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Evaluation of anti-arthritic potential of *Leonotis nepetifolia* (L.)R.Br. against Freund's adjuvant induced arthritis

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ABSTRACT

Background: *Leonotis nepetifolia* (L.)R.Br. (LN) belonging to Lamiaceae family is a tall erect annual weed native to Southern India and tropical Africa used by tribals and folklore traditions in India for cough, fever, stomach ache, skin ailments, kidney diseases, rheumatism and dysmenorrhoea. The purpose of the study was to evaluate the anti-arthritic activity of the traditional dosage form (decoction) as used by the tribals in comparison to a modified dosage form (dry aqueous extract) of whole plant of LN in experimental animal models. **Materials and Methods:** Thirty wistar strain albino rats were selected and randomly divided into five groups. Arthritis was induced by Freund's complete adjuvant (FCA) and then treated with either the decoction of whole plant of LN or the dry aqueous extract for 30 days. The various parameters like paw volume, ponderal changes, serum biochemical parameters and histopathological changes were assessed. The data was analyzed by employing one-way ANOVA followed by Dunnett's multiple 't' test for unpaired data to determine significant difference between groups at $P < 0.05$. **Results:** In the present study it was observed that dry aqueous extract form of the test drug is having weak activity against primary oedema whereas decoction form did not show any effect on primary oedema. Both forms of test drug have comparable values as standard drug on 25th day in secondary oedema. **Conclusion:** The findings suggest the beneficial effect of the drug against chronic inflammation and inhibition of peri-arthritis and osteogenic activity.

Key words: *Leonotis nepetifolia*, *Granthiparni*, Anti-arthritic activity, ethnomedical, chronic inflammation.

INTRODUCTION

To identify a drug that is safe, affordable and effective is a challenge to modern medicine today. Current estimates are that it may cost as much as over a billion dollar to develop a drug by a pharmaceutical

company.^[1] Drug discovery strategies based on natural products and traditional medicine are re-emerging as attractive options.^[2] A folk medicine or Ayurvedic drug which has already been in use for many years having anecdotal evidence of efficacy for the treatment of a disease (which is also presumed to be safe) can be tested for efficacy in a clinical trial. This method has been described as 'reverse pharmacology'.^[3] The drugs commonly used to treat inflammation and arthritis include glucocorticoids like cortisone and prednisone, NSAIDs like Ibuprofen and naproxen etc., disease-modifying anti-inflammatory and anti-rheumatic drugs like Methotrexate (MTX) and leflunomide etc., and newer therapies such as biological response modifiers like tumor necrosis factor, alpha blocking agents, anti-CD 20 therapy (rituximab) and abatacept which are often required to inhibit or halt the underlying immune processes.

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However, besides high costs, all of these drugs are associated with numerous side effects, severe adverse reactions and toxicity, including some risk of infections in subsets of patients who are being treated with biological response modifiers.^{[4],[5]} *Leonotis nepetifolia* (L.)R.Br. belonging to Lamiaceae family, native to Southern India and tropical Africa is used by tribals and folklore traditions in India. LN roots are considered as the source plant for *Granthiparni* according to The Ayurvedic Pharmacopoeia of India, Part 1, Volume 3. At least, 23 ethnomedical claims are available on its use in various ailments from different parts of India.^[6] One such claims reported from Andhra Pradesh is on its use in joint complaints. The decoction made of 20 g of whole plant of LN (*Seerintalocal* name) in 50 ml of water and given once a day is known to relieve patients with joint pain.^[7] Few extracts of the plant have been explored for its analgesic, anti-inflammatory and anti-arthritic potential. The most noted studies are by Hortensia Parra-Delgado *et al*, (2004) on several extracts of aerial parts of LN showing anti-inflammatory activity on TPA-induced edema model in mice. Leonotinine was identified as the active constituent with marked anti inflammatory activity.^[8] Manocha N *et al*, (2012) reported the anti-inflammatory and anti-rheumatic activities of methanolic extract of capitulum (flowering head) of LN.^[9] Stigmasterol and Leonotinin^[10] isolated from the plant has shown significant anti-inflammatory activity. Flavonoids present in the plant are also said to attribute anti-inflammatory effect.

