The Journal of Phytopharmacology 2015; 4(2): 68-72
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2015; 4(2): 38-72
March- April

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Anti-inflammatory activity of *Nelsonia canescens* (Lam)Spreng. root in albino rats.

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Abstract

Nelsonia canescens (Lam).Spreng. from Acanthaceae family is traditionally known as Bada-rasna in the western part of Odisha, India. Ethnic people use root of this plant for its anti-inflammatory properties in the name of Rasna, a well known Ayurvedic analgesic and anti-inflammatory drug. Hence, present study was undertaken to evaluate anti-inflammatory activity of the root powder of *Nelsonia canescens* (Lam).Spreng. In this study two dose levels selected (270 mg/kg and 540 mg/kg) from root powder of *Nelsonia canescens* for evaluating the Acute and chronic anti-inflammatory activity in Wistar albino rats, using animal models of carrageenan-induced paw oedema, formaldehyde-induced hind paw oedema and cotton pellet-induced granuloma formation respectively. Both dose levels Therapeutic Equivalent Dose (TED) and TED×2 showed significant anti-inflammatory against chronic inflammation. In Carrageenan-induced paw oedema, treatment with *Nelsonia canescens* root at the TED dose level inhibited inflammation at marginal level, while at TED×2 did not show any impact. Furthermore, test drug at TED dose level significantly inhibited formalin induced paw oedema inflammation and interestingly, the effect was even better than result of standard drug. The study indicate that drug is having anti-inflammatory effect on animal models and According to results we can conclude that the root of *Nelsonia canescens* has an effect on sub-acute inflammation and mild to moderate effect against acute inflammation.

Keywords: Ayurveda, folklore medicinal plant, Inflammation, *Nelsonia canescens*.

Introduction

The World Health Organization (WHO) has defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today.[1] Herbal medicines offer safe and effective treatment and are in great demand in the developed world for primary health care. Now a days, they are also used as therapeutic drugs for age-related disorders such as memory loss, osteoporosis, immune disorders for which no modern medicine is available in the market.[2] Herbal medicines are the synthesis of therapeutic experiences from generations of traditional healers, and in many countries, the plant drugs are still in use in its traditional way and modern health care systems for treatment of different disorder and to improve the health.

Inflammation is one of the types of disease which is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells and/or irritants.[3] It is a body defense reaction in order to eliminate the spread of injurious agent as well as to remove the consequent necrosed cells and tissues. Inflammation helps to clear the infections and along with repair, makes wound healing possible, both have considerable potential to cause harm. For example, inflammatory reactions underlie life-threatening anaphylactic responses to insect bites or drugs. An initial inflammatory stimulus triggers the release of chemical mediators from plasma or cells, which then regulate the subsequent vascular and cellular responses.[4]

Nelsonia canescens (Lam).Spreng. is one of the medicinal plant grow abundantly in various parts of Odisha, India[5] and also found in the Gujarat,[6] Assam,[7] and Madras[8]. Traditionally, the root of this plant is used by the traditional healers of Odisha (in the local name of *Badarasna-Rasna*) for the treatment of various inflammatory and painful conditions, especially in arthritic conditions.[9, 10] In spite of its reputation in treating these ailments, till date no pharmacological work to support these claims has been reported. Hence, the present study was designed to evaluate the anti-inflammatory activity of root of *Nelsonia canescens* in experimental rats.

Materials and Methods

Chemicals and drugs

Dexomethasone (DEXONA, Cadila Healthcare Ltd, India), diclofenac sodium (VOVERAN-D, Novartis India Ltd). Phenylbutazoneand carrageenan solutions (Wilson Laboratories, Mumbai) Formaldehyde solution (37-41%) (Himedia laboratories Ltd, Mumbai). All other chemicals were of the highest purity commercially available.

