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Research Article

ISOLATION OF PIPERIDINE ALKALOIDS FROM THE ROOTS OF LOBELIA NICOTIANAFOLIA AND ITS ANTI INFLAMMATORY ACTIVITY

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ABSTRACT

Several important piperidine alkaloids lobeline, lobelidine, lobelanine, nor lobeline, lobelanidine, isolobeline have been isolated from the various parts of plant Lobelia nicotianafolia of family companulaceae. Though in traditional, siddha and ayurvedic system of medicine and tribal medicine in India and other countries, multiple clinical applications like aphrodisiac, diuretic, antispasmodic, anti-septic, anti-asthmatic, anti-bacterial and scorpion bite has been indicated for this plant. In view of the prevalent use mentioned by the folklore in the treatment of inflammation and jaundice. This gave us an impetus to isolate piper dine alkaloids from lobelia nicotianafolia and to test for anti-inflammatory activity. Ethylacetate extract was prepared from the roots of Lobelia nicotianafolia and chromotographed over a column of silica gel by gradient elution technique. During the study the phytochemical investigation reveals that there was an existence of new compound which is coded as LN1, hence we felt worthwhile to investigate the new compound for its structural elucidation and anti-inflammatory activity.

Keywords: Lobelia nicotianafolia , Piperidine alkaloids and structural elucidation.

INTRODUCTION

The use of natural products with therapeutic properties is ancient as human civilization for a long time, mineral, plant and animal products were the main source of drugs[1].With the rapid progress in various fields of human activity, the field of medicine and allied sciences as rapid strides. Several available potent drugs used today are extracted from plants and synthetic drugs are made from lead molecules which are derived from plants. In European and American countries up to 25% of all prescriptions include plant products or plant derivatives [2]. In developing countries, medicinal plants continue to be the main source of medication. Most of the drugs that are currently used in different countries,77% of them are being obtained from plants in traditional medicine.[3]Inflammation is the common symptom of various diseases having disturbance in the normal phy siological function of the body it can be caused by physical trauma, noxious chemicals or microbiological agents which may leads to accumulation of plasma fluid and the blood cells.[4]A number of inflammation mediators such as pro inflammatory cytokines , interleukins(IL -1 , IL- 6, IL-12, IL-18), Tumor necrosis factor(TNF),Interferon(INF) and the granulocyte – macrophage colony stimulating factors are involved[5]. Conventionally, inflammatory conditions are treated by non steroidal anti-inflammatory drugs(NSAIDS) but they produce serious side effects like gastric lesion[6]

Lobelia nicotianafolia an herbaceous plant , family companulaceae native to India, especially western Ghats of south canara region where it is used to treat infectious diseases. the literature survey indicates that the plant was used for the treatment of jaundice and inflammatory conditions. T he plant and its extracts are reported to possess aphrodisiac, diuretic, stomachic, cure kapha, strangury, and disease of blood, the heart, the uterus, the vagina, cure burning sensation. An Infusion of leaves are used as antspasmodic[7] it also used as antiseptic[8] anti asthmatic[9] and antibacterial[10]. All the reported work of the mentioned plant gave us interest to focus for

its further studies. It has been well accepted concept that all pain, whether acute or chronic, peripheral or central, originates from inflammation and inflammatory response[11]The carragenan model is a well established paradigm for studying an inflammatory pain[12]. The main objective is to isolate and characterize the lead molecule which might show anti-inflammatory activity from the roots of Lobelia nicotianafolia.

MATERIALS AND METHODS

COLLECTION AND AUTHENTICATION

The plant Lobelia nicotianafolia was collected from the western Ghats of south canara, cleaned and dried at room temperature in shade. And kept away from direct sunlight and it was authenticated by Dr.venkaiah, taxonomist Andhra university.(voucher specimen BGR-3-6-2006).

Extraction& Isolation

Shade dried root powder was extracted in sox let apparatus for 6 hours with ethyl acetate and concentrated to dryness under reduced pressure.[13]

Preliminary phytochemical analysis of the crude extract was conducted and ethyl acetate soluble extractive found 8.75%. The TLC was carried out using benzene: ethylacetate:diethylamine 6:3:1 and developed TLC plate was sprayed with dragendorffs reagent and heated at 100c spots will be developed. It was observed under UV 365 nm yellow, dark blue spots and with dragendorffs reagent it gives orange spots. Extract of ethyl acetate gave dark green crystals which was coded as LN1, subjected for further spectral studies for the determination of structural parameters.

