Research Article

Evaluation of analgesic and antipyretic activity of *Nigella sativa*: an experimental study

Harshal N Pise[1], Sushma S Jadhav[2]

[1]Department of Pharmacology, SRTR Govt. Medical College, Ambajogai, Beed, Maharashtra, India.
[2]Department of Physiology, Government Medical College, Latur, Maharashtra, India.

Correspondence to: Harshal N Pise, E-mail: drharshalpise@gmail.com

Received February 7, 2016. Accepted February 22, 2016

Abstract

Background: Drugs commonly used in modern medicine for suppression of pain and fever provide only symptomatic relief, and long-term use of these drugs is associated with serious adverse effects. Recently, some evidences suggest that *Nigella sativa* inhibit eicosanoid generation in leukocytes and lipid peroxidation. They are reported to inhibit both cyclooxygenase and 5-lipooxygenase pathways of arachidonic acid metabolism (Houghton et al. Planta Med 1995;61:33–6). **Aims and Objectives:** To investigate the analgesic and antipyretic activity of *N. sativa* seed fixed oil and compare it with control and aspirin. **Materials and Methods:** Albino Wistar rats of either sex weighing 180–200 g and Swiss mice weighing 25–30 g were used. The study was conducted after approval from the Institutional Animal Ethics Committee. The tail flick method in rats described by D'Amour and Smith and acetic acid-induced writhing in mice were used for evaluation of analgesic activity and baker's yeast-induced pyrexia method was used to evaluate antipyretic activity. **Result:** In tail flick method of analgesia, *N. sativa* showed analgesic activity, which was comparable with aspirin. In acetic acid-induced writhing model of analgesia, the action of *N. sativa* was significantly greater than the control group, and it was comparable with aspirin. In baker's yeast-induced pyrexia method, *N. sativa* group did not show any significant reduction in the rectal temperature at any hour interval. The changes in the rectal temperature in *N. sativa* group were comparable with control group ($p > 0.05$). **Conclusion:** *Nigella sativa* fixed oil has significant analgesic activity in both tail flick and acetic acid-induced method of analgesia. But, it does not have any significant antipyretic activity in baker's yeast-induced pyrexia method.

KEY WORDS: Analgesic; Antipyretic; Nigella Sativa; Fixed Oil; Pain

Introduction

Pain is the subjective experience hard to define exactly, even though we all know what we mean by it. International Association for the Study of Pain defines pain "as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."[1] The role of medicine is to protect and reestablish health and to ease distress. Knowledge of pain is vital to both these goals. Fever has been defined as the elevation of core body temperature above normal; in normal adults, the average oral temperature is 37°C (98.6°F).[2] Although host defense mechanism gets increased by fever, other aspects also warrant concern, such as the patient's comfort and physiologic responses. Drugs commonly used in modern medicine for suppression of pain and fever such as nonsteroidal anti-inflammatory drugs and corticosteroids provide only symptomatic relief, and long-term use of these drugs is associated with serious adverse effects. Hence, the search for a new, safe analgesic and anti-inflammatory drug is always going on.

Access this article online	
Website: http://www.njppp.com	Quick Response Code:
DOI: 10.5455/njppp.2016.6.07022016124	

Traditional medicine and folk medicine have offered us with significant drugs in the therapy of various diseases and are more and more subjected to scientific research. Salicylate had their origin in the willow bark of herbal medicine or morphine had its origin in *Papaver somniferum*. *Nigella sativa* is one such herb extensively used in Unani, Ayurvedic, and Siddha systems for centuries for various indications including pain, inflammation, and fever.[3]

Recently, some evidences suggest that *N. sativa* inhibit eicosanoid generation in leukocytes and lipid peroxidation. They are reported to inhibit both cyclooxygenase (COX) and 5-lipooxygenase (LOX) pathways of arachidonic acid metabolism.[4] So, they may possess analgesic and antipyretic activity. Hence, we considered it is worthwhile to find out whether it exhibits antipyretic activity and to confirm its analgesic activity.

Materials and Methods

This study was carried out in SRTR Government Medical College, Ambajogai, Maharashtra.

Animals

Albino Wistar rats of either sex weighing 180–200 g and Swiss mice weighing 25–30 g were used. The study was conducted after approval from the Institutional Animal Ethics Committee of our institute, which is an approved body by CPCSEA, New Delhi. The rats and mice were grouped in separate cages with six animals in each cage. They had free access to food and water.

Chemicals

Aspirin and carboxymethylcellulose (CMC) were obtained as kind gift from Medley Pharmaceuticals, Mumbai, India. *N. sativa* seed fixed oil was received as gift sample from Manish Herbals, Mandsaur, Madhya Pradesh. Carrageenan (1% in 0.9% saline) and tramadol were obtained from commercial sources.