Plant material

Nelsonia canescens (Lam.)Spreng. Identified, authenticated and collected from its natural habitat of Gandhamardana hills range, Bolangir, Odisha, India. A herbarium with a voucher specimen (Phm no. 6002/11) prepared and deposit in the pharmacognosy laboratory for future references. The roots dried and made to the fine powder. The powder dissolved in distilled water subjected to shaking before each administration time. Doses of the drug were fixed by extrapolating the human dose in laboratory animals as per the method described by Paget and Barnes.[11] Experiments were carried out at two dose levels TED (270 mg/kg) and TED×2 (540 mg/kg).

Animals

The Wistar albino rats of either sex 220 ± 20g were obtained from the central animal house of the institute. Animals maintained with standard pellet diet (Amrut, Pranav Agro Industries Ltd., India) and tap water was given adlibitum. The animals were housed in a five rats per cage under well controlled conditions of temperature (25 ± 1 °C), humidity (55 ± 5%) was kept at optimum and animals were exposed to 12 hr light-dark cycles (Lights on 0700). The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC/08-11/04-M.Sc) and all the experimental procedures were performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal welfare division, Government of India, New Delhi.

In present study different protocols carried out based on acute inflammatory models (Carrageenan and formaldehyde-induced paw edema) and chronic inflammatory model (Granuloma pouch technique).

Acute inflammatory models

Carrageenan-induced paw oedema

The effect of test drug on Carrageenan-induced paw oedema in rats was studied as per the method described by Winter *et al*.[12] Wistar albino rats of either sex divided into four groups of six rats each. Group I served with tap water as a control. Groups II and III were treated with the test drug in TED and TED×2, respectively by oral route. The test drug was administered once daily for five consecutive days before carrageenan injection. The Group IV administered with the standard drug phenylbutazone (100 mg/kg, p.o.). On 5th day prior to carrageenan injection the initial paw volume of the left hind paw was measured using a Plethysmometer (Electronic-IITC, California).

Formaldehyde-induced paw oedema

The procedure of Brownlee (1950) as described by Nataraj employed.[13] The rats were divided into four groups of six each irrespective of weight and sex. Group I treated orally with tap water. Group II and Group III were treated with the test drug in TED and TED × 2, respectively. The drug was administered once daily for five consecutive days. The Group IV administered with the standard drug diclofenac sodium (5 mg/kg, p.o.). On 5th day, initial left hind paw volumes were recorded with the help of Plethysmometer. Subsequently, formaldehyde solution (0.1 ml of 2% v/v) was injected by subcutaneous (S.C.) route in the plantar aponeurosis into the left hind paw, one hr after the drug administration. Paw edema volumes were again measured in 24 hr and 48 hr after formaldehyde injection.

Chronic inflammatory model

Cotton pellet-induced granuloma formation

The effect of test drug on cotton pellet-induced granuloma formation in rats was studied as per the method described by Garcia *et al*.[14] Wistar albino rats of either sex divided into four groups of six each. The rats were anaesthetized with ether. Dorsum was shaved and swabbed with 70% (v/v) alcohol. Midline incision of 1 cm was made in the intrascapular region. A small tunnel was made on either side of the incision with the help of small blunt forceps. One sterile cotton pellet weighing 50 mg (prepared by rolling a cotton piece of 50 mg and sterilized by autoclaving for 30 min under 15 lb pressure) inserted per tunnel and closed the incision with interrupted sutures after expelling the air from the tunnel.

Group I, treated with tap water and considered as a control group. Group II and III were administered with the test drug in TED and TED×2, respectively, for 7 consecutive days starting from the day of implantation. The Group IV was administered with the standard Dexomethasone (100 µg/kg p.o). The rats were sacrificed on 8th day and dissected for collection of thymus, spleen, liver, adrenal glands and cotton pellets were removed and cleaned of extraneous tissue and dried on hot air oven overnight at 80°C and then weighed. The difference between the initial and the final weight of the pellet after drying was taken as the granuloma tissue weight. The result was expressed as mg granulation tissue formed per 100 g body weight.