Experimental Animals

Wistar albino rats of either sex (150-220 g) procured from Mahaveer Enterprises, Hyderabad, India. and were used to study the anti-inflammatory activity. The rats were randomly distributed into groups and housed in cages (5 per cage). The animals were maintained under standard laboratory conditions (light period of 12h/day and temperature $22^0C \pm 2^0C$), with free access to standard rodent pellet diet (Ratan brothers, India) and water ad libitium. The experiment was cleared by Institutional Animal Ethical Committee and regulatory body of Government (Regd. No.516/01/A/CPCSEA).

Drugs And Chemicals

Carrageenan, sodium car boxy methyl cellulose, ibuprofen were obtained from Sigma-Aldrich, India. All the other chemicals were of analytical grade.

Anti-Inflammatory Activity of the Extract & Isolated Compound

Carrageenan-Induced Paw Oedema in Rats

Anti-inflammatory activity was assessed by the method described by Winter et al., The rats were divided into 5 groups of five animals each.

Oedema was induced by sub plantar injection of 0.1 ml of 1% w/v freshly prepared suspension of Carrageenan to each animal of 5 groups. The ethyl acetate extract was suspended in 1% sodium CMC solution. The test groups received the extract (200, 400 and 800 mg/kg, p.o), LN1 (2.5×10^{-5} moles/kg), the standard group received Ibuprofen (2.5×10^{-5} moles/kg, p.o), and the control group received drug vehicle (1% sodium CMC) only. All the doses were given orally 2 hours prior to the injection of Carrageenan.

Group I: Received 1% sodium CMC

Group II: Received Ibuprofen (2.5×10^{-5} moles/kg). p.o

Group III: Received ethyl acetate extract of Lobelia nicotianafolia 200 mg/kg, p.o

Group IV: Received ethyl acetate of Lobelia nicotianafolia 400 mg/kg, p.o

Group V: Received ethyl acetate of Lobelia nicotianafolia 800 mg/kg, p.o

Group VI : Received isolated compound LN1 (2.5×10^{-5} moles/kg). p.o

The paw thickness of each rat was measured by using Zetlin's apparatus [14] before and at 1, 2, 3, 4, 5 and 6th hour after Carrageenan injection. The percentage inhibition of paw oedema was calculated by using the following formula.

% Increase in paw thickness =

$$\frac{Y_t - Y_0}{Y_0} \times 100$$

Where,

Y_t = Paw thickness at time t (1, 2, 3, 4, 5 and 6th) after injection

Y_o = Paw thickness at 0 hr (before injection).

STATISTICAL ANALYSIS

Results of the study were expressed as Mean ± S.E.M. ANOVA followed by Dennett's test were used to determine significant differences between groups. P- values less than 0.05 and 0.01 were considered as indicative of significance.

RESULTS AND DISCUSSION

The ethyl acetate fraction of Lobelia nicotianafolia was subjected to column chromatography over silica gel. Column and

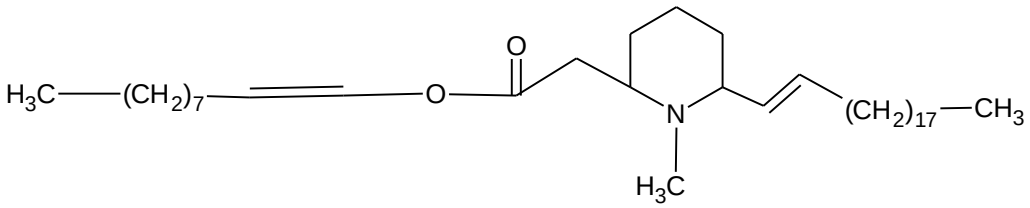
fractions were monitored using silica gel TLC to assess the homogeneity of the compound and the fractions showed similar spots were mixed together. the isolated LN1 compound was identified & Named as ***Nicotianafoline.***

