International Journal of Drug Development & Research
| April-June 2012 | Vol. 4 | Issue 2 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.03 & H index 2

Evaluation of the anti arthritic potential of the Genus Strobilanthus

Ms. Sarpate Rupali*, Dr. Tupkari Suresh
Smt. Sharadchandrika Suresh Patil College of Pharmacy, Pharmacognosy and Phytochemistry Department, Chopada, Jalgoan, Maharashtra, India.

Abstract

Adjuvant induced arthritis is a chronic crippling, skeleton-muscular disorder having nearest approximation to human rheumatoid arthritis for which there is currently no medicine available effecting a permanent cure. Even modern drugs used for the amelioration of the symptoms, offer only temporary relief and also produce severe side effects. In the indigenous system of medicine, the genus Strobilanthus is believed to be is useful in the treatment of inflammation and arthritis. In the present study the two chemical constituents RVS-A (Lupeol) and RVS-C (Doctriacantone) isolated from the two species of genus Strobilanthus namely *Strobilanthus callosus* Nees and *Strobilanthus ixiocephala* Benth were evaluated for its anti-arthritic potential. In the present study, anti-arthritic activity is done by Freund's adjuvant induced arthritis model using Prednisolone as the standard drug. We used various haematological parameters to assess the effectiveness of the treatment. The results of the Fruend's adjuvant induced arthritis model, indicated that the isolated components RVS-A (20 mg/kg) and RVS-C (20 mg/kg) both by oral route shows percent inhibition by 81.71% and 84.05% after 21 days respectively, where as the Standard Prednisolone 5mg/kg showed percent inhibition by 95.68% after 21 days ($P<0.01$). The results of the current investigation concluded, that both the plants of genus Strobilanthus namely *Strobilanthus callosus* Nees, *Strobilanthus ixiocephala* possess a significant anti-arthritic activity against adjuvant induced arthritis and justifying its therapeutic role in arthritic condition.

*Corresponding author, Mailing address:
Ms. Sarpate Rupali
E-mail: rupali_sarpate@yahoo.co.in

Key words:
Strobilanthus callosus Nees, *Strobilanthus ixiocephala* Benth, Anti arthritic activity, Fruend's adjuvant

How to Cite this Paper:
Ms. Sarpate Rupali*, Dr. Tupkari Suresh "Evaluation of the antiathritic potential of the Genus Strobilanthus", Int. J. Drug Dev. & Res., April-June 2012, 4(2): 230-236

Article History:----------
Date of Submission: 08-04-2012
Date of Acceptance: 22-04-2012
Conflict of Interest: NIL
Source of Support: NONE

INTRODUCTION

Plants that bloom after long intervals belonging to the Genus *Strobilanthes* e.g. *Strobilanthus callosus* Nees, *Strobilanthus ixiocephala* Benth are known as plietesials. These both species flower once in seven years.[1] *Strobilanthes callosus* Nees (Synonym: Carvia callosa (Nees) Bremek, family Acanthaceae) is

a shrub found mainly in the lower hills of the western ghats all along the west coast of India.[2] The shrub is locally known as Karvi sometimes written in English as Karvy. [3] The leaves of *Strobilanthes callosus* Nees are poisonous, toxic and unfit for human consumption it is used as a traditional medicine herb by the local adivasi tribals and villagers for the treatment of inflammatory disorders. [4] The stem bark of *Strobilanthes callosus* is used as an emollient in formulations for painful and ineffectual attempts to urinate or defecate.[5] It is used externally for mumps and flowers are used as a vulnerary and to treat arthritis. [6,7]

