, oleanolic acids and ursolic acid, triterpenoid lactone, linoleic acid, olelic acid[3-7]. There are many biological reports of this plant for its anti-inflammatory activity[8,9], antifungal[10] and prostaglandin inhibitory and antioxidant[11]. However systematic studies were not carried out in detail. Hence, the present investigation is taken up to study the anti-inflammatory activity of various solvent extracts of *leucas aspera* spreng. The ethanolic extract of *leucas aspera* spreng showed significant anti-inflammatory activity and COX-II enzyme inhibition studies.

## MATERIALS AND METHODS

### Plant material

The fresh whole plant of *Leucas aspera* was collected during the month of August and September in and around Gulbarga University campus and authenti-

Full Paper

cated at the Herbarium (Voucher No. 530), Department of Botany, Gulbarga University, Gulbarga.

### Preparation and characterization of the extract

Collected plant materials were immediately sprayed with ethanol to cease the enzymatic degradation of secondary metabolites. The shade-dried plant was chopped into small pieces and powdered. The powdered plant material was extracted with petroleum ether, chloroform, and ethanol successively in a Soxhlet extractor. The extracts were concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (40-50°C).

### Animals

Wistar rats either sex weighing 150-200g were procured from the National Institute of Nutrition, Hyderabad, India. They were acclimatized to laboratory conditions for a week prior to the initiation of the experiment. They were fed on standard rat feed and given free access to water.

### Drugs

The following chemicals used: Carrageenan (Sigma, Spain), Pet ether, Chloroform, Methanol and Diclofenac sodium (Ranbaxy Fine Chemicals, India). Lipopolysaccharide (LPS) and N, N, N, N-tetramethyl p-phenylenediamine (TMPD) were purchased from Sigma chemical Co. Ltd. Enzyme inhibition assay kit was purchased from Assay Designs, Inc. (Ann Arbor, MI, USA). Recombinant human COX-2 was a generous gift from Shozo yamamoto (School of Medicine, University of Tokushima, Japan).

### Paw edema method (Acute model)

The carrageenan-induced[12] rat hind paw edema was carried out as per by Winter et al. The rats were divided into six groups of six animals each. Group I received Tween-80 (1%), which served as a control. Group II received Diclofenac sodium at a dose of 50mg/kg body weight and used as standard. Groups III, IV and V were treated with Petroleum ether, Chloroform and Ethanol extracts of *Leucas aspera* respectively at a dose of 100 mg/kg body weight, orally. A mark was made on both the hind paws just below the tibio-tarsal junction so that every time the paw could be dipped in the mercury column of plethysmograph up to the mark to ensure constant paw volume. After 30 min. of above treatment an inflammatory edema was induced in the left hind paw by injection 0.1 ml of carrageenan (1%, w/v) in the planter tissue of the paw of all experimental rats. The right paw served as a reference to non-inflamed paw for comparison. The initial paw volume was measured plethysmographically within 30 seconds of the injection. The relative increase in the paw volume was measured in control, standard and treated group rats at an intervals 0, 1/2, 1, 3 and 5$^{th}$ hour after carrageenan injection. The increase in paw volume over the initial reading was measured. The change in paw volume in animals treated with standard drug and the three extracts of *Leucas aspera* were compared with that of untreated control animals after 5 hours. Thus, percent inhibition of paw volume in treated animals was used for calculating the percent inhibition of edema of the control group using the formula;

$$\% \text{ Inhibition} = \left(1 - \frac{Vt}{Vc}\right) \times 100$$

where Vt and Vc are mean relative changes in the paw volume of the test and control respectively.

