ORIGINAL ARTICLE

Evaluation of anti-inflammatory activity of *Strobilanthus callosus* Nees and *Strobilanthus ixiocephala* Benth

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

Context: *Strobilanthus callosus* Nees and *Strobilanthus ixiocephala* Benth belongs to family Acanthaceae. The plants have been the subject of scientific research which confirms its use in folk medicine as anti-inflammatory drugs showing potent anti-rheumatic effects. Previous research claims the anti-inflammatory and anti-arthritic activities of Lupeol and 19α-H Lupeol isolated from *Strobilanthus callosus* and *Strobilanthus ixiocephala* roots. Based on the literature cited, the unexplored parts stems and leaves of the two species were selected for the present study. **Aim:** The present study is designed to isolate steroidal and alkaloidal components from the two species *Strobilanthus callosus* and *Strobilanthus ixiocephala* using the unexplored parts viz. stems and leaves and to investigate its anti-inflammatory effect. **Settings and Design:** The anti-inflammatory effect was investigated employing subacute anti-inflammatory models namely cotton pellet granuloma and carrageenan-induced rat paw edema. **Materials and Methods:** Anti-inflammatory activity was carried out using isolated test components RVS-A (Lupeol), RVS-C (Doctriacantone) and standard drug Diclofenac sodium (10 mg/kg). **Results:** The present study has dealt up with isolation of two phytoconstituents Lupeol and Dotriacontane which gave marked anti-inflammatory activity at the dose 20 mg/kg in both the models Carrageenan induced rat paw edema and Cotton pellet granuloma. **Conclusion:** The results confirm that the mechanism of the anti-inflammatory effect of RVS-A (Lupeol) and RVS-C (Doctriacantone) involves reduction of prostaglandins through inhibition of cyclooxygenase and suppression of proliferative phase of sub acute inflammation. Thus the steroidal and alkaloidal components Lupeol and Doctriacantone isolated from *Strobilanthus callosus* Nees and *Strobilanthus ixiocephala* Benth shows marked anti-inflammatory activity.

Key words: Acanthaceae, anti-inflammatory, *Strobilanthus callosus* Nees, *Strobilanthus ixiocephala* Benth

INTRODUCTION

Plants that bloom after long intervals are known as plietesials. The species of belonging to the Genus *Strobilanthus* e.g., *Strobilanthus callosus* Nees, *Strobilanthus ixiocephala* Benth are of the same category and these both species flower once in seven years.[1]

Strobilanthus callosus Nees (Synonym: *Carvia callosa* (Nees) Bremek, family Acanthaceae) is a shrub found mainly in the low 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 *Strobilanthus callosus* Nees are poisonous, toxic and unfit for human consumption it is used as a traditional medicine herb by the local adivasi tribal's and villagers for the treatment of inflammatory disorders.[4] The stem bark of *Strobilanthus callosus* is used as an emollient in formulations for painful and ineffectual attempts to urinate or defecate.[5] Flowers are used as a vulnerary and to treat arthritis.[6,7]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.104931

Strobilanthus ixiocephala Benth, Family Acanthaceae (Ruellia family) is a small straggling shrub found in Konkan, the 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 *Strobilanthus ixiocephala* and Synonym is *Thelepaepale ixiocephala*. Its common name is Sky Blue Karvy and in Marathi it is called as Patri, Waiti. Traditionally over the ages, the tribal's 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]

Scientific information on their pharmacognosy, phytochemistry is very scarce. The authors Agarwal RB and Rangari VD have worked on the phytochemical investigation and evaluation of anti-inflammatory and anti-arthritic activities of essential oil isolated

Address for correspondence: Prof. Rupali Vitthal Sarpate, Flat No.3, Siddharth Building, Opposite Roplas Company, Pimpri, Pune, Maharashtra State, India. E-mail: rupali_sarpate@yahoo.co.in
Received: 06-04-2012; **Accepted:** 17-08-2012

