Acta Pharmaceutica Turcica
46: 131-139 (2004)

Antinociceptive and Anti-inflammatory Effects of the Standardized Oil of Indian *Callistemon lanceolatus* Leaves in Experimental Animals

M. Sudhakar[1*], Ch. V. Rao[2], A. Lakshman Rao[1] and D. B. Raju[1]

[1]Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram 534 202 (A.P, India).
[2]National Botanical Research Institute, Ranapratap marg, Lucknow 226 001 (U.P, India).

Abstract

The effect of *Callistemon lanceolatus* (Family: Myrtaceae) leaf oil was studied for the antinociceptive and anti-inflammatory activity in experimental animals. *C. lanceolatus*, 25 – 100 mg/kg administered orally for 3 days exhibited graded dose response equivalent to 21.95% - 89.90% protection in the tail flick latent test in rat. The *C. lanceolatus* oil (50 and 100 mg/kg, p.o X 3 days) was effective in hot plate reaction time (64.05% and 112.97%, $p< 0.01$ and $p< 0.001$), analgesymeter induced mechanical pain (28.17% and 54.42%, $p < 0.01$ and $p < 0.001$) and acetic acid - induced writhing (26.68% and 51.79%, $p < 0.5$ and $p < 0.05$) in mice. The oil of *C. lanceolatus* potentiated the analgesic activity with pentazocine (10 mg/kg, i.p.) and aspirin (25 mg/kg, i.p.). In the carrageenan- induced paw edema *C. lanceolatus* oil (50 and 100 mg/kg, p.o X 3 days) decreased paw volume significantly (26.68% and 51.79%) and dose dependent anti-inflammatory activity in 1-3 hour time interval and potentiated with nimesulide (50 mg/kg, p.o.). This study demonstrates that leaf oil of *C. lanceolatus* has significant antinociceptive and anti-inflammatory activity.

Key words: *Callistemon lanceolatus*, volatile oil, pain, inflammation.

Introduction

Callistemon lanceolatus (Family: Myrtaceae) is an ornamental plant indigenous to Australia. Owing to their crimson red colored spikes, the 2-5 m high shrubs are popularly known as bottlebrush. *C. lanceolatus* is now commonly available in Indian gardens as an ornamental tree. The light petroleum extract of the leaves of Egyptian *C. lanceolatus* yielded ursolic and oleanolic acid (Younes, 1975) A chemical composition of the oil of mature leaves of *C. lanceolatus* reveals that it is comprised of monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes (Misra *et al.*, 1997). The Lambadi tribals of north telangana districts of Andhra Pradesh use this plant for the treatment of pain, gastrointestinal disorders and infectious diseases. There are several reports of the oil exhibited fungitoxicity, inhibiting the growth of fusarium oxysporium, cowpea mosaic virus, mung bean mosaic virus, bean common mosaic virus and southern bean mosaic virus (Pandey *et al.*, 1982; Rao *et al.*, 1986; Singh *et al.*, 1997). The ethnic tribal communities have been using the *C. lanceolatus* from many generations and information regarding the efficacy remains primarily anecdotal. There is no previous record and research work available on the traditional medicinal values of *C.*

* *Corresponding author. E-mail:* muvvala1963@yahoo.co.in

lanceolatus. Most of the ancient knowledge systems continued to survive by oral communication from generation to generation in rural as well as in tribal communities. Therefore, the present study was undertaken to demonstrate scientifically the antinociceptive and anti-inflammatory activities of the standardized oil of *C. lanceolatus* leaves in experimental animals.

Materials and methods

Plant material: C. lanceolatus was marked and the leaves were collected from the medicinal plant garden, Shri Vishnu Educational Society, Bhimavaram, Andhra Pradesh in November, 2002. The plant material was identified and authenticated taxonomically by Prof. Gopala Raju, Dept. of Botany, DNR College of Science, Bhimavaram. A voucher specimen of the collected sample was also deposited in the herbarium of SVCP for the future reference.