### Granuloma method (Sub acute model)

Foreign body induced Granuloma method[13], which was developed by D'Arey et al.[13] was adopted. Healthy rats of either sex weighing between 150-200 g were selected and hair from the axillae and groin region were removed with the help of clips. A small incision was made in axillae groin region under light ether anesthesia. Sterile cotton pellet weighing 10 mg and two sterile grass piths (25 × 2mm) each was implanted subcutaneously. The incisions were sutured and animals were caged individually after recovery from anesthesia. Aseptic precautions were taken throughout the experiment. Cotton pellet and grass pith implanted rats were divided into six groups of six rats. Group I received Tween-80% (1%) solution and served as control. Group II received Diclofenac sodium solution at a dose of 50mg/kg body weight and treated as reference standard. Group III, IV and V received petroleum, ether, chloroform and ethanol extracts respectively at a dose of 100mg/kg body weight. The treatment was started on the day of implantation of foreign body and continued for successive ten days.

On the 11$^{th}$ day, 24 hours after treatment of drug,

the rats were sacrificed with an over dose of anesthesia to remove cotton pellets and gross piths. Cotton pellets were taken out, freed from extraneous tissue, dried overnight at 60°C and their dry weights were recorded. Net Granuloma formation was calculated by subtracting initial weight of cotton pellets (10mg) from recorded weights.

The mean granuloma dry weight was calculated and expressed as mg/100g body weight.

**Ulcerogenic effect in rats (Sub acute inflammation)**

Study of ulcerogenic potential by scoring the gastric lesions[14] (Vogel H. Gerhard). Rats were divided into six groups of six animals each. Group I received Tween-80 (1%) solution and served as control. Group II received Diclofenac sodium solution at a dose of 50mg/kg body weight and served as reference standard. Groups III, IV, V and VI received petroleum ether, chloroform and ethanol extracts respectively at a dose of 100mg/kg body weight. The treatment was started on the day of implantation of cotton pellet and grass piths to continue for successive ten days.

On 11th day, the rats were scarified with an over dose of anesthesia and stomach were opened along the greater curvature and the mucosa was gently washed with normal saline avoiding injuries to the mucosa, the number of ulcers in all the groups were counted with the help of magnifying glass and severity was calculated by using obituary system as described was followed:

Denuded epithelium = 10
Petechial and frank haemorrhages = 20
One or two ulcers = 30
Multiple ulcers = 40
Perforated ulcers = 50

**COX-2 enzyme inhibition assay of various extracts**

The assay was carried out as previously described by Brideau et al.[15], with little modification. The animals were separated into seven groups of five animals each. Group I received 5% gum acacia solution and served as a control. Group II received Celecoxib at a dose of 50mg/kg body weight and used as reference standard. Groups III, IV and V received petroleum ether, chloroform and ethanol extracts of *Leucas aspera* respectively at a dose of 100mg/kg body weight.

All the treatments were given per orally and after one hour of the treatment, animals were sacrificed. Whole blood of all the experimental rats was collected by venipuncture into heparinised tubes. 1ml aliquots of whole blood were incubated both in presence and absence of Lipopolysaccharide (LPS) obtained from *E. coli* in a concentration of 10-100µl. The contribution of platelet COX-1 was suppressed by the addition of 200µM of aspirin. The resulting mixture of 200µM of the chromogen i.e. TMPD. After incubation, the plasma from the samples was separated by centrifugation of the whole blood samples at 1000 rpm for 10-15 min.

The optical density of the resulting plasma was recorded by using spectrophotometer at a wavelength of 611 nm. The UV-visible spectra of Phycocyanobilin was recorded between 280-500 nm in methanol 2% HCl and the concentration was estimated at 374 nm using extinction coefficient of 47.900 $M^{-1}$ $cm^{-1}$.

The results obtained were calculated as A611/ 0.00826$\mu m^{-1}$ × 0.21 / 0.01 / 2* = n mole/ml (U/ml) (*It takes 2 molecules of TMPD to reduce $PGG_2$ to $PGH_2$). The data so obtained were subjected to statistical analysis.

**Statistical analysis**

Results were analyzed using student's 't' test and One-Way ANOVA followed by Dunnett's 't' test.