from *Strobilanthus ixiocephala* Benth. Additionally, anti-inflammatory and anti-arthritic activities of Lupeol and 19 α-H Lupeol isolated from *Strobilanthus callosus* and *Strobilanthus ixiocephala* roots, is reported in different research papers. Studies have been conducted on isolation of steroidal components from roots of *Strobilanthus callosus* and roots, flowering tops of *Strobilanthus ixiocephala*.[9,10] These claims instigate our piece of research on the isolation of steroidal and alkaloidal components from the same plants but different parts viz. stems and leaves. On the stand of literatures cited it was confirmed that the plants belonging to the genus *Strobilanthus* are drugs with potent anti-inflammatory activity. So, the two species *Strobilanthus callosus* and *Strobilanthus ixiocephala* were selected for the present study. The present study emphasize on the extraction, isolation of the chemical components from *Strobilanthus ixiocephala* Benth and *Strobilanthus callosus* Nees using the unexplored parts of both the plants on which until no research work has been done. The work is then accentuated on the anti-inflammatory activity. The anti-inflammatory effect is investigated employing, subacute anti-inflammatory model namely cotton pellet granuloma and carrageenan-induced rat paw edema. This study is done with an objective of drawing an attention on those scarce species of the plietesials category as well as a reference for further scientific investigations. The work of the present research gives the initiative for the development of the potent anti-inflammatory and anti rheumatic drugs of the plant origin of no side effects which can be further commercialized.

MATERIALS AND METHODS

Plant Material

The plant *Strobilanthus callosus* Nees and *Strobilanthus ixiocephala* Benth was procured from Trimbakeshwar, Nasik, India and authenticated in Botanical Survey of India (BSI) and voucher specimen is RSC-I and RSI-1 was kept at departmental herbarium of BSI, Pune. Fresh material (leaves and stems) of both the plants were shade dried and made into 60 mesh powder and then were used for extraction.

Extraction and Phytochemical Screening

The dried material of stems and leaves of both the plants *Strobilanthus callosus* Nees and *Strobilanthus ixiocephala* Benth was used for extraction purpose**.** Air dried material (stem and leaves) of *Strobilanthus callosus* nearly about 5.0 kg was extracted successively with solvents of increasing polarity like pet ether (40-60°C), chloroform and methanol (48 hours each solvent). The extracts were dried under vacuum in a rotary evaporator. Extracts were stored in refrigerator. The yield obtained in grams for 5.0 kg of the drug material is 98.2 g for pet-ether (40-60°C), 402.3 g for chloroform and 508.3 g for methanol.

Air dried material (stem and leaves) of *Strobilanthus ixiocephala* nearly about 5.0 kg was extracted successively with solvent of increasing polarity like Pet ether (40-60°C), Chloroform and Methanol (48 hours each solvent). The extracts were dried under vacuum in a rotary evaporator. Extracts were stored in refrigerator. The yield obtained in grams for 5 kg of the drug material is 92.7 g for pet ether, 201.2 g for chloroform and 603.8 g for methanol. This six extract which were obtained by successive extraction from both the plants were undergone phytochemical investigations. The results of phytochemical investigations illustrated steroids, alkaloids, flavanoids, and glycosides tests positive. On the basis of phytochemical investigations and the aforementioned research claims, the present work was focused on isolation of steroidal and alkaloidal components and evaluation of its anti-inflammatory activity.

Isolation of Chemical Constituents

Isolation of phytoconstituents-I from Strobilanthus callosus Nees

Ensuing the successive extraction, isolation was done. The yield obtained for petether was 98.2 g for 5 kg material. The percent yield obtained was 1.97% w/w. Out of this; 50 g of dried petether extract was then saponified with alcoholic KOH to remove the fatty material, yielded nearly about 22 g of unsaponified material. Nearly about 12 g 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 resolved was dark violet in colour.[11-13] A total of 0.82 g (820 mg) of phytoconstituents-I was isolated and it was coded as RVS-A.

Extraction and isolation of phytoconstituents-II fromStrobilanthus ixiocephala Benth

Ensuing the successive extraction, isolation was done. The yield obtained for Chloroform extract was 201.2 g for 5 kg material. The percent yield obtained was 5.03% w/w. A portion of Chloroform extract 10 g was chromatographed on the Merk silica gel column (15 cm × 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 (2.5 g), Dichloromethane (3.2 g) and Methanol (5.2 g). The fraction eluted by hexane was dried and nearly about 1.00 g was re-chromatographed on silica gel (60) column (13 cm×2 i.d.). This column was eluted with pure n-hexane and yield obtained of Phytoconstituents-II was 120 mg as a semisolid fatty component.[14] This component was coded as RVS-C.