Analysis of essential oil: C. lanceolatus leaves (500g) were subjected to hydrodistillation in a Clevenger apparatus for 4.5 hours. In different batches of distillation the yield of the oil varied from 0.65 – 0.8 per cent on fresh weight basis. The oil is colourless with a characteristic aroma having the specific gravity at 29.5° is 0.9074. Optical rotations were measured using a JASCO DIP 181 polarimeter and it is +6.9°. Refractive index at 30° is 1.4604 determined using an Abbe's refractometer. The oil was subjected to gas chromatographic examination on a gas chromatograph equipped with thermal conductivity detector and a stainless steel column (6'X1/4") packed with C22 firebrick (42-60) having 30% coating of carbowax 1000. The apparatus was run with two different isothermal temperatures of 160°C and 100°C (Bhagat, 1975; Misra *et al.*, 1997). It revealed the presence of 17 components in the oil, the major constituents were 1,8, cineole (41.5%), β-pinene (4.2%), α-pinene (4.1%), α-terpineol (7.3%), limonene (6.0%).

Test animals: Charles-Foster (CF) albino rats (110-125 gm) and Wistar strain mice (16-18 gm) of either sex were obtained from the animal house of Dr. B. V. Raju foundation, Bhimavaram. They were kept in the departmental animal house at 25 ± 2°C and relative humidity 45 – 51.5%, light and dark cycles of 10 and 14 h respectively for 1 week before and during the experiments. The animals were provided with standard rodent pellet diet (Hind lever) and water was allowed *ad libitum*. Rearing up of animals in the experimental period and there upkeep during the entire experimental span confirmed to ethical guidelines laid down by Institutional Animal Ethical Committee (IAEC of SVCP, India).

Drug treatment: The essential oil of the leaves of *C. lanceolatus* (suspended in 0.5% carboxy methyl cellulose in distilled water) in doses of 25 - 100 mg/kg was administered once daily for three consecutive days. Nimesulide (Cipla, India) in the dose of 50 mg/kg, p.o was used as the standard anti-inflammatory agent, where as pentazocine (Ranbaxy, India) 10 mg/kg, i.p. and aspirin (Astra – IDL Ltd, India) 25 mg/kg, i.p. were used as standard analgesic agents. All the reference drugs were administered 30 minutes before the experiment. Control group of animals received suspension of 0.5% carboxy methyl cellulose in distilled water. Experiments were conducted on day 3, one hour after last drug or vehicle administration.

Pharmacological tests

Antinociceptive activity: Tail flick latent period: The technique described by Davies *et al.*, (1946) was adopted, using a techno analgesiometer. The rat was placed in a rat holder with its tail coming out through a slit in the lid. The tail was kept on the bridge of the analgesiometer, called jacket with an electrically heated nichrome wire underneath. The tail received radiant

heat from the wire, heated by passing current by 6 mA. The time taken for the withdrawal of the tail after switching on the current, was taken as a latent period, in seconds of tail flicking response and was considered as the index of nociception. The cut off time for determination of latent period was taken at 30 seconds to avoid injury to the skin (Bhattacharya *et al.,* 1971). Three tail flick latencies were measured per rat at each time interval and the means of the tail flick latencies were used for statistical analysis. Pentazocine (10 mg/kg, i.p.) was used as a standard reference.

Hot plate reaction time in mice: Mice were screened by placing them on a hot plate maintained at 55 ± 1°C and recording the reaction time in seconds for fore paw licking or jumping. Only mice which reacted within fifteen seconds and which did not show large variation when tested on four separate occasions, each fifteen minutes apart, were taken for the test. Pentazocine (10 mg/kg i.p) was used as reference standard. The time for fore paw licking or jumping on the heated plate of the analgesiometer was taken as a reaction time (Woolfe and MacDonald, 1944).