## RESULTS AND DISCUSSION

The anti-inflammatory activity results of *Leucas aspera* extracts against Carrageenan-induced paw edema are presented in TABLE 1. The results showed that, the ethanolic extract at 100mg/kg exhibited significant anti-inflammatory activity comparable to that of standard anti-inflammatory drug Diclofenac sodium. Other extracts, petroleum ether and chloroform extract showed moderate activity. The maximum inhibition (45.78%) was observed with 100mg/ kg of crude ethanol extract

The results of the cotton-pellet granuloma model of inflammation are presented in TABLE 2 showed that the ethanol extract showed significant inhibition of the granuloma weight and other extracts are showed moderate activity. Here 100mg/kg ethanol extract produced a maximum inhibition of granuloma weight (48.15%),

# Full Paper

**TABLE 1 : Data showing the antiinflammatory activity of various extracts of *Leucas aspera* spreng by carrageenan induced paw edema method**

| Group | Dose (mg/kg) | Mean paw volume in ml ± S.E. | | | | |
|---|---|---|---|---|---|---|
| | | 0 hr | ½ hr | 1 hr | 3 hr | 5 hr |
| Control (Tween-80) | 1 ml (1%) | 1.48 ± 0.016 | 1.48 ± 0.016 | 1.56 ± 0.021 | 1.76 ± 0.021 | 1.66 ± 0.021 |
| Standard (Diclofenac sodium) | 50 | 1.46 ± 0.021 | 1.21 ± 0.021 | 1.05 ± 0.02* | 0.99±0.016** | 0.88±0.021** |
| Petroleum Ether extract | 100 | 1.45 ± 0.022 | 1.33 ± 0.03 | 1.28 ± 0.03* | 1.23 ± 0.021* | 1.23 ± 0.017* |
| Chloroform extract | 100 | 1.45 ± 0.022 | 1.39 ± 0.025 | 1.20 ± 0.03* | 1.15±0.042** | 1.12 ± 0.03** |
| Ethanol extract | 100 | 1.45 ± 0.022 | 1.28 ± 0.016* | 1.11±0.022** | 1.00±0.042** | 0.90±0.042** |

**Data are expressed in mean ± SEM, N=6 animals in each group, Data's were analyzed by ANOVA followed by Dunnett's 't' test. *P <0.05 when compared with control, ** P <0.01 when compared with control**

**TABLE 2 : Effect of various treatments on cotton wool pellet granuloma**

| Groups | Drugs & Dose mg/kg B.W. | Granuloma dry weight (mg/100g B.W.) Mean ± S.E. | % Inhibition |
|---|---|---|---|
| 1 | Control | 19.50 ± 0.42 | - |
| 2 | Diclofenac sodium 50 | 9.83 ± 0.47** | 49.58 |
| 3 | Petroleum Ether 100 | 17.00 ± 0.57 | 12.82 |
| 4 | Chloroform100 | 15.50 ± 0.42* | 20.5 |
| 5 | Alcohol 100 | 10.11 ± 0.49** | 48.15 |

**Data are expressed in mean ± SEM, N=6 animals in each group, Data's were analyzed by ANOVA followed by Dunnett's 't' test. *P <0.05 when compared with control, ** P <0.01 when compared with control**

**TABLE 4 : Evaluation of cyclooxygenase-2 activity in whole blood (LPS method) UNITS/ML**

| | Control | Celecoxib | Pet. ether | Chloroform | Alcohol |
|---|---|---|---|---|---|
| | 10.58 | 65.05 | 20.59 | 10.3 | 32.27 |
| | 15.2 | 55.25 | 15.88 | 16.69 | 39.84 |
| | 20.2 | 48.25 | 15.25 | 21.77 | 44.67 |
| Mean±SEM | 15.33±2.77 | 56.18±4.87* | 17.24±1.68 | 20.59±1.99 | 38.93±7.46* |

while compared to Standard Diclofenac sodium (49.58%).