Histopathological and biochemical investigation

The adrenal glands, spleen, liver and thymus were collected and weighed. In addition, blood samples were collected from neck blood vessels and the separated serum was utilized for the estimation of orosomucoid content. The tissue sections of the 5-6 µm thickness were cut, washed and stained. The slides were viewed under binocular research Carl-Zeiss microscope (Germany) at various magnifications to note down the changes in microscopic features and histopathological architecture.

Statistical analyses

All the data are presented as Mean ± S.E.M. The significance of differences in the mean between control and treated animals for different parameters was determined by using One-way Analysis of variance (ANOVA) followed by multiple comparisons Dunnett's test. A value of $P<0.05$ was considered as statistically significant.

Result

Acute inflammatory models

Carrageenan-induced paw oedema

The Nelso*nia canescens* root showed an apparent and statistically non-significant decrease in paw oedema observed in TED-treated group at both 3hr and 6hr when compared to control group. In contrast to this, TED×2 failed to show any impact on carrageenan-induced paw oedema. In Reference Standard(RS)-treated group revealed highly significant inhibition inoedema formation as compared to control group at both time intervals (Table-1).

Formaldehyde-induced paw oedema

Administration of test drug at both dose levels leads to moderate oedema suppression at 24hr, but in non-significant manner comparison to control group. After 48 hr an apparent inhibition in paw oedema was observed in all the treated groups. However, the observed

inhibition in TED and RS-treated groups were found to be statistically non-significant (Table-2).

Chronic inflammatory model

Effect on body weight

Marginal increase in body weight was observed in the cotton pellet control group when compared to its initial values. In treated groups an apparent increase in body weight was observed in comparison to both initial values as well as control group. However, only RS-treated group showed significantly increased in body weight as compared to control group. Further, among two dose levels of test drug TED showed better effect.

Effect on different organ weight

Administration of test drug and RS did not affect the weight of liver to a significant extent when compared to control group. Weight of spleen in TED×2-treated group, was marginally increased.

Administration of the test and RS drugs showed marginally but non-significantly decreased in the weight of thymus. Moderately decreased in adrenal weight in TED and TED×2-treated group observed, but the effect was statistically non-significant. Administration of test drugs decreased formation of granulation tissue weight in a dose dependant manner. RS-treated group showed significantly decreased granulation tissue weight in comparison to control group. The effect observed in TED×2-treated group was almost equal to that of RS-treated group (Table-3).

Biochemical estimations

Increase marked observed in total protein content of the liver homogenate in both test as well as RS-treated groups as compared to control group. Administration of test drugs and RS did not affect the lipid peroxidation in liver tissue homogenate to a significant extent. catalase activity of liver homogenate decreased after administration of test and Rs drugs when compared to control group. However, the effect was non-significant. Serum orosomucoid content of TED and TED×2-treated groups were found to be apparently decreased, where as RS-treated group showed increases in serum orosomucoid content when compared to control group. However, the observed changes in all the treated groups are found to be statistically non-significant. The test drug failed to produce any significant effect on the adrenal ascorbic acid level at both the dose levels (Table-4).

Table 1: Effect of *Nelsonia canescens* on Carrageenan induced paw oedema in rats

Groups	Dose (mg/kg)	% increase in paw volume at different time interval after carrageenan injection			
		3 hour	% Inhibition	6 hour	% Inhibition
Control	Q.S.	64.82 ± 6.51	---	57.747 ± 10.26	----
TED	270	59.63 ± 6.93	08.00 ↓	49.648 ± 05.13	14.02 ↓
TED×2	540	65.42 ± 3.88	--	57.600 ± 02.15	--
R.S	100	11.01±04.28***	83.02 ↓	15.710±4.56**	72.80 ↓

Data: Mean ± SEM, ↓ - Decrease, **P<0.01, ***P<0.001,
Group I animals received vehicle (water), Groups II (TED) and III (TED×2) received the test drug. Group IV administered with the standard drug phenylbutazone (100 mg/kg, p.o.).