An analysis of the tribal claims and previous pharmacological works indicates the potential of the plant to be an anti arthritic drug which needs to be validated through preclinical, safety and efficacy trials. Hence the present pharmacological study was designed to evaluate the efficacy of the decoction (traditional dosage form) and dry aqueous extract (modified dosage form) of LN in suitable animal experimental models for its anti-arthritic activity.

MATERIALS AND METHODS

Animals

Wistar strain albino rats of either sex weighing between 170 to 250 g were used for the

experiments. The selected animals were kept under acclimatization for 7 days before dosing. The experimental protocols were approved by Institutional Animal Ethics Committee (Ph.D./IAEC/10/2012/07) in accordance with the guidelines formulated by CPCSEA, India.

Plant material

Collection of Plant material

The whole plant of LN was collected during its flowering season in the month of November from Mankarai region, Coimbatore, Tamil Nadu. It was authenticated (No.BSI/SRC/5/23/12-13 Tech/1757) at the Botanical Survey of India, Southern Regional Centre, Coimbatore and a voucher specimen (No. IPGT&RA/6066/12-13) was deposited at the Pharmacognosy Lab, Institute for Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar for future reference.

Preparation of Dosage form

For pharmacological evaluation, coarse powder of the test drug was used to prepare decoction as per classical method. One part of drug to 16 parts of water and reduced to quarter part. Aqueous extract of the whole plant was prepared with the total yield of 16.4%. Decoction was administered without diluting it, while for extract; stock solution of suitable concentration was prepared freshly with distilled water just prior to administration.

Dose Fixation

Dose of the drug was calculated by extrapolating the human therapeutic dose to rat on the basis of body surface area ratio (conversion factor 0.018 for rat) by referring to the table of Pagets and Barnes (1964).^[11]

Human dose of LN decoction is 50 ml per day as used by the tribals, hence the dose for rat was calculated as 4.5 ml/kg body weight of rat. Similarly the human dose of extract was fixed as 1000 mg/day based on which the dose for rat was decided as 90 mg/kg body weight of rat.

Route of administration

The test drugs suspension administered according to the body weight of the animals by oral route with the help of oral feeding canula.

Chemicals

Freund's complete adjuvant (FCA) was purchased from Sigma Aldrich (Product no. F5881). Dexamethasone IP (Batch No. LM 1399) obtained from Cadila Healthcare Limited, Ahmedabad. All chemicals or reagents used in the experimental study were procured from standard and reputed firms and were generally and whenever available are of analytical grade regularly used in the laboratory.

Evaluation of test drug effect on Freund's complete adjuvant induced arthritis in rats

The selected animals were grouped into five groups of 6 rats each. First group (normal control) was administered tap water. Second group (arthritic control) was administered tap water orally and injected with FCA. Third group (Dry aqueous extract) was administered with 90mg/kg body weight dry aqueous extract of LN orally (p.o.). Fourth group (Decoction) was treated with 4.5ml/kg body weight decoction of LN p.o. The fifth group (reference standard [RS]) was administered with the standard drug Dexamethasone (100 µg/kg). The test drugs and RS were administered for 30 consecutive days. On day 1, the complete FCA was made into fine emulsion with the help of a syringe and 0.1 ml of it was injected beneath the plantar aponeurosis in the left hind paw and 0.05 ml subcutaneously into the root of the tail. The volumes of both the hind paws were measured with the help of digital plethysmometer just before the adjuvant injection (initial). Paw volumes of both hind limbs were recorded on the day of adjuvant injection and again measured on 2nd, 5th, 10th, and 15th day, and 15th, 20th, 25th, and 30th day for primary and secondary oedema respectively. Paw volume of the 0 (initial) days were taken as the reference value for determining the increase in paw volume on the subsequent days. The animals were observed daily for the appearance of secondary lesion. On 30th day after one hour of drug administration, animals were weighed again and anaesthetized by anaesthetic ether and blood was collected from retro orbital plexus by capillary puncturing and used for estimation of serum

biochemical parameters. Parameters such as blood urea,^[12] serum creatinine,^[13] serum glutamate oxaloacetate transaminase,^[14] serum glutamate pyruvate transaminase,^[15] and serum alkaline phosphatase^[16] were estimated by feeding requisite quantity of serum to the auto analyzer (Fully Automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai, Maharashtra, India) which was automatically drawn in to the instrument for estimating different parameters. References given in the kit literature mentioning the basis of the methods on which test procedures was carried out.^[17] Further both right and left synovial joints were dissected out and the histopathological slides were prepared by referring to standard procedure. The slides were viewed under trinocular research Carl-Zeiss's microscope at various magnifications to note down the changes in the microscopic features.