Animals

Healthy albino rats (wistar strains) were used for the study. All the animals were housed under standard conditions of temperature (27 ± 1°C) and constant humidity (60%), 12 hours light/dark cycles and fed with standard pellet diet and water *ad libitum*. The experiment was carried out according to Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines and Institutional Animal Ethical Committee approved all the procedures. Experimental studies were undertaken according to their rules and regulations.

Anti-Inflammatory Activity

Carrageenan induced rat paw edema

The anti-inflammatory activity of RVS-A and RVS-C was determined using a carrageenan-induced paw edema test according to the method of Winter *et al*.[15] Six male wistar rats (100-150 g) were randomly divided into four groups consisting six animals each and fasted overnight before the experiment with free access to water. Test drug viz. RVS-A (20 mg/kg), RVS-C (20 mg/kg) was freshly prepared as a fine homogenized suspension in Tween-80 (2% w/v). Diclofenac sodium (10 mg/kg) was used as a standard drug. All the groups were treated with their respective treatment. Test drug and standard drug was administered orally to rats one hour before subcutaneous injection of Carrageenan (1% in NSS) into the plantar surface of the left hind paw. The control group received an equivalent volume of vehicle (Tween-80) and the positive-control group received Diclofenac sodium dissolved in NSS (10 mg/kg). After the carrageenan injection, the paw volumes were measured at 0, 1, 2, 3 and 4 hours using a plethysmometer (Model 7150, UGO Basile, Italy). Edema was expressed as the mean increase in paw volume relative to control animals.

The percentage inhibition of edema was calculated by the following equation:

% inhibition of edema = 100 (1−Vt/Vc),

Where, Vc is the edema volume in the control group and Vt is the edema volume in tested group.

Cotton pellet granuloma induced inflammation in rats

Animals were divided into four groups of six each. Test drug viz. RVS-A (20 mg/kg), RVS-C (20 mg/kg) was freshly prepared as a fine homogenized suspension in tween-80 (2% w/v). Diclofenac sodium (10 mg/kg) was used as a standard drug.

Sub-acute inflammation was produced by cotton pellet induced granuloma model in rats.[16,17] On day 1, with aseptic precautions sterile cotton pellets (50 ± 1 mg) were implanted subcutaneously, along the flanks of axillae and groins bilaterally under ether anaesthesia. All drugs were given orally to the respective group of rats daily for six consecutive days from day one. The animals were sacrificed on the 7th day. The granulation tissue with cotton pellet was dried at 60^{o}C overnight and then the dry weight was taken. Weight of the cotton pellet before implantation was subtracted from weight of the dissected dried pellets. Only dry weight of the granuloma formed was used for statistical analysis.

Data Analysis

Data are expressed as Mean ± S.E.M. Statistical analyses was performed by one-way ANOVA followed by Dunnett's test. *P* values <0.01 were considered significant.

RESULTS

The successive extraction carried out for both the plants yielded pet ether, chloroform and methanolic extracts. From the plant *Strobilanthus callosus* Nees the % yield of pet ether (40-60°C) extract is 1.96%, that of chloroform is 8.04% and of methanol is 10.2% and from the plant *Strobilanthus ixiocephala* Benth the % yield of pet ether extract is 2.32% that of chloroform is 5.03% and of methanol is 15.10 %. The phytochemical investigation showed positive test for steroids, alkaloids, flavanoids and glycosides. The isolation work was dealt up with isolation of two phytoconstituents RVS-A and RVS-C. The TLC, HPTLC characterization and spectral analysis concluded this component RVS-A as Lupeol and RVS-C as Dotriacontane. The carrageenan-induced rat paw edema test was performed to scrutinize the anti-inflammatory effect of RVS-A (Lupeol) and RVS-C (Dotriacontane). The results indicated that Lupeol (20 mg/kg) and Dotriacontane (20 mg/kg) significantly decreased the paw volume (P<0.01) at 4 hours after carrageenan administration compared to vehicle control. At each hour after carrageenan administration, Lupeol (20 mg/kg) and Dotriacontane (20 mg/kg) showed the inhibition of edema which is notified in Table 1.

In Carrageenan induced rat paw edema model, the test compound Lupeol (20 mg/kg), Dotriacontane (20 mg/kg) and standard drug Diclofenac sodium (10 mg/kg) shows mean percent inhibition as 11.92%, 12.13% and 13.27% respectively, when compared with control group. Details notified in Table 2.