Table 2: Effect of *Nelsonia canescens* on formaldehyde induced paw oedema in rats.

Groups	Dose (mg/kg)	% increase in paw volume at different time intervals after formaldehyde injection.			
		24 hour	% change	48 hour	% change
Control	Q.S.	29.06 ± 2.80	--	25.263 ±3.37	--
TED	270	21.663± 3.34	25.45↓	8.968 ± 1.46**	64.50↓
TED×2	540	27.075± 2.17	06.82↓	15.655± 2.82	38.03↓
RS	5	23.128± 1.76	20.23↓	17.103± 1.52*	28.29↓

Data: Mean ± SEM, ↓ - Decrease,**P<0.01
Group I animals received vehicle (water), Groups II (TED) and III (TED×2) received the test drug. Group IV administered with the standard drug diclofenacsodium (5 mg/kg, p.o.).

Table 3: Effect of *Nelsonia canescens* on granulation tissue weight in albino rats

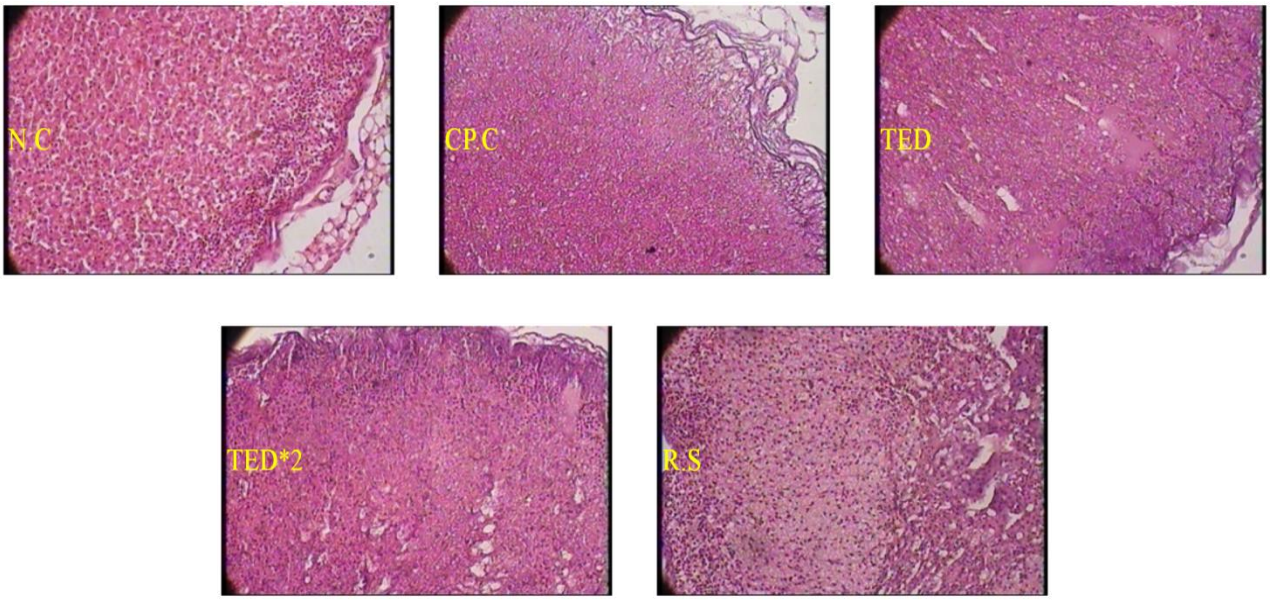
Groups	Dose (mg/kg)	Absolute wt (mg)	Relative wt (mg/100g body wt)	% change
Control	Q.S.	385.500 ± 10.076	158.168 ± 12.695	---
TED	270	271.833 ± 11.794	109.314 ± 10.345*	30.89 ↓
TED×2	540	229.167 ± 23.694	91.421 ± 07.229**	42.20 ↓
Dexomethasone	0.1	189.333 ± 7.843	87.800 ± 07.220***	44.49 ↓

Data: Mean ± SEM, ↓ - Decrease,*P<0.05, **P<0.01, ***P<0.001
Group I animals received vehicle (water), Groups II (TED) and III (TED×2) received the test drug. Group IV administered with the standard drug Dexomethasone (100 µg/kg p.o).