Strobilanthus ixiocephala Benth, Family Acanthaceae (Ruellia family) is a small straggling shrub found in Konkan, Deccan and Kanara in India. It is scarcely found in Khandala and Brahmagiri hills of Nashik in Maharashtra at an altitude of 500-900 m. Its Botanical name is *Strobilanthes ixiocephala* and Synonym is *Thelepaepale ixiocephala*. Its common name is Sky Blue Karvy and in Marathi it is called as Patri, Waiti. Scientific information on their pharmacognosy, phytochemistry is very scant. Traditionally over the ages, the tribals have used the roots of these plants for the treatment of inflammatory disorders. The roots of Karvi in the form of Lepa are reported to reduce the inflammation.[3,8] But, there were no reports on well controlled experiment trials depicting the anti-arthritic potential of these two plants of genus Strobilanthus namely *Strobilanthus callosus* Nees, *Strobilanthus ixiocephala*. The present study encompasses the anti-arthritic potential of both this species by means of Fruend's adjuvant induced arthritis model.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (1½ month old, 150-200 g weight) were used. Breeding and maintenance of animals were done according to the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA) and animal welfare division, Government of India for the use of laboratory animals. All the animals were housed in polypropylene cage using paddy husk bedding at 28 ± 1°C temperatures and 50 ± 5% humidity. Animals were fed on laboratory feed (Gold mohur; Lipton India LTD) and water *ad libitum*.

Extraction and Isolation of Chemical Constituents

A) Extraction and Isolation of Lupeol (RVS-A) from *Strobilanthus callosus* Nees [9,10, 11]

Nearly about 5 kg of the dried drug material (stem) was undergone successive extraction with solvent of increasing polarity like pet ether, chloroform and methanol (48hours each solvent). The yield obtained for pet ether was 98.2gms for 5 kg material. The percent yield obtained was 1.97% w/w. Out of this; 50 gm of dried pet ether extract was then saponified with alcoholic KOH to remove the fatty material, yielded nearly about 22 gm of unsaponified material. Nearly about 12 gms of unsaponified matter was subjected to column chromatography on silica gel (60-120 mesh) as a stationary phase. Gradient elution was performed using Toluene:Methanol (10:0; 9:1 up to 0:10) as the mobile phase. 120 fractions were collected in the test tube. On evaporation of mobile phase' pure white crystals were obtained in the test tubes of Toluene: Methanol (9:1) fractions. Single spot was resolved at Rf 0.70 using Toluene: Methanol (9:1) as mobile phase. The spot resoled was dark violet in colour. A total of 0.82 gms (820 mg) Lupeol was isolated.

B) Extraction and Isolation of Dotriacontane (RVS-C) from *Strobilanthus ixiocephala* Benth[12]

Nearly about 5 kg of the dried drug material (stem) was undergone successive extraction with solvent of

increasing polarity like Pet-ether, Chloroform and Methanol (48hours each solvent). The yield obtained for Chloroform extract was 201.2 gm for 5 kg material. The percent yield obtained was 5.03% w/w. A portion of Chloroform extract 10 gm was chromatographed on the Merk silica gel column (15cm x 6.0 i.d.) in order to separate the compounds according to polarity. The column was eluted sequentially with hexane then dichloromethane and finally with methanol. Evaporation of the solvents yielded the dry elutes from Hexane (2.5gm), Dichloromethane (3.2 gm) and Methanol (5.2 gm). The fraction eluted by hexane was dried and nearly about 1.00 gm was re-chromatographed on silica gel (60) column (13cm x 2 i.d.). This column was eluted with pure n-hexane and yield obtained of RVS-C was 120 mg in semisolid fatty component.

Acute Toxicity Test and Dose Selection

Groups of six albino male Wistar rats were administered the isolated components RVS-A and RVS-C in the doses of 0.25 and 0.5 g/kg body weight respectively. [13] Rats were continuously observed for mortality and behavioral responses for 48 h. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions. We selected 20mg/kg as the primary dose for the experiment to avoid toxicity.