Analytical IR, NMR, MASS Spectral Data

LN1(nicotianafolin) C37H71O2N,mol.weight =573,m.p=180,Rf=0.82,Rt=18.5 min, IR(KBR)Vmax:1732(c=o stretching), H^1 NMR spectrum indicates the presence the unsaturated protons by exhibiting peeks at δ 5.3. The signals at δ 4.1, 3.8, 3.4 & 3.2 indicate the presences of protons in which the carbon atoms are connected to oxygen atom are nitrogen atom. The multiple signals at δ 0.86 indicate the presence of methyl groups attached to methylene groups. The proton signals between 2.0 & 2.7 indicates that some methylene protons are attached to carbonyl groups or unsaturated systems. The strong signal at δ 1.25 shows the presence of long chain methylene groups. It was further conformed by its 13C NMR spectrum by exhibiting a signal at δ 189.62 indicates the presence of carbonyl group, the signals at δ 108.24, 127.96, 130.07 & 126.58 indicated the presence of two double bonds, the strong signal at δ 29.38, was due to the carbon atoms of long chain methylene groups ,The signals at δ 14.12 & 14.27 were due to two methyl carbon atoms , the signals at δ 37.36 & 44.93 may be due the carbons attached to the N. atom, the other signals at δ 22.64, 25.04, 31.95, 32.83, 33.75 suggest the presence of the other methylene carbons connected to unsaturated systems and carbonyl groups

The – Ve mode APCL - MS indicates an ion at M/Z 573 and the mode APCL-MS also

Based on the above data, the compound(**LN-1**) is confirmed as nicotianafoline and reported for the first time from our findings.