Table 4: Effect of *Nelsonia canescens* on, Adrenal ascorbic acid content, Total protein content, liver tissue lipid peroxidation and liver tissue catalase activity

Groups	Dose (per kg)	Ascorbic acid (µg/mg tissue)	Total protein (mg protein/g tissue)	Lipid Peroxidation (µ mole MDA released/g tissue)	Catalase activity (µ moles H_2O_2consumed/min./mg protein)
Control	Q.S.	2.10 ± 0.28	2.63 ± 0.92	37.57 ± 10.54	29.53 ±13.18
TED	270	2.70 ± 0.48	3.47± 0.96	40.94 ± 08.64	15.90 ± 04.16
TED×2	540	2.68 ±0.45	3.57± 0.84	35.55 ± 10.56	11.53 ± 02.01
Dexomethasone	0.1	1.15 ± 0.27*	4.50 ± 0.67	27.30 ± 01.75	04.42 ± 01.59

Data: Mean ± SEM, ↑- Increase, ↓ - Decrease, *P<0.05
Group I animals received vehicle (water), Groups II (TED) and III (TED×2) received the test drug. Group IV administered with the standard drug Dexomethasone (100 µg/kg p.o).



NC- Normal control group, with Normal cyto architecture; **CP.C**- Cotton pellet control group, with Features of decreased cellullarity; **TED**- Therapeutic equivalent dose, with Normal cytoarchitecture; **TED×2**- Therapeutic equivalent ×2, with Features of cortical stimulation; **R.S**-Reference Standard group with Features of cortical stimulation.

Figure 1: Photomicrographs of section of adrenal gland from different groups, *(1× 200 magnification)

Discussion

In carrageenan induced paw edema model, treatment with Nelsonia canescens root at TED dose level inhibited carrageenan induced inflammation at marginal level, while at TED×2 did not show any impact on carrageenan induced paw oedema inflammation.

In formaldehyde induced paw oedema model administration of test drug at both dose levels leads to moderate oedema suppression at 24 hr. However at 48 hr test drug at TED dose level significantly inhibited formalin induced paw oedema and the observed effect is even better than that of standard drug. Hence, it can be predicted that the drug is having an effect on sub-acute inflammation and the mechanism may involved by suppressing the mediator which is unrelated to 5-hydroxytryptamine.

On effect of cotton pellet induced granuloman formation dexomethasone (RS) group shows marked and statistically significant decrease in granulation tissue weight in comparison to control group, this indicates that dexomethasone suppress the proliferative phase in a significant manner and it has better anti-proliferative activity. Administration of test drug decreased formation of granulation tissue weight in a dose dependant manner and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

Marginal increase in body weight was observed in cotton pellet control group in comparison to its initial values. It may be due to inflammation induced degenerative changes in the body. The animals from TED dose treated group showed an apparent gain in body weight, may be the ability of test drug to prevent the inflammation induced changes in the body. Surprisingly, the same effect was not observed in TED×2 treated groups. Further, in reference standard treated group significant gain of body weight was observed.

There is no significant change on weight of liver, thymus, spleen and adrenals, which have a role in the inflammatory process.

Serum orosomucoid content of TED and TED×2 treated groups were found to be apparently decreased and in the reference standard treated group it was increased in comparison to control group. Further, liver tissue homogenate biochemical parameters were not affected to a significant extent.