Anti-Arthritic Activity

Freud's adjuvant induced arthritis [14] model was used to assess the anti-arthritic activity in albino rats. The animals were divided into four groups consisting six animals each. Test drugs were freshly prepared as a fine homogenized suspension in Tween-80 (2% w/v). Prednisolone (5 mg/kg) was used as a standard drug. All the groups were treated with their respective treatment as described in table no. 01. Freund's adjuvant induced arthritis was induced by injecting 0.1 ml (0.1% w/v) suspension of killed Mycobacterium tuberculosis bacteria homogenized in liquid paraffin into the left hind paw. Drug treatment was started from the initial day i.e. from the day of adjuvant injection (0 day), 30 minute before adjuvant injection and continued till 21st day. Paw volume was measured on 3rd and 21st day by using plethismometer. The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days and % inhibition of paw edema with respect to untreated group was calculated using following formula:

Percentage inhibition of paw edema = [1- (mean change in paw volume of treated rat/ mean change in paw volume of untreated rat)] x 100.

The changes in body weight were recorded daily. On the 21st day, blood was withdrawn through retro-orbital vein puncture of all groups by anaesthetizing the animals with diethyl ether and the biochemical parameters such as hemoglobin %, total WBC count, differential WBC count, ESR and total protein content (albumin and globulin content) were analyzed.

Table 1: Grouping and Treatment Schedule for Fruend's Adjuvant Induced Arthritis Model

Group No.	Drug Treatment	Dose and route	Treatment Schedule
Group I	Control Tween-80	10 ml/kg, p.o.*	Drug treatment was given to each group one hour before s.c. injection of carageenan
Group II	Prednisolone	5 mg/kg, p.o. *	
Group III	RVS-A	20 mg/kg, p.o. *	
Group IV	RVS-C	20 mg/kg, p.o. *	

* p.o.; per oral

BIOCHEMICAL ASSAYS

Haemoglobin content was estimated by the method of Austin and Drabkin.[15] Red blood cell (RBC) and white blood cell (WBC) counts were estimated according to the method of Chesbrough and Mc Arthur [16] in an improved Neubauer chamber. Estimation of erythrocyte sedimentation rate (ESR) was carried out by the method of Westergren. [17]

STATISTICAL ANALYSIS

Results were expressed as mean ± SD. The significance of difference among the groups was assessed using One way analysis of variance (ANOVA) followed by Dunnet's test. P<0.05 was considered significant.

RESULTS

Plants that bloom after long intervals belonging to the Genus *Strobilanthes* e.g. *Strobilanthus callosus* Nees, *Strobilanthus ixiocephala* Benth were selected for present study with the intention of exploring the scant species.[1] The results of the preliminary phytochemical screening of the methanol extract of both the plants revealed the presence of phytoconstituents such as alkaloids, steroids, flavonoids, phenolic compounds, tannins and glycosides. In acute toxicity studies, no toxic symptoms or mortality up to the dose level of 0.5 g/kg body weight in rats, and hence the extract was considered to be safe and non-toxic for further pharmacological screening. In adjuvant induced arthritis model, rats developed a chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodeling. These inflammatory changes ultimately result in the complete destruction of joint integrity and functions in the affected animal.[18] In the present study, the rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid disease. The determination of rat paw swelling is apparently simple, sensitive and one of the quick procedures for evaluating the degree of inflammation and the therapeutic effects of test drugs. [19] The results of the Fruend's adjuvant induced arthritis model, indicated that the isolated components RVS-A (20 mg/kg) and RVS-C (20 mg/kg) both by oral route shows percent inhibition by 81.71% and 84.05% after 21 days respectively, where as the Standard Prednisolone (5mg/kg) showed percent inhibition by 95.68% after 21 days (P<0.01) as shown in the Table No 02. The body weight was also estimated daily from the day of induction of arthritis to the 21st day. It was observed that the weight which was decreased after induction of arthritis was counteracted during the drug and the standard treatment due to the restoration of the absorption capacity of the intestine. The results of the changes in the body weight are shown in Table No. 03.

Table 2: Effect Of RVS-A and RVS-C Obtained from *S. Callosus* and *S. Ixiocephala* Respectively, On Fruend's Adjuvant Induced Arthritis Model in Rats.