We have established the anti-inflammatory activity of extracts of *Lucas aspera*. It is evident that carrageenan-induced oedema is commonly used as an experimental *in vivo* model for evaluating the anti-inflammatory potential of plant extracts[16,17] and is believed to be biphasic. The initial phase is mediated by the early release of histamine and serotonin followed by the release of kinin and finally through the release of bradykinin, prostaglandin and lysosome[18]. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents[19].

Therefore, it may be suggested that the anti-inflammatory activity of Leucas *aspera* extract is due to its

**TABLE 3 : Effect of various treatments on ulcer index**

| Groups | Drug & Dose (mg/kg) body weight | Mean Ulcer index S.E.M. |
|---|---|---|
| 1 | Control | 10.33± 0.98 |
| 2 | Standard Diclofenac sodium 50 | 35.00 ± 3.16 |
| 3 | Petroleum Ether 100 | 9.50 ± 0.42 |
| 4 | Chloroform 100 | 8.50 ± 0.42 |
| 5 | Alcohol 100 | 6.50 ± 0.56 |

**Data are expressed in mean ± SEM, N=6 animals in each group, Data's were analyzed by ANOVA followed by Dunnett's 't' test. *P <0.05 when compared with control, ** P <0.01 when compared with control**

antihistamine or antiserotonin nature. The crude ethanol extract exhibited significant anti-inflammatory activity in the cotton-pellet granuloma test. This reflected its efficacy to inhibit the proliferative phase of the inflammation process, i.e. increase in the number of fibroblasts synthesis of collagen and mucopolysaccharides during granuloma tissue formation[20].

The irritative and ulcerative effect of the examined ethanol extracts is lower than that of standard Diclofenac sodium. (TABLE 3).

The conventional NSAIDs cause gastric mucosal damage[21,22]. COX-I is the major COX isozyme in the gastrointestinal tract of many species[23]. The inhibition of COX-I activity is associated with the ulcerogenic potential of NSAIDs[24-25]. In the study, chronic treatment with Diclofenac sodium damaged the gastric mucosa where ethanol extract did not causes gastric lesions at 100mg/kg body weight. These results suggest that PG production in this chronic inflammatory model is mediated by the activity of the inducible COX-II, and that the selective COX-II inhibitor. The selective inhibitor ethanol extract, which does not cause rat gastric damage, may be more useful clinically than conventional NSAIDs in the treatment of chronic inflamma-

tory diseases.

In order to study the anti-inflammatory activity of three extracts of *Leucas aspera*, it is essential to carryout the COX enzyme inhibition assay as shown in TABLE 4. The chemical molecule(s) present in them are responsible for the inhibition of COX-2 enzyme that present in the blood plasma of experimental rats.

## CONCLUSION

Preliminary phytochemical analysis performed in this study shows the presence of triterpenoids, long chain compounds, aliphatic ketols and phenols in the whole plant extracts of *Leucas aspera*. The anti-inflammatory action of triterpenoids has been reported by many researchers[26-28]. Therefore, it seems that anti-inflammatory profile of *Leucas aspera* might be related to the triterpenoids and phenols. In conclusion, the whole plant extract of *Leucas aspera*, which contains triterpenoids, phenols, possesses anti-inflammatory effects. However, the exact mechanism(s) and the active compound(s) involved in these effects need to be clarified in future studies.

In fact, this result does not seem to be the pharmacological profile of these inhibitors of the prostaglandin biosynthesis. On the basis of such experimental results, it is clear that a great deal of work will be necessary to propose the clear mode of action, or rather, the multiple modes of action.

However, it is important to determine the specific compound(s) responsible for the anti-inflammatory activity as well as to establish the mechanism of action of the extract to come to a definite conclusion is in progressed.