Methods

Evaluation of analgesic activity. Animals are divided into following three groups:

1. Control group: normal saline, dose: 2 mL/kg (p.o.);
2. Standard group (aspirin group): aspirin, dose: 300 mg/kg (p.o.);
3. Test group (N. sativa group): N. sativa fixed oil, dose: 10 mL/kg (p.o.)[5]

Tail flick method in rats[6]. Antinociceptive activity was assessed by tail flick response method by analgesiometer; originally described by D'Amour and Smith in 1941. Rats, positioned on the analgesiometer with tail freely projecting out of the holder, were used for recording the observations. The current's strength transmitted via the naked nichrome wire was maintained constant at 6 amps. Readings were noted by positioning the middle part of the tail on the radiant heat supply. Squeak (utterance of high-pitch sounds) or a quick removal of the tail called as "tail flick response" was considered as the endpoint of the test. The time from positioning the tail of the rat on the radiant heat supply and the quick removal of the tail was noted as "reaction time."

To avoid thermal injury, a cutoff time of 10 s was forced as maximum latency in all the experiments performed. In all the groups, tail flick test was done before giving the drug and at 30, 60, 90, and 120 min after giving the drug, and the estimation of reaction time at each time interval (test latency) was performed.

Percentage maximum possible effect (%MPE; analgesia) was estimated by using the formula:

$$\%\text{MPE} = \frac{\text{Test Latency} - \text{Basal Latency}}{\text{Maximum latency} - \text{Basal Latency}} \times 100$$

Acetic acid induced writhing in mice[7]. Being a chemical nociceptive test, the basis of the writhing model depends on the stimulation of peritonitis-like situation in animals by administering irritant material intraperitoneally (i.p.). Swiss albino mice were placed separately in the test cage before acetic acid injection and accustomed for 30 min.

After 30 min of drug injecting, mice were injected with 0.1 ml of 1% acetic acid solution (i.p.). Individual positioning of mice into glass beakers was followed by a 5-min elapse time. Then, the mice watched for about 10 min, and the numbers of writhes were noted for every animal. For scoring reasons, a writhe is specified by abdomen stretching with concurrent stretching of at least one hind limb. The following formula was used to calculate percentage inhibition:

$$\%\ \text{Inhibition} = \frac{\text{No. of writhings in control group} - \text{no. of writhings intest group}}{\text{No. of writhings in control group}} \times 100$$

Evaluation of Antipyretic Activity. *Baker's Yeast Induced Pyrexia Method*[8]: Pyrexia was induced in rats by administering freeze-dried baker's yeast as 20% suspension in 0.9% saline (1 g/kg s.c.) in the nape of neck. The temperature was estimated by inserting a 3 cm digital thermometer coated with glycerine into rectum. All the study groups were treated 4 h after injection of baker's yeast. Rectal temperature was measured at 0, 3, 4, and 6 h using a thermometer. For reducing the impact of stress related to handling and injections on rectal temperature, all rats were accustomed to the estimating method for two successive days. During these training periods, the above-mentioned temperature estimating method was followed in all the animals.

Statistical Analysis

Data were analyzed by using Graph pad Prism software, version 5.01. Comparison between different groups was done

by one-way ANOVA, followed by Bonferroni posttest. The '*p*' value less than 0.05 was considered statistically significant.

Results

Analgesic Activity in Rats

Tail-flick method. Effects of different drugs on nociception in tail flick model of analgesia in rats are as shown in Table 1.

The analgesic effect of *N. sativa* oil was seen at 30 min as evident from 30-min latency, which was significantly more than control group ($p < 0.05$) but less when compared with aspirin and tramadol groups. The analgesic activity of *N. sativa* went on increasing till 90-min interval; thereafter, it decreased.

At 60 min, the analgesic activity of *N. sativa* oil was significantly more than control group and was comparable with aspirin group but significantly less than that of tramadol group. At 90 min, the analgesic activity of *N. sativa* group was comparable with aspirin group and then decreased by 120 min as can be seen by 120-min latency. Again at 120 min, *N. sativa* oil has shown significant analgesic activity when compared with control and was comparable with aspirin and tramadol groups.

The analgesic effect of *N. sativa* oil was comparable with aspirin at all time intervals except at 30-min interval.

The MPE of drugs in tail flick method of analgesia in rats is as shown in Table 2.

At 30 min, the MPE of *N. sativa* oil was less than tramadol and aspirin groups. At 60-min interval, the MPE of *N. sativa* oil was comparable with aspirin group but was less than tramadol group. The MPE of aspirin was also less when compared with tramadol group. At 90 min, the MPE of *N. sativa* oil was significantly more ($p < 0.05$) when compared with aspirin group and was comparable ($p > 0.05$) with tramadol group.

At 120-min interval, the difference between mean MPE was comparable in aspirin, tramadol, and *N. sativa* groups.

Acetic acid-induced writhing method. Effect of different drugs in acetic acid-induced writhing models in mice is as shown in Table 3.

The total number of writhes in 10 min was highest in control group and lowest in aspirin group. Number of writhes in *N. sativa* group was significantly less ($p < 0.05$) when compared with control and was comparable with aspirin group ($p > 0.05$). Number of writhes in aspirin group was lowest and significantly less when compared with control. The number of writhes in aspirin group was less when compared with *N. sativa* group but the difference was not statistically significant ($p > 0.05$). The percentage analgesia was at maximum in aspirin group (79.72%), whereas in *N. sativa* group, it was 69.92%.

Antipyretic Activity of *N. sativa*

The effect of different drugs on rectal temperature in baker's yeast-induced pyrexia model in rats is as shown in Table 4.

In the control group, there was consistent rise