Group No.	Group Specification	Mean paw volume			Mean difference		Percent Inhibition	
		Initial	After 3	After 21	After 3	After	After 3	After
Group I	Control Tween-80	1.131±	1.968±	2.014±	0.837±0.0	0.883±0.0		--
Group II	Prednisolone 5	1.080±	1.252±	1.118±	0.173±	0.038±	79.387%	95.678%
Group	RVS-A 20 mg/kg	1.111±	1.313±	1.273±	0.202±	0.162±	75.921%	81.714%
Group	RVS-C 20 mg/kg	1.154±	1.335±	1.295±	0.181±	0.141±	78.351%	84.054%

**P < 0.01

Table 3: Changes in the Body Weight in Adjuvant-Induced Arthritis Model in Rat

Groups	Before induction (gm)	On 21st day (gm)	Mean changes in the body weight
Group I	155.4	143.2	3.2±3.78
Group II	152	172	20±3.67
Group III	153.8	166.4	12.6±2.51
Group IV	154.2	165.6	11.4±3.21

The biochemical parameters reveal the following results. The effect of RVS-A and RVS-C on biochemical parameters studied in adjuvant induced arthritis are described in Table No.04.

(a) Hemoglobin:

The RVS-A , RVS-C and the standard drug Prednisolone showed increase in hemoglobin as compared to the adjuvant positive controlled group (P<0.01).

(b) Total WBC count:

The Total WBC count in adjuvant positive controlled group is 10893.67±30.42 per cu.mm was significantly suppressed by RVS-A , RVS-C and Standard Prednisolone showing suppression as 8606.17±41.77, 8910.00±53.41and 8504.17±46.73 per cu.mm, respectively.

(c) Differential WBC count:

The decreased neutrophile, eosinophils and monocyte count and the increased lymphocyte count in the adjuvant positive controlled group was significantly restored back to normal count by RVS-A, RVS-C and standard Prednisolone. (P<0.01).

(d) ESR:

Erythrocyte Sedimentation Rate of adjuvant positive control group is 7.72±0.42; it was restored to normal by Standard Prednisolone , RVS-A and RVS-C at 3.39 ±0.20, 4.26 ±0.03 and 4.73 ±0.59 for both Test group and standard group, respectively.

(e) Total Protein content:

The increase total Protein content of adjuvant positive controlled group **(7.72**±0.42 gm %) was significantly decreased by the standard Prednisolone. The RVS-A and RVS-C did not show any significant effect on total protein content but significantly decreased only the serum albumin content as compared to adjuvant positive controlled group. The serum globulin value of the positive controlled group is 4.67±0.19 gm%, the test component RVS-A and RVS-C and standard Prednisolone value shows significant decrease for serum globulin. The biochemical parameters studied in adjuvant induced arthritis is shown in Table No.03

Table 4: Effect Of RVS-A and RVS-C on Biochemical Parameters Studied in Adjuvant Induced Arthritis.

Sr. No.		Parameters	Control Tween-80 (2% (w/v))	Standard Prednisolone (5 mg/kg)	RVS-A (20 mg/kg)	RVS-C (20 mg/kg)
1		Heamoglobine (g.dL)	10.67 ± 0.62	12.61 ± 0.48**	11.87 ± 0.50**	11.67 ± 0.20**
2		Total WBC count (per cu.mm)	10893.67 ± 30.42	8504.17 ± 46.73**	8606.17 ± 41.77**	8910.00 ± 53.41**
3		Differential WBC count				
	i.	Neutrophiles (%)	17.44 ± 0.89	45.28 ± 0.71**	42.18 ± 1.41**	41.55 ± 1.42**
	ii.	Lymphocytes (%)	81.35 ± 1.07	53.35 ± 1.32**	55.35 ± 0.51**	56.26 ± 0.62**
	iii.	Eosinophilles (%)	1.59 ± 0.25	2.88 ± 0.10**	2.47 ± 0.32**	2.48 ± 0.32**
	iv.	Monocytes (%)	0.52 ± 0.01	1.49 ± 0.28**	0.82 ± 0.01*	0.79 ± 0.01*
	v.	Basophiles (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4		ESR (mm after 1 hour)	7.72 ± 0.42	3.39 ± 0.20**	4.26 ± 0.03**	4.73 ± 0.59**
5		Total Protein content (gm %)	7.96 ± 0.14	5.6 ± 0.05**	6.74 ± 0.21**	6.75 ± 0.25**
	i.	Serum albumin (gm %)	3.29 ± 0.11	2.15 ± 0.02**	2.53 ± 0.26**	2.45 ± 0.25**
	ii.	Serum globulin (gm %)	4.67 ± 0.19	3.47 ± 0.05**	4.21 ± 0.07**	4.29 ± 0.14**