Analgesy-metre induced pain: The analgesic effect of *C.lanceolatus* was tested in mice of either sex using an Ugo Basile analgesy-metre. This method involves the application of force to the paw of the mice using the analgesy-metre which exerts a force that increases at a constant rate. The mice were gently placed between the plinth and plunger. The instrument was switched on and constant motor rate was used to drive the plunger on to the paw of mice. When the mice struggles the instrument is switched off and force at which the animal felt pain was read on a scale calibrated in gram X 10 by pointer (Rodriguez Alia, 1990).

Acetic acid induced writhing response in mice: Acetic acid solution at a dose of 10ml/kg (0.6%) was injected i.p. and the number of writhes during the following 15 minutes period was observed (Witkin *et al.,* 1961). Significant reductions in number of writhes by drug treatment as compared to vehicle treatment animals were considered as a positive analgesic response. The percent inhibition of writhing was calculated.

Anti-inflammatory activity: Carrageenan-induced paw edema: Rats were injected with 0.1 ml of 1 % carrageenan into the subplantar region of the left hind paw (Winter *et al.,* 1962). The paw was marked with ink at the level of lateral malleolus and dipped in perspex cell up to the mark. The paw volume was measured with Ugo Basile Plethysmometer (No: 6142, 7140 Comerio-varese, Italy) before and 60, 120 and 180 minute's after injecting the carrageenan suspension.

Statistical analysis: The values are expressed as mean ± SEM. Statistical significance of the differences between control and treated groups was calculated using unpaired Students' t test followed by Mann-Whitney U-test (two tailed). A value of $p < 0.05$ was considered to be significant.

Results

Pentazocine increased the reaction time of *C. lanceolatus* to 238.20% and 257.30% at 50 and 100 mg/kg when compared to the control group. It indicated that the protection by the oil at *Tail flick latent period*- The oil of *C. lanceolatus* at the dose levels of 25, 50, 100 mg/kg exhibited graded dose response equivalent to 21.95% - 89.90% protection. Pretreatment with pentazocine significantly potentiated the antinociceptive effect of *C. lanceolatus* at the dose of 50 and 100 mg/kg producing 115.21% and 144.48% protection when compared to the control group. It may be even stated that the protection by the oil at the dose of 50 and 100 mg/kg, was further potentiated in the presence of pentazocine by 42.98% and 28.75% respectively (Table 1).

Hot plate reaction time in mice- C. lanceolatus (50 and 100 mg/kg) significantly increased the reaction time and the percent protection is equivalent to 64.05% and 112.97% respectivelythe

dose of 50 and 100 mg/kg, was further potentiated in the presence of pentazocine by 106.15% and 67.77% respectively (Table 2).

Analgesy-meter induced pain- The data (Table 3) indicates that the oil of *C. lanceolatus* treated mice exhibited resistance (28.17% and 54.42%) against mechanical pain after 30 minutes. The weight that indicates pain after treatment was dose dependent and significantly synergies the activity of aspirin.

Table 1. Effect of *C. lanceolatus* oil on tail flick latent period in rats.

Treatment	Dose (mg/kg)	Mean latent period of tail flick response (sec)	
		Initial	After 30 min
Control	_	8.05 ± 1.31	8.61 ± 1.42
C. lanceolatus	25	9.09 ± 1.02	10.50 ± 1.00
C. lanceolatus	50	10.01 ±1.00	12.61 ± 1.13[a]
C. lanceolatus	100	9.97 ± 1.35	16.35 ± 1.42[b]
Pentazocine	10	10.12 ±1.25	16.30 ± 1.25[b]
C. lanceolatus + Pentazocine	50 + 10	9.81 ± 0.93	18.53 ± 1.71[b]
C. lanceolatus + Pentazocine	100 + 10	10.10 ± 1.01	21.05 ± 1.25[c]

Values are mean ± SEM for six rats.
P: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to control group.