Photomicrographs from, (Figure-1) shows that there was a normal cytoarchitecture of the adrenal gland, while sections of adrenal from

cotton pellet control group showed mild decrease in cellullarity as compared to a normal control group. Sections of the adrenal from TED-treated group showed cytoarchitecture similar to that of control group, whereas TED×2-treated group showed features of cortical stimulation as compared to normal group. Adrenal sections of the dexomethasone group showed features of adrenal stimulation. Mild stimulation was also observed in high dose of test drug treated group. However, there were no remarkable changes which could be corroborated by findings of either of ponderal and biochemical parameters recorded during the study this factor needs further detailed investigations. These investigations from folk medicines are valuable in the concept of reveres pharmacology and leads to identifying the compounds for anti-inflammatory drugs with less or no side effect in comparing to NSAIDs and corticosteroids and other chemical anti inflammatory drugs.

Conclusion

Based on the result of the present study, it can be concluded that the roots of *Nelsonia canescens* have a mild to moderate anti-inflammatory effect on acute inflammation and significant anti-inflammatory effect on chronic inflammation. Hence, it demonstrated scientific rationale for the folk use of the plant in the treatment of pain and inflammation.

Conflict of Interest

: None to declare.

Author contribution

B.M, performed the experiments of the study and drafts the manuscript. A.B.K and V.J.S, were the supervisors and designed the study. R.A, provide the materials and guided to manuscript writing.

Acknowledgments

Author acknowledges the support of institute for post graduate teaching and research in ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India.

References

1. Fahn A. Plant Anatomy. Pergamon Press, Oxford, 1989.

2. Kamboj.V. P.. Herbal medicine. General articles. current science. Vol. 78, No. 1, 10 JANUARY 2000. 35

3. Ferrero.M. L., Nielsen O.H., Andersen P.S., Girardin S.E."Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. British Society for Immunology, Clinical and Experimental Immunology. 2007;147:227–235

4. Kumar V., Abbas A.K., Fausto N. Robbins & Cotron.. Pathologic basis of disease. 7th ed. Philadelphia: Saunders; 2004. p. 47-118, 1304-11. Downloaded from www.ppjoy.tripod.com/BKmedicinalplants.

5. Saxena H. O., Brahman.M.. The flora of Orissa. vol. III, Regional Research Laboratory, Orissa Forest Development Corporation Ltd., Bhubaneswar, Orissa.(India); Dec, 1995; Page no:1323, 1372-1374.

6. Shah.G. L.. Flora of Gujarat State. Part-I, Shri. K. A. Amin, Registrar, Sardar Patel University, Vallabh Vidyanagar; February, 1978; Page no: 525-527, 549-550.

7. Kanjila. U.N.. The flora of Assam. vol.III, Osmos publications, New Delhi (India); 1997; Page no: 411.

8. Gamble. J. S.. Flora presidency of Madras. vol. II, Bishen Singh Mahendra Pal Singh, Dehradun, India; (London); 1993; Page no:1010.

9. Brahman. M., Saxena.H.O.. Ethnobotany of Gandhmardan Hills. Some Noteworthy Folk-Medicinal Uses. Ethnobotany, Vol.2, 1990.Page no.71-79.

10. Mohaddesi B., Dwivedi R., Ashok B.K., Aghera H., Acharya R., Shukla V.J.. Analgesic activity of *Nelsonia canescens* (Lam.)Spreng. root in albino rats.AYU, Apr-Jun 2013; 34-2: 226

11. Paget G.E., Barnes J.M.. Evaluation of drug activities. In: Lawrence DR, Bacharach AL, editors. Pharmacometrics.Vol.1. New York: Academic Press; 1964;161.

12. Winter, C. A., Risley, E. A. and Nuss, G. W.. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. (N.Y.). 1962; 111:544-547.

13. Brownlee G. Effect of deoxycortone and ascorbic acid on formaldehyde-induced arthritis in normal and adrenalec-tomized rats. Lancet. 1950;1:157–9.

14. Swingle K.F., Shideman F.E.. Phases of the inflammatory response to subcutaneous im-plantation of a cotton pellet and their modification by certain anti-inflammatory agents. J Pharmacol Exp Ther. 1972;183:226-34.