** P<0.01 * P<0.05

DISCUSSION

Scientific information on this two species *Strobilanthus callosus* Nees, *Strobilanthus ixiocephala* Benth is very scant and so this species were explored in the present study. The two chemical constituents namely RVS-A (Lupeol) and RVS-C (Doctriacantone) isolated from the two species of genus Strobilanthus namely *Strobilanthus callosus*

Nees, *Strobilanthus ixiocephala* Benth are scrutinized for its anti-arthritic potential. In the present study, anti-arthritic activity is done by Freund's adjuvant induced arthritis model using Prednisolone as the standard drug. The chronic inflammation involves the release of number of mediators like cytokines, GM-CSF, interferons and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can leads to severe disability However, the standard drug, Prednisolone and the isolated components RVS-A and RVS-B significantly suppressed the swelling of the rat paws.

From the results observed in the current investigation, it may be concluded that the RVS-A and RVS-C at the dose of 20 mg/kg body weight displays a significant anti-arthritic activity which may due to the presence of phytoconstituents such as alkaloids, steroids, and glycosides. Several studies indicate that aforementioned phytoconstituents possess significant anti-arthritic activity.[20] The biochemical parameters such as hemoglobin content, total WBC, RBC, erythrocyte and sedimentation rate which were fluctuated in the arthritic condition were remarkably counteracted in the standard Prednisolone and the test groups i.e. Group 03 and 04 treated with the compounds RVS-A and RVS-C and thus justifying its significant role in the arthritic conditions. Changes in body weight have also been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs. [21] As the incidence and severity of arthritis increased, the changes in the body weights of the rats also occurred during the course of the experimental period. The loss of the body weight during arthritic condition was also supported by earlier observations, [22] on alterations in the metabolic activities of diseased rats. The body weight was increased during the drug and the standard treatment due to the restoration of the absorption capacity of the intestine. From the aforementioned results in the current investigation, it may be concluded that the isolated components RVS-A (20 mg/kg) and RVS-C (20 mg/kg) displays a significant anti-arthritic activity. In arthritic condition, there is a mild to moderate rise in WBC count due to the release of IL-IB inflammatory response, IL-IB increases the production of both granulocyte and macrophages colony stimulating factors. [23] In the present study, the migration of leucocytes into the inflamed area is significantly suppressed by RVS-A and RVS-C when compared to standard drug Prednisolone, as seen from the significant reduction in the total WBC count.

Erythrocyte Sedimentation Rate (ESR) is an estimate of the suspension stability of RBC's in plasma. It is related to the number and size of the red cells and to the relative concentration of plasma proteins, especially fibrinogen, alpha and beta globulins. Increase in the rate, is an indication of active but obscure disease processes. The acute phase proteins in ESR and C-Reactive Proteins (CRP) share the property of showing elevations in the concentration in response to stress or inflammations like injection, injury, surgery and tissue necrosis. The ESR count significantly increased in arthritic control group, whereas these counts were remarkably counteracted in the standard, Prednisolone and RVS-A, RVS-C and thus justifying its significant role in the arthritic conditions. [24]

The results of the current investigation concluded, that both the plants of genus Strobilanthus namely *Strobilanthus callosus* Nees, *Strobilanthus ixiocephala* possess a significant anti-arthritic activity against adjuvant induced arthritis and justifying its therapeutic role in arthritic condition.