LN-1

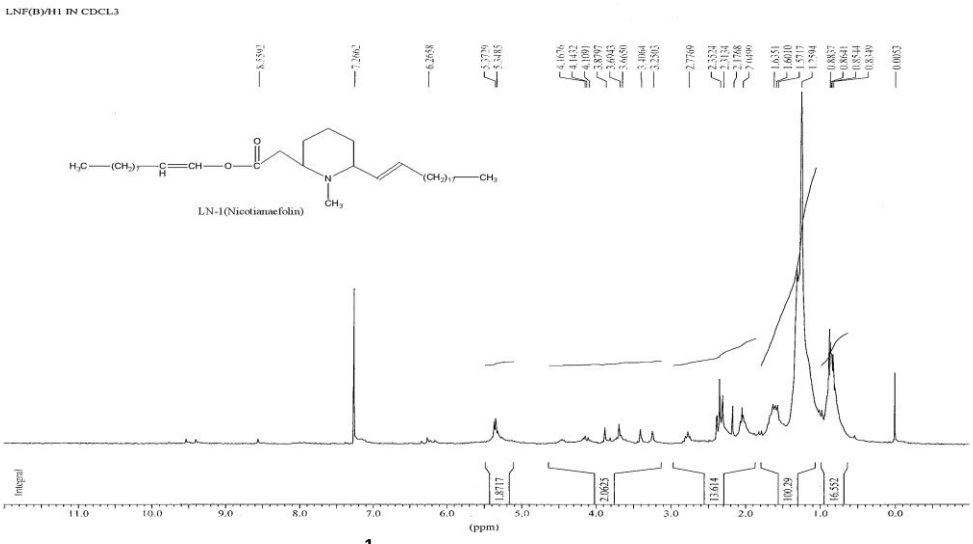


Fig. 1: ^{1}H NMR Spectrum of LN-1

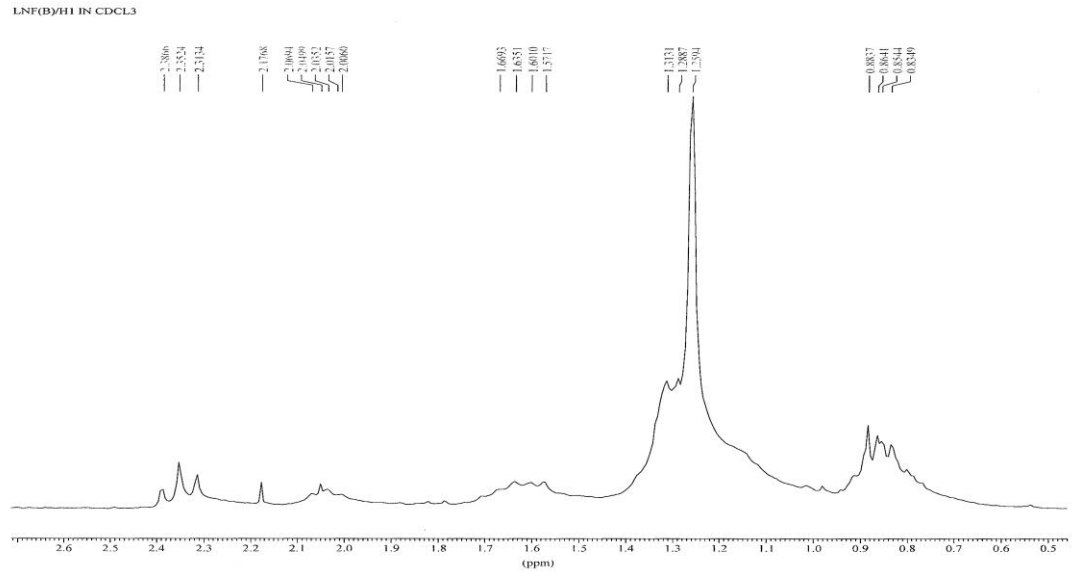


Fig. 2: ^{1}H NMR Spectrum of LN-1

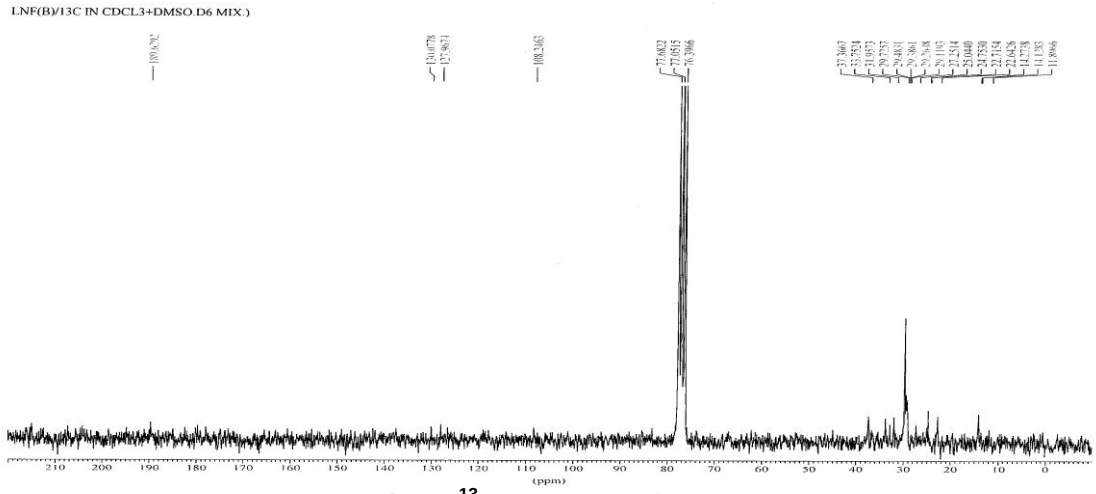
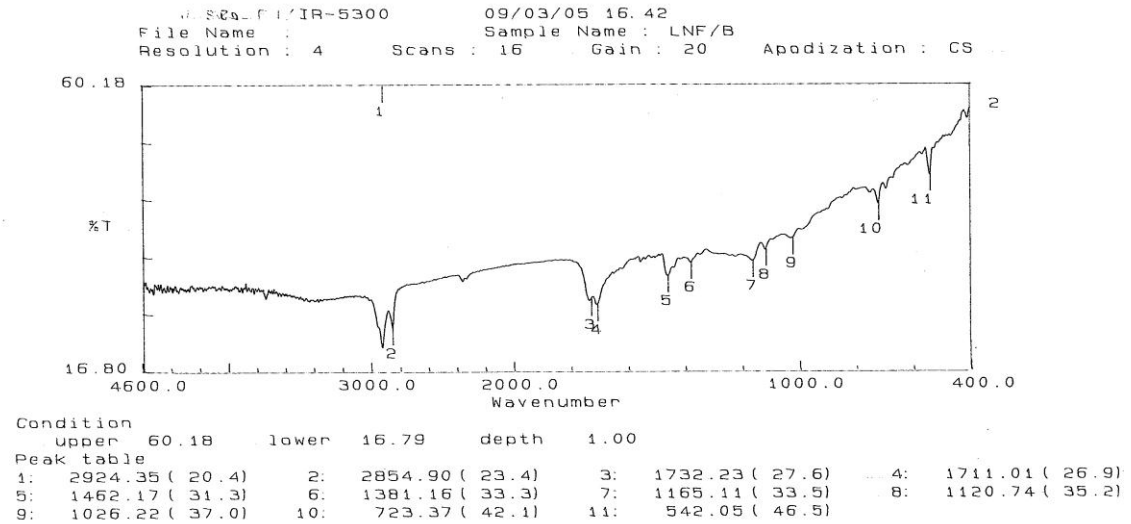