In Cotton pellet granuloma model the test compound Lupeol (20 mg/kg) and Dotriacontane (20 mg/kg) both when administered orally showed 32.95% and 38.16% inhibition of granuloma as compared to the standard Diclofenac sodium 10 mg/kg which produces 52.92% inhibition of granuloma (P<0.01). Details are notified in Table 3.

Table 1: Effect of RVS-A and RVS-C obtained from *S. callosus* and *S. Ixiocephala* respectively, on carrageenan induced rat paw edema

Group	Group specification	0 hour	1 hours	2 hours	3 hours	4 hours
I	Control tween-80 (20%)	0.96±0.04	0.98±0.04	0.99±0.04	1.03±0.02	1.04±0.02
II	Diclofenac sodium 10 mg/kg	0.93±0.01	0.91±0.01*	0.88±0.02*	0.86±0.02*	0.84±0.01*
III	RVS-A 20 mg/kg	0.94±0.04	0.91±0.02*	0.89±0.02*	0.88±0.02*	0.86±0.02*
IV	RVS-C 20 mg/kg	0.93±0.03	0.91±0.03*	0.89±0.03*	0.88±0.03*	0.86±0.03*

*$P<0.01$

Table 2: Percentage inhibition (%) at various time intervals

Group	Group specification	Percentage inhibition (%) at various times intervals				
		1 hours	2 hours	3 hours	4 hours	Mean of % inhibition
II	Diclofenac sodium 10 mg/kg	6.32	11.17	16.61	18.97	13.27
III	RVS-A 20 mg/kg	6.50	9.64	14.84	16.72	11.92
IV	RVS-C 20 mg/kg	6.32	9.98	15.16	17.04	12.13

Table 3: Effect of RVS-A and RVS-C obtained from *S. callosus* and *S. Ixiocephala* respectively, on Cotton pellet granuloma induced inflammation in rats

Group	Group specification	Initial weight (mg)	Weight after implantation (mg)	Difference in weight (mg)	Percent inhibition
I	Control tween-80 (20%)	51.5±1.5166	240.17±1.6021	188.67±1.211	—
II	Diclofenac sodium (10 mg/kg)	51.0±0.8944	139.83±1.4720	88.83±1.7224*	52.92
III	RVS-A (20 mg/kg)	50.33±1.3663	176.83±2.3166	126.50±2.2583*	32.95
IV	RVS-C (20 mg/kg)	50.83±1.1690	167.50±2.1679	116.67±1.2111*	38.16

*$P<0.01$

DISCUSSION

Carrageenan-induced paw edema is a suitable experimental animal model for evaluating an anti-edematous effect. Edema developed after injection of carrageenan serves as an index of acute inflammatory changes, can be determined from differences in the paw volume measured immediately after carrageenan injection and then every hour for 4 hours. Edema induced by carrageenan is believed to be biphasic: The first phase (1 hour) involves the release of serotonin and histamine and the second phase (over 1 hour) is mediated by prostaglandins, cyclooxygenase products. Continuity between the two phases is provided by kinins.[18,19]

The results of Carrageenan-induced paw edema suggests that RVS-A (Lupeol) and RVS-C (Dotriacontane) produces an anti-edematous effect during the second phase, similarly to Diclofenac sodium. Data from preclinical models suggest that isolated components Lupeol and Dotriacontane are able to produce anti-inflammatory effect through cyclooxygenase inhibition and consequent reduction of prostaglandins.[20] Therefore, our results confirm that the mechanism of the anti-inflammatory effect of steroidal and alkaloidal components Lupeol and Dotriacontane involves reduction of prostaglandins through inhibition of cyclooxygenase.

Cotton pellet granuloma model was used to evaluate the anti-inflammatory activity of RVS-A (Lupeol) and RVS-C (Dotriacontane) in sub acute inflammation. Three phases of the inflammatory response to a subcutaneously implanted cotton pellet in the rats have been described: (a) a transudative phase, that occurs during the first 3 h; (b) an exudative phase, occurring between 3 and 72 h after implanting the pellet; (c) a proliferative phase, measured as the increase in dry weight of the granuloma that occurs between 3 and 6 days after implantation.[21] The suppression of proliferative phase of sub acute inflammation could result in decrease in the weight of granuloma formation.[22] The dry weight of cotton pellet granuloma was significantly reduced ($P<0.01$) by Lupeol and Dotriacontane, however the antiproliferative effect of Lupeol (20 mg/kg) and Dotriacontane (20 mg/kg) was lesser than that of the standard drug.