Statistical Analysis

The data generated during the study were analyzed by employing Student 't' test and one-way ANOVA followed by Dunnet's multiple 't' test for unpaired data to determine significant difference between groups at $P < 0.05$.

RESULTS

FCA induced rat paw oedema

The values at different time intervals were compared with initial paw volume of respective group and the percentage increase in paw volume was calculated. Suppression in primary paw (left paw) volume was not observed in either of the test drug groups on 2nd and 5th day compared to the arthritic control group. Insignificant decrease was observed in primary oedema in both test drug administered groups carried out on 10th and 15th day in comparison to arthritic control group. A significant suppression in primary oedema was observed in dexamethasone treated groups on 10th ($p < 0.05$) and 15th day ($p < 0.01$) of arthritis induction in comparison to arthritic control group. (Table 1)

Table 1: Effect of test drugs on Primary paw oedema (oedema of left hind paw)

Groups	Percentage increase in paw edema compared to initial paw volume			
	2 nd day	5 th day	10 th day	15 th day
Arthritic Control	28.06 ± 4.33	33.52 ± 4.13	37.22 ± 4.78	55.43 ± 8.53
LN dry aqueous extract form	28.41 ± 3.13	33.91 ± 1.91	33.42 ± 2.71	44.20 ± 6.06
LN decoction form	31.55 ± 3.88	36.83 ± 3.96	36.61 ± 2.45	51.18 ± 5.47
Dexamethasone	22.99 ± 4.54	30.38 ± 2.10	24.50 ± 2.50*	20.50 ± 6.64**

Data: Mean ± SEM; *P<0.05, **P<0.01 (comparison to arthritic control group, unpaired t-test) SEM= Standard error of the mean

Freund's adjuvant induced arthritis produced increase in secondary oedema (i.e. right paw oedema) after 12th day in all rats. Arthritic control group showed maximum secondary oedema on 20th day. Marked suppression of secondary paw oedema was observed on 20th and 25th day in both extract treated and decoction treated groups in comparison to arthritic control group. However only extract treated group showed statistically significant (p<0.05) result in suppression of secondary oedema in comparison to arthritic control group. Reference standard group showed significant decrease in secondary paw oedema on 20th and 25th day in comparison to control group. (Table 2)

Table 2: Effect of test drugs on Secondary paw oedema (oedema of right hind paw)

Groups	Percentage increase in paw edema compared to initial paw volume			
	15 th day	20 th day	25 th day	30 th day
Arthritic Control	11.42 ± 2.10	18.69 ± 2.81	13.50 ± 1.76	4.45 ± 1.18

LN dry aqueous extract form	10.32 ± 2.50	10.50 ± 2.64*	6.99 ± 0.02*	6.30 ± 2.46
LN decoction form	12.10 ± 3.80	10.56 ± 2.99	7.44 ± 2.39	4.23 ± 2.28
Dexamethasone	8.36 ± 0.90	6.30 ± 0.50**	6.60 ± 1.90*	5.06 ± 1.17

Data: Mean ± SEM; *P<0.05, **P<0.01 (comparison to arthritic control group, unpaired t-test) SEM= Standard error of the mean

Ponderal changes

Table 3 illustrates the effect of test drugs on body weight of Freund's adjuvant induced arthritic rats. Normal control rats showed progressive increase in body weight of rats. Arthritic control rats showed significant decrease (25.20%) in body weight in comparison to initial values. Test and standard drugs also showed decrease in body weight but magnitude was less as compared to arthritic control group. (Table 3)

Table 3: Effect of test drugs on body weight

Groups	Initial Body weight (g)	Final Body weight (g)	Actual Change in body weight	% Change in comparison to initial
Control	200.20 ± 08.00	220.50 ± 06.92	18.80 ± 04.22	10.13↑
Arthritic Control	212.50 ± 13.15	187.00 ± 11.50	25.20 ± 6.60*	12.00↓
LN dry aqueous extract form	203.00 ± 15.26	191.66 ± 14.70	11.33 ± 6.76	5.58↓
LN decoction form	204.28 ± 12.28	192.75 ± 07.05	11.42 ± 11.59	5.64↓
Dexamethasone	214.50 ± 08.10	208.50 ± 09.22	06.00 ± 2.86	2.79↓