Table 2. Effect of *C. lanceolatus* oil on hot plate reaction time in mice.

Treatment	Dose (mg/kg)	Mean latent period (sec)	
		Initial	After 30 min
Control	_	10.96 ± 1.10	11.10 ± 1.12
C. lanceolatus	50	11.15 ± 1.15	18.21 ± 2.15[a]
C. lanceolatus	100	11.93 ±1.32	23.64 ± 2.89[b]
Pentazocine	10	11.45 ± 1.39	32.33 ± 4.10[c]

C. lanceolatus + Pentazocine	50 + 10	10.40 ± 1.05	37.54 ± 3.54[c]
C. lanceolatus + Pentazocine	100 + 10	10.69 ± 1.19	39.66 ± 3.93[c]

Values are mean ± SEM for six mice.
P: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to control group.

Table 3. Effect of *C. lanceolatus* oil on force induced pain in mice.

Treatment	Dose mg/kg)	Weight causing pain (g)	
		Before administration	After administration
C. lanceolatus	50	85.9 ± 4.72	110.1 ± 5.99[a]
C. lanceolatus	100	86.0 ± 5.61	132.8 ± 7.05[b]
Aspirin	25	86.2 ±6.20	131.4 ± 7.07[b]
C. lanceolatus + Aspirin	50 + 25	83.3 ± 4.35	142.0 ± 7.13[b]
C. lanceolatus + Aspirin	100+ 25	85.1 ± 5.17	148.0 ± 8.32[b]

Values are mean ± SEM for six mice.
P: [a]< 0.01 and [b]< 0.001 compared to respective before administrative group.

Acetic acid induced writhing- The oil of *C. lanceolatus* showed a significant decrease in writhing response induced by acetic acid and the degree of percent inhibition was 26.68% and 51.79% at 50 and 100 mg/kg. Under the same experimental condition the analgesic effect of *C. lanceolatus* potentiated the analgesic activity of aspirin as shown by further decrease in the writhing response prevented the abdominal cramping, when given in combination. Or it may be stated that the protection by the oil at the dose of 50 and 100 mg/kg, was further potentiated in the presence of pentazocine by 51.85% and 37.22% respectively (Table 4).

Table 4. Effect of *C. lanceolatus* oil on acetic acid-induced writhing in mice.

Treatment	Dose (mg/kg)	Number of writhing	% Inhibition
Control	-	25.41 ± 3.11	
C. lanceolatus	50	18.63 ± 2.33	26.68

C. lanceolatus	100	12.25 ± 2.85[a]	51.79
Aspirin	25	10.55 ± 1.94[b]	58.48
C. lanceolatus + Aspirin	50 + 25	8.97 ± 1.51[c]	64.69
C. lanceolatus +Aspirin	100 + 25	7.69 ± 1.21[c]	69.74

Values are mean ± SEM for six mice
P: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to control group

Carrageenan induced paw edema- Treatment with different doses of *C. lanceolatus* oil at 50 and 100 mg/kg showed a significant decrease in paw volume (26.68% and 51.79%) and dose dependent anti-inflammatory activity with time interval 1-3 hours. The effect was similar to nimesulide and potentiated the activity of *C. lanceolatus* (Table 5).

Table 5. Effect of *C. lanceolatus* oil on carrageenan induced paw edema in rats

Treatment	Dose (mg/kg)	Paw volume (ml) at		
		60 min	120 min	180 min
Control	-	0.89± 0.03	1.05± 0.04	1.06± 0.03
C. lanceolatus	50	0.85± 0.03	0.94± 0.03	0.85± 0.02[c]
C. lanceolatus	100	0.83± 0.02	0.88± 0.02[b]	0.63± 0.01[c]
Nimesulide	50	0.75± 0.02[b]	0.64± 0.02[c]	0.62± 0.01[c]
C. lanceolatus + Nimesulide	50 + 50	0.80± 0.01[a]	0.52± 0.02[c]	0.48± 0.01[c]
C.lanceolatus+ Nimesulide	100 + 50	0.77± 0.01[b]	0.47± 0.01[c]	0.41± 0.01[c]