ACKNOWLEDGMENT

We thanks to the laboratory facilities provided by Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopada, Jalgoan.

REFERENCES

1. Sagreiya KP, Singh B. Botanical and Standardised Hindi Names of Important and Common Forest Plants of Madhya Pradesh. Gwalior Government: Regional press; 1958,525.
2. Hooker JD. The Flora of British India. London: Reer and Co; 1875, Vol. 1;1875;527.
3. Anonymous. The Wealth of India, Raw Materials. New Delhi:

Publication and Information Directorate, CSIR; 1976, Vol. 10; 57-8.
4. Kirtikar KR. The Poisonous Plants of Bombay. vol. VI. Jodhpur: India Scientific Publishers: 2003,300.
5. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed, vol. I. New Delhi: Published by Bishen Singh; 1933, 340-5.
6. Nadkarni KM. Indian Materia Medica. Bombay: Popular Book Depot; 1954, Vol. 1; 1172.
7. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: PID; 1956, 235.
8. Santapau H. The flora of Khandala on the western ghats of India. 2nd ed, vol. 16. Calcutta: Govt. of India Press; 1960, 1-3.
9. Agarwal RB, Rangari VD. Phytochemical investigation and evaluation of anti-inflammatory and anti-arthritic activities of essential oil of *Strobilanthus ixiocephala* Benth. Indian J Exp Biol 2003;41:890-4.
10. Agarwal RB, Rangari VD. Antiinflammatory and antiarthritic activities of Lupeol and 19α-H Lupeol isolated from *Strobilanthus callosus* and *Strobilanthus ixiocephala* Roots. Indian J Pharmacol 2003;35:384-7.
11. Pandey DJ, Anand IS. Isolation and high-performance thin layer chromatographic estimation of Lupeol from *Oxystelma esculentum.* Pharm Methods 2011;2:99-5.
12. Jamal AK, Yaacob WA, Din LB. A Chemical study on *Phyllanthus reticulates.* J Phys Sci 2008;19:45-0.
13. Elvira E, Kovač B, Kemal D, Zdenka K, Emin S. Identification And Isolation of Pharmacologically Active Triterpenes in *Betuale cortex*, *Betula pendula* Roth., Betulaceae. Bosn J Basic Med Sci 2009; 9:31-8.
14. 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 2011;2011:128349.
15. Winter CA, Risley EA, Nuss GW. Carrageenin-induced oedemas in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962;111:544-7.
16. Winter CA, Porter CC. Effect of alteration inside chain upon anti-inflammatory and liver glycogen activities of hydrocortisone ester. J Am Pharm Assoc Am Pharm Assoc (Baltim) 1957;46:515-9.
17. Turner RA. Screening Methods in Pharmacology. vol. 1. U.S.A (NY): Academic press; 1965; 105-8.
18. Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. J Ethnopharmacol 2006;104:410-4.
19. Vinegar R, Scheirber W, Hugo R. Biphasic development of carageenin edema in rat. J Pharmacol Exp Ther 1969;166:96-3.
20. Fernandes JC, Spindola H, DeSousa V, Santos-Silva A, Pintado ME, Malcata FX, *et al.* Anti-Inflammatory Activity of Chitooligosaccharides *in vivo*. Mar Drugs 2010;8:1763-8.
21. Swingle KF, Shideman FE. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain antiinflammatory agents. J Pharmacol Exp Ther 1972;183:226-4.
22. Kavimani S, Vetrichelvan T, Ilango R, Jaykar B. Antiinflammatory activity of the volatile oil of *Toddalia asiatica*. Indian J Pharm Sci 1996;58:67-70.

How to cite this article: Sarpate RV, Tupkari SV. Evaluation of anti-inflammatory activity of *Strobilanthus callosus* Nees and *Strobilanthus ixiocephala* Benth. Int J Green Pharm 2012;6:199-203.

Source of Support: Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopada, Jalgoan, Maharashtra, India. **Conflict of Interest:** None declared.