Biochemical estimation

Out of all the biochemical parameters studied, significant increase was found in blood urea and non-significant increase in alkaline phosphatase level in Freund's arthritic control group in comparison to the normal control group. Serum creatinine was found decreased significantly in the decoction group in comparison to the arthritic control. All other parameters were found unaffected in drug and standard drugs treated groups in comparison to the control group and arthritic control group. (Table 4)

Table 4: Effect of test drugs on serum biochemical parameters

Parameters	Cont rol	Arthr itic contr ol	LN dry aque ous extra ct form	LN decoc tion form	Dexameth asone
B. sugar (mg/dL)	82.0 6 ± 04.8 8	92.50 ± 4.92	92.00 ± 3.90	83.16 ± 2.75	78.20 ± 6.32
Urea(mg/d L)	46.2 5 ± 08.1 8	67.75 ± 4.23*	68.83 ± 7.27	65.00 ± 2.12	64.30 ± 04.44
Creatinine(mg/dl)	0.60 ± 00.0 4	0.57 ± 0.04	0.58 ± 0.03	0.50 ± 0.04*	0.68 ± 00.09
SGPT (IU/L)	58.0 0 ± 06.7 4	54.25 ± 5.25	54.33 ± 4.34	51.66 ± 3.32	62.25 ± 12.12
SGOT (IU/L)	160. 20 ± 16.5 0	170.7 5 ± 21.75	145.6 6 ± 10.17	166.6 7 ± 14.35	180.40 ± 22.60

Histopathological study

In Freund's adjuvant arthritis control rat remarkable degenerative changes in the form of bone and cartilage erosion and synovial membrane proliferation were observed in both the joints. These changes were found to be very much decreased in both the dosage of test drug and reference standard administered group (Plate 1a, 1b, 1c and 1d).

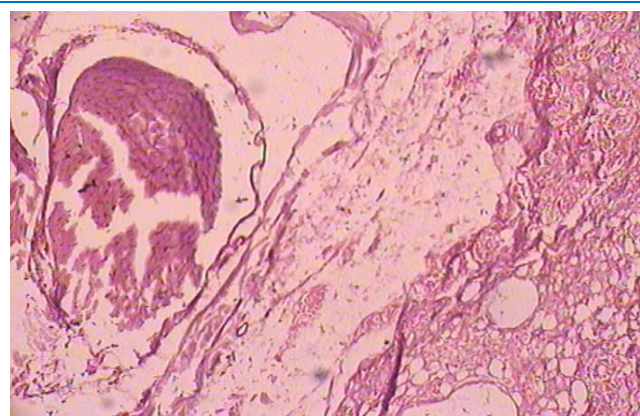


Plate 1a



Plate 1b



Plate 1c

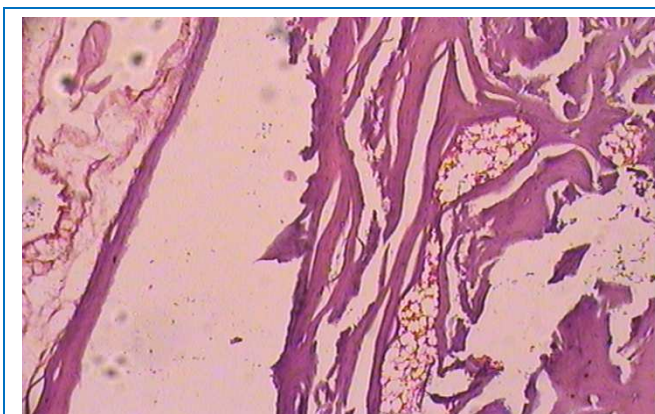


Plate 1d

DISCUSSION

The Freund's adjuvant induced arthritis model in rats is frequently used to evaluate anti-arthritic activity of new drugs as it closely resembles clinical arthritis.^[18] Therefore, this model is used with a relatively high degree of validity for evaluating agents with potential anti-arthritic activity. Four phases of arthritis are established by researchers on the basis of biochemical markers of arthritis viz., days 1–4 with acute local inflammation and systemic effects, days 7–12 with remission of acute inflammation and peri-arthritis, days 12–28 with chronic inflammation, peri-arthritis, and osteogenic activity and day 35 onward with permanent articular deformity and minimal inflammation.^[19] This model has been used to study subchronic or chronic inflammation in rats and is of considerable relevance to understand patho-physiology and pharmacological control of inflammatory processes.