REFERENCES

1) Sagreiya KP, Singh B. Botanical and Standardised Hindi Names of Important and Common Forest Plants of Madhya Pradesh. Gwalior Government: Regional Press, 1958, pp 525.

2) Hooker JD. The Flora of British India. London, Reer and Co., 1875, pp 527.
3) Anonymous. The Wealth of India, Raw Materials. New Delhi, Publication and Information Directorate, CSIR, 1976, pp 57-58.
4) Kirtikar KR. The Poisonous Plants of Bombay. Vol. VI, India Scientific Publishers, 2003, pp 300.
5) Kirtikar KR, Basu BD. Indian Medicinal Plants, ed 2, Vol. I, New Delhi, Published by Bishen Singh, 1933, pp. 340-343.
6) Nadkarni KM. Indian Materia Medica. Bombay, Popular Book Depot, 1954, pp 1172.
7) Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi, PID, 1956, pp 235.
8) Santapau H. The Flora of Khandala on the Western Ghats of India. ed 2, Vol. 16, Calcutta, Govt of India Press, 1960, pp 1-3.
9) Pandey DJ, Anand IS. Isolation and high-performance thin layer chromatographic estimation of Lupeol from Oxystelma esculentum. Pharmaceutical Methods 2011; 2: 99-95.
10) Jamal AK, Yaacob WA, Din LB. A Chemical Study on Phyllanthus reticulates. Journal of Physical Science 2008; 19: 45-50.
11) Elvira E, Kovač B, Kemal D, Zdenka K, Emin S. Identification And Isolation of Pharmacologically Active Triterpenes in Betuale Cortex, Betula Pendula Roth., Betulaceae. Bosnian Journal Of Basic Medical Sciences 2009; 9: 31-38.
12) Higuchi CT, Sannomiya M, Pavan FR, Leite SRA, Sato DN, Franzblau SG, et. al. Byrsonima fagifolia Niedenzu Apolar Compounds with Antitubercular Activity. Evid Based Complement Alternat Med 2008; 1-5.
13) Ecobichon DJ. The basis of toxicology testing, ed 2, New York, CRC press, 1999, pp 43-46.
14) Newbould BB. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. Br J Pharmacol Chemother 1963; 21(1):127-136.
15) Austin JH, Drabkin DL. Estimation of haemoglobin. J Biol Chem 1935; 112: 67-88.
16) Chesbrough M, Mc Arthur J. A Laboratory Manual of Rural Tropical Hospitals. The English Language Book Society and Churchill Livingstone, Edinburgh, 1972.
17) David G, Sykes AJ. Westergren and Wintrobe methods of estimating ESR compared. Br Med J 1951; 2: 1496-1497.
18) Carl MP. Experimental joint disease observations on adjuvant induced arthritis. J Chronic Dis 1963; 16(8):863-864.
19) Singh S, Majumdar DK. Effect of fixed oils of Ocimum sanctum against experimentally induced arthritis and joint edema in laboratory animals. J Pharmacogn 1996; 34(3):218-222.
20) Rajendran R, Krishnakumar E. Anti-Arthritic Activity of Premna serratifolia Linn., Wood against Adjuvant Induced Arthritis. Avicenna J Med Biotech 2010; 2(2): 101-106.
21) Winder CV, Lembke LA, Stephens MD. Comparative bioassay of drugs in adjuvant induced arthritis in rats: flufenamic acid, mefenamic acid and phenylbutazone. Arthritis Rheum 2005; 12(5):472-482.
22) Walz DT, Dimartino MJ, Misher A. Adjuvant induced arthritis in rats. II. Drug effects on physiologic, biochemical and immunologic parameters. J Pharmacol Exp Ther 1971; 178(1):223-231.
23) Eric GB, Lawrence JL. Rheumatoid arthritis and its therapy, The Textbook of therapeutic drugs and disease management, ed 6, Baltimore, Williams and Wilkins Company; 1996, pp 579-595.
24) William JK. Arthritis and allied condition. A Text-book of rheumatology, Vol. 1, ed 3, Baltimore, Tokyo, A Waverly Company, 1996, pp 207-226.