## REFERENCES


**[1]** K.R.Kirtikar, B.D.Basu; 'Indian Medicinal Plants', Deharadun: Lalit Mohan Basu, **3**, **(1999)**.

**[2]** R.N.Chopra, S.L.Nayar, I.C.Chopra; 'Glossary of Indian Medicinal Plants', India, CSIR, **(1956)**.

**[3]** T.N.Misra, R.S.Singh, H.S.Pandey, S.Singh; Phytochem., **31**, 1809 **(1992)**.

**[4]** T.N.Misra, R.S.Singh, C.Prasad, S.Singh; Phytochem., **32**, 199 **(1993)**.

**[5]** T.N.Misra, R.S.Singh, H.S.Pandey, S.Singh; Indian J.Chem.B, **34**, 1108-10 **(1995)**.

**[6]** A.N.Choudary, D.Ghosh; J Indian Chem.Soc., **46**, 95 **(1969)**.

**[7]** B.P.Pradhan, O.K.Chakraborty; Phytochem., **29**, 1693 **(1990)**.

**[8]** N.M.Goudgaon, N.R.Basavaraja, A.Vijayalaxmi; Indian J.Pharmacol., **35**, 397 **(2003)**.

**[9]** K.Srinivas, M.E.B.Rao, S.S.Rao; Indian J.Pharmacol., **32**, 37 **(2000)**.

**[10]** D.K.Thakur, S.K.Misra, P.C.Choudhuri; Indian J.Anim.Health, **26**, 31 **(1987)**.

**[11]** S.K.Sadhu, Emi Okuyama, HaruhiroFujimoto, Masami Ishabashi; Chem.Pharm.Bull., **51**, 595 **(2003)**.

**[12]** C.A.Winter, E.A.Risely, G.W.Nuss; Exp.Bio.Med. (NY)., **111**, 544 **(1962)**.

**[13]** P.F.D'Arey, E.M.Haward, P.W.Muggleton, S.B.Townsend; J.Pharm.Pharmacol., **12**, 659 **(1960)**.

**[14]** H.G.Vogel; 'Drug Discovery and Evaluation', Germany; Springer-Verlag Berlin Heidelberg, **(2002)**.

**[15]** C.Brideau, S.Kargman, S.Liu, A.L.Dallob, E.W.Ehrich, I.W.Rodger; Inflame Res., **45**, 68 **(1996)**.

**[16]** L.A.Della, A.P.Tubaro, C.Zilli, N.P.Del; Clin.Biol. Res., **213**, 481 **(1986)**.

**[17]** M.J.Alcaraz, M.J.Jimenez; Fitoterapia., **59**, 25 **(1998)**.

**[18]** J.Castro, H.Sasame, H.Sussaman, P.Buttette; Life Sci., **7**, 129 **(1968)**.

**[19]** E.Smucker, E.Arrhenius, T.Hilton; Bioc.J., **103**, 55 **(1967)**.

**[20]** E.Arrigoni-Martellie; New York, Spectrum Publications, **(1977)**.

**[21]** D.Riendeau, M.D.Percival, S.Boyce; Brit.J. Pharmacol., **121**, 105 **(1997)**.

**[22]** H.Hirata, H.Ukawa, M.Kitamura; Life Sci., **11**, 445 **(1997)**.

**[23]** S.Kargman, S.Charleson, M.Cartwright; Gastroenterology., **111**, 445 **(1996)**.

**[24]** J.L.Masferrer, B.S.Zweifel, P.T.Manning; Proc. Natal.Acad.Sci.USA, **91**, 3228 **(1994)**.

**[25]** K.Seibert, Y.Zhang, K.Leahy; Proc.Natal.Acad.Sci USA, **91**, 120 **(1994)**.

**[26]** B.Vazquez, G.Avila, D.Segura, B.Escalante; J.Ethnopharmacol., **55**, 69 **(1996)**.

**[27]** U.Huss, T.Ringbom, P.Perera, L.Bohlin, M.Vasange; J.Nat.Prod., **65**, 1517 **(2002)**.

**[28]** N.Suh, T.Honda, H.J.Finaly, A.Barchowsky, C.Williams, N.E.Benoit; Can.Res., **58**, 717 **(1998)**.