Values are mean ± SEM for six rats.
P: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to respective control group

Discussion

C. lanceolatus showed significant antinociceptive and anti-inflammatory effects on the experimental animal models. The oil of *C. lanceolatus* was found to increase significantly the tail flick reaction time. This test is useful for discriminating between centrally acting opiate and non opiate analgesics, giving positive response with the former only (Kulkarni, 1993). The essential oil of *C. lanceolatus* exhibited analgesic activity in rats and potentiated in the presence of pentazocine. Hot plate reaction time in mice method was originally described by Woolfe and

MacDonald (1944). This test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance (Plummer *et al.*, ,1991). The significant results indicate that *C. lanceolatus* may be acting centrally. Analgesy-meter induced pain is the force applied to the paw by the plinth increases at a constant rate, being the motor synchronous with mains frequency, its speed (60rpm) is constant, unaffected by friction and wear. The force measured on the scale is in 10 gram steps by a pointer riveted to the slide. *C. lanceolatus* significantly alleviated the pain threshold. This offers new perspectives in the treatment of pain, as there is evidence that a symptom of vital pain varies in intensity with central and peripheral somato sensory pathways (Alcaraz and Jimenez, 1988). In the acetic acid induced writhing response, *C. lanceolatus* significantly inhibited the abdominal constriction and potentiated the activity of aspirin in mice. Acetic acid causes an increase in peritoneal fluids of PGE_2 and $PGF_2\alpha$ involving in part, peritoneal receptors (Deraedt *et al.*, 1980; Bentley *et al.*, 1983) and is very sensitive method of screening antinociceptive effect of compounds (Collier *et al.*, 1964). The capacity of prostaglandins to sensitize pain receptors to mechanical and chemical stimulation appears to result from a lowering of the threshold of the polymodal nociceptors of C fibres (Goodman and Gilman, 1996).

Similarly leaf oil of *C. lanceolatus* exhibited significant anti-inflammatory activity in carrageenan- induced edema in rats. It is evident that carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) commonly used to induce acute inflammation and it is believed to be biphasic. The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome (Castro *et al.*, 1968). It has been reported that the second phase of edema is sensitive to the most clinically effective anti-inflammatory drugs, which has been used frequently to access the anti-edematous effect of natural product (Della Loggia *et al.*, 1968; Alcaraz and Jimenez, 1988). Prostaglandin plays a major role in the development of second phase of reaction that is measured at three-hour time (DiRosa, 1972). Based on these reports it can be inferred that the inhibitory effect *C. lanceolatus* on carrageenan- induced inflammation in rats might be due to inhibition of mediators responsible for inflammation and pain. Thus, the present observation indicates the antinociceptive and anti-inflammatory activity of *C. lanceolatus*.

Acknowledgement

The authors thank Dr C.M.Gupta, Director, CDRI, Lucknow, India for the help extended to record the GC, GC/MS of the *Callistemon lanceolatus* leaf oil.

References

Alcaraz, M. J. and Jimenez, M. J. (1988). Flavonoid an anti-inflammatory agent. *Fitoterapia.* 59: 25–38.

Bentley, G. A., Newton, S. H. and Starr, J. (1983). Studies on the antinociceptive action of α-agonist drugs and their interaction with opioid mechanisms. *Br. J. Pharmacol.* 79: 125-134.

Bhagat, S. D. (1975). Oil of *Callistemon lanceolatus*: Chemical constituents and commercial prospects. *The Indian J. Pharmacy* 37: 158-160.

Bhattacharya, S. K., Raina, M. K., Banerjee, D. and Neogy, N. C. (1971). Potentiation of morphine and pethidine analgesia by some monoamine oxidase inhibitor. *Indian J. Exp. Biol.* 9: 257-262.