Fig. 3: ^{13}C Spectrum of LN-1



Condition
upper 60.18 lower 16.79 depth 1.00

Peak table

1:	2924.35 (20.4)	2:	2854.90 (23.4)	3:	1732.23 (27.6)	4:	1711.01 (26.9)
5:	1462.17 (31.3)	6:	1381.16 (33.3)	7:	1165.11 (33.5)	8:	1120.74 (35.2)
9:	1026.22 (37.0)	10:	723.37 (42.1)	11:	542.05 (46.5)		

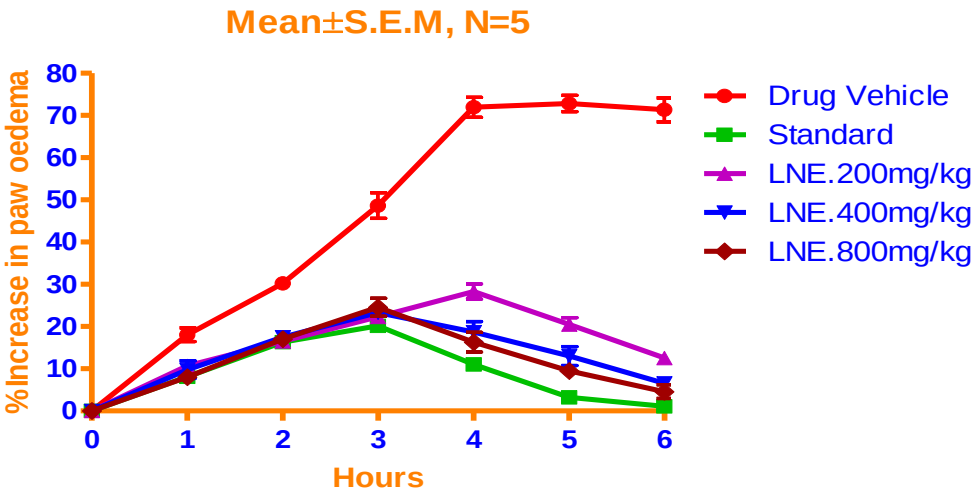
Fig. 4: ^{13}C Spectrum of LN-1

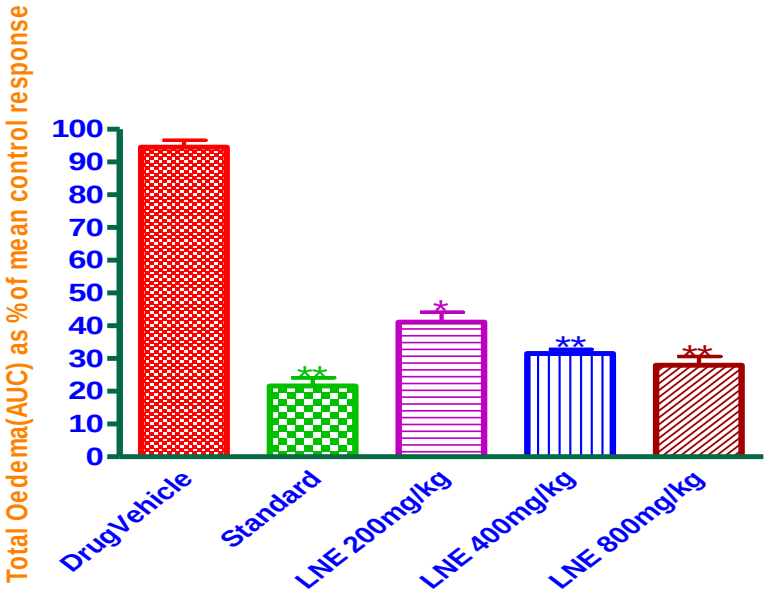
The anti-inflammatory activity of ethyl acetate extract of Lobelia nicotianafolia roots against carrageenan induced paw oedema is present in table 1. The results suggested that the standard drug Ibuprofen significantly inhibited paw oedema, where as the ethyl acetate extract of *L.nicotianaefolia* produced significant and dose dependant effect in reducing paw oedema at the two phases of inflammation when compared to the drug vehicle treated group. The extract at 400 mg/kg, p.o, showed maximum inhibition of oedema as that of 200 and 800 mg/kg. The results obtained indicates that the extract found to have significant (P<0.01) anti-inflammatory activity in rats.