The determination of paw swelling is considered a simple, sensitive, and quick procedure for evaluating the degree of inflammation and assessing the therapeutic effects of drugs. In this study, rats were selected as an animal model since they develop a chronic swelling in multiple joints with an influence of inflammatory cells and followed by erosion of cartilage in joints and destruction of bones. Paw volumes of both hind limbs were recorded on the day of adjuvant injection and again measured on 2nd, 5th, 10th, and 15th day, and 15th, 20th, 25th and 30th day for primary and secondary oedema respectively. The

15th day measurement is indicative of primary lesions and then onward measurement aids in estimating secondary lesions. On 21st day, the secondary phase of rheumatoid arthritis becomes more evident and inflammatory changes spread systemically and become observable in the limb not injected with Freund's adjuvant. This is because of the manifestation of cell mediated immunity.^[20]

In the present study it was observed that LN extract form is having weak activity against primary oedema whereas LN decoction form did not show any effect on primary oedema. RS group showed significant decrease in primary oedema in rats on 10th and 15th day compared to FA control group. This indicates presence of weak anti-inflammatory activity in LN extract form and significant anti-inflammatory activity in reference standard.

The symptoms of secondary lesion, such as swelling of the non-injected hind foot, of the ears, of the nose, and on the tail were observed at the 12th day, after injection of Freund's adjuvant. The test drug in both the dosage forms showed effect on secondary oedema. Dry aqueous extract treated group showed significant decrease in secondary paw oedema on 20th day and 25th day. Decoction form of LN also showed the similar values and inhibitory effect as shown by extract form in secondary oedema. Both forms of test drug have comparable values as standard drug on 25th day in secondary oedema. In dexamethasone treated group significant suppression of secondary oedema was observed on 20th and 25th day compared to FA control group. Since secondary oedema represents cell mediated immunity it is possible that there is an immunomodulatory component in the observed anti-arthritic activity in test formulations. The findings suggest the beneficial effect of the drug against chronic inflammation and inhibition of peri-arthritis and osteogenic activity.

This observation was further evidenced by histopathological study where joints from both dosage forms of LN and RS treated animals showed remarkable protection against Freund's adjuvant induced degenerative changes in the form of cartilage

erosion, synovial membrane proliferation and hyperplasia in both the joints.

Table 5: Effect of test drugs on synovial joints (histopathological observations)

Organ	Arthritic control	LN dry aqueous extract form	LN decoction form	Dexamethasone
Left synovial joint	Bone and cartilage erosion and synovial membrane proliferation	Almost normal joint structure	Almost normal joint structure	Almost normal joint structure
Right synovial joint	Bone and cartilage erosion and synovial membrane proliferation	Almost normal joint structure	Almost normal joint structure	Almost normal joint structure

Among the serum biochemical parameters studied only one parameter was affected to significant extent by injection of Freund's adjuvant. Statistically significant increase in blood urea was observed in arthritic control group in comparison to normal control group. Increased blood urea level was reported in arthritic rats and it was hypothesized that substantial fraction of blood urea in arthritic rats comes from arginine synthesized in the kidney.^[21] The test formulations and RS did not have any significant influence on this parameter.

Body weight is considered as an indirect index of health status and recovery from disease. A change in body weight of rats is measured as one of the parameter to assess the course of the disease and the response to therapy of anti-inflammatory and

anti-arthritic drugs. As the incidence and severity of arthritis is increased, a decrease in body weights of the rats occurs during the course of the experimental period and this observation is by the findings of previous study on alterations in the metabolic activities of diseased rats.^[22] It has been suggested that, the decrease in body weight during inflammation is due to deficient absorption of nutrients through the intestine and that treatment with anti-inflammatory drugs normalizes the process of absorption.^[23] In the present study, none of the groups showed weight gain except the normal control group. Arthritic control group showed marked decrease in body weight in comparison to initial values. Weight loss in the test drugs and standard groups were found to be lower in comparison to arthritic control group.

CONCLUSION

The overall assessment of pharmacological data revealed that folklore claim on the use of whole plant of *L. nepetifolia* in joint complaint is valid. Pharmacological studies suggest the beneficial effect of both dosage forms of *L. nepetifolia* against chronic inflammation and inhibition of periartthritis and osteogenic activity.

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