Castro, J., Saseme, H., Sussman, H. and Bullette, P. (1968). Diverse effect of SKF 52 and antioxidants on CCL_4 induced changes in liver microsomal P-450 content and ethylmorphine metabolism. *Life Sciences* 7: 129-136.

Collier, H. O. J., Dinneon, L. C., Johnson, C. A. and Schneider, C. (1964). The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br. J. Pharmacol.* 32: 295-310.

Davies, O. L., Raventos, J. and Walpole, A. L. (1946). A method for evaluation of analgesic activity using rats. *Br. J. Pharmacol.* 1: 255-261.

Della Loggia, A., Tubaro, A., Dri, P., Zilli, C. and Del Negro, P. (1968). The role of flavonoids in the anti-inflammatory activity of *Chamomilla recutita. Clinical Biol. Res.* 213: 481–486.

Deraedt, R., Jougney, S., Delevalacee, Fig. and Falthour, M. (1980). Release of prostaglandin E and F in algogenic reaction and its inhibition. *Eur. J. Pharmacol.* 51: 17-24.

Di Rosa, M. (1972). Biological properties of carrageenan. *J. Pharmacy Pharmacol.* 24: 89–102.

Goodman and Gilman's The Pharmacological Basis of Therapeutics (1996). (eds Hardman JG, Limbird LE) McGrawhill, New York. 9th edition: 620.

Kulkarni, S. K. (1993). Hand Book of Experimental Pharmacology, Vallabh Prakashan. New Delhi, 2nd edition. pp.49-56.

Misra, L. N., Huq, Fig., Ahmad, A. and Dixit A. K. (1997). Chemical composition of the essential oils of *Callistemon lanceolatus* DC and *C. Polandi.* F. M. Bailey. *J. Essential Oil Res.* 9: 625-628.

Pandey, D. K., Chandra, H. and Tripathi, N. N. (1982) Volatile fungitoxic activity of some higher plants with special reference to that of *Callistemon lanceolatus* DC. *Phytopathologische. Zeitschrift.* 105: 175-182.

Plummer, J. L., Cmiellewski, P. l., Gourlay, G. K., Owen, H. and Cousins, M. (1991). Assessment of antinocipective drug effects in the presence of impaired motor performance. *J. Pharmacol. Meth.* 26: 79- 83.

Rao, G. P., Pandey, A. K. and Shukla, K. (1986). Essential oils of some higher plants *vis-a-vis* some legume viruses. *Ind. Perfumer* 30: 483-486.

Rodriguez Alia, R. E. (1990). Antinociceptive activity of glycosidic enkephalin analogues. *Psychopharmacol.* 101: 222–225.

Singh, S. P., Mishra, M., Rao, G. P., Singh, M., Upadhyaya, P. P. and Singh, M. (1997). Efficacy of essential oils of aromatic angiosperms against plant viruses. *J. Living World* 4: 39-44.

Winter, C. A., Risley, E. A. and Nus, G. *N.* (1962). Carrageenan induced edema in the hind paw of the rats as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 111: 544-547.

Witkin, L. B., Huebner, C. F., Galdi, F. O., Keefe, E., Spitaletta, P. and Plumer, A. J. (1961). Pharmacology of 2 amino-indane hydrochloride (SU 8629); A potent non-narcotic analgesic. *J. Pharmacol. Exp. Therapeutics* 133: 400–408.

Woolfe, G. and MacDonald, A. D. (1944). The evaluation of the analgesic action of pethidine hydrochloride (DEMEROL). *J. Pharmacol. Exp. Therapeutics* 80: 300-305.

Younes, M. E. (1975). Chemical examination of local plants. XIV Triterpenoids from the leaves of Egyptian *Callistemon lanceolatus*. *Australian J. Chem.* 28: 217-220.

Received: 10.03.2004
Accepted: 01.04.2004