Table 1: Percentage inhibition of carrageenan induced paw oedema in rats by prophylactic treatment with the ethylacetate extract of *L.nicotianaefolia* and ibuprofen

Treatment	Percentage inhibition of the maximal paw oedema during 6h.	Percentage inhibition of total AUC paws oedema during 6h.
Group I	0.0 ± 1.95	0.0 ± 7.47
Group II	72.41 ± 1.49**	79.65 ± 2.29**
Group III	61.22 ± 1.78*	60.42 ± 1.06*
Group IV	68.06 ± 0.80**	68.50 ± 0.45**
Group V	66.34 ± 2.15**	71.28 ± 1.26**

Significance:*P<0.05, **P<0.01





Influence of purified molecule LN-1 from *L.nicotianaefolia* on carrageenan-induced rat paw oedema

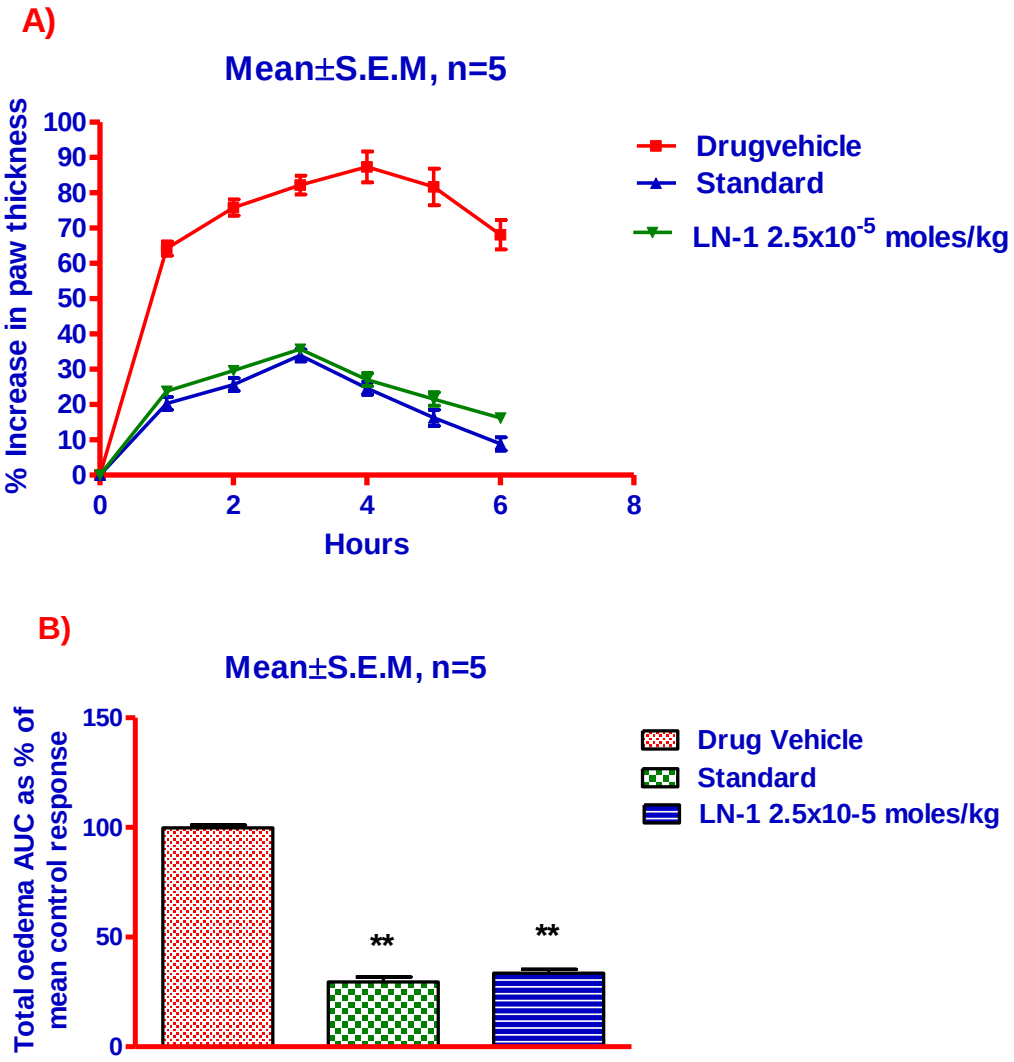
Table 2 shows that the Pure compound LN-1 significantly inhibited the maximal oedema response by 61.32 ±1.72 and 59.26 ± 1.40 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 70.50 ± 1.83 and 66.18 ± 1.93 respectively over 6 h. The pure molecule exhibited significant reduction in reducing paw oedema when compared to the control group at all evaluated intervals of time.

The pure molecule exhibited almost 97% equipotency compared to standard drug treated group.

Table 2: Percentage inhibition of carrageenan induced paw oedema in rats by prophylactic treatment with the pure isolated molecule LN-1and ibuprofen

Treatment	Percentage inhibition of the maximal paw oedema during 6h.	Percentage inhibition of total AUC paw oedema during 6h.
Group I	0.0 ± 4.39	0.0 ± 5.49
Group II	61.32 ± 1.72**	70.5 ± 1.83**
Group VI	59.26 ± 1.40**	66.18 ± 1.93**

Significance:*P<0.05, **P<0.01.



Effect of the pure molecule isolated from *L.nicotianaefolia* along with ibuprofen (2.5 x 10^{-5}moles/kg body wt.) on A) the maximal and B) the total paw oedema in carrageenan induced rats

Significance:*P<0.05, **P<0.01

SUMMARY

In summary, our research findings showed that the ethyl acetate extract of Lobelia nicotiana folia roots as well as our isolated new compound Nicotianafoline had relevant, beneficial Antiinflamatory activity. Further studies need to be done to know the exact potency of the isolated compound nicotianafoline.

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REFERENCES

1. Pasquale De. Pharmacology: The oldest modern science. J of Ethnopharmacol. 1984;11(1):1-16.
2. Jaroszewski JW. Natural products and drug development, Pharmacy. 1984;5:27-28
3. Fransworth NR, Akerele AS and Bingel. In New Natural products and plant drugs with pharmacological, biological or therapetuical activity. Eds. H Wagner and P Wolff. 1977;61-73.
4. Radoslaw sobota. Biochem.biophys Res.commun. 2000;267:329-333.
5. Babu NP. J ethnopharmacol. 2009;125:356-360.
6. Tarnawskias and Caves TC. Gastroenterol. 2004;127:341-343.
7. Kiritikar KR and Basu BD. Indian Medicinal Plants 2nd edition, International book publishers, Dehradun. 1999;2:1452-1454.
8. The Useful plants of India. National institute of science communication, New Delhi. 2000.
9. Krishnamaraju Alluri V, Tayi IVN Rao, Sunderaraju D and Vanisree M. International Journal of Applied Science Engineering. 2006;4(2):115-125.
10. Natchimuthu Karmegam, Karuppusamy, Maniprakash, Manijaykumar, Kuppuraju and Rajasekar. International journal of Bio Medical and Pharmaceutical Sciences. 2000;2(2).
11. Omoigui S. The biochemical origin of Pain: the origin of all pain is inflammation and the inflammatory response. Part 2 of the 3-inflammatory profile of pain syndromes. Med Hypotheses. 2007;69:1169-78.
12. Winter CA, Risley EA and Nuss GW. Carrageenan induced oedema in the hind paw of the rats as an assay for anti-inflammatory drug. Proc Soc Exptl Biol Med. 1962;111:544-547.
13. Harborne JB. Phytochemical Methods, Chapman and Hall; London. 1973;5-8.
14. Battu GR, Zeitlin IJ, Gray AI and Waterman PG. Brit J Pharmacol. 1999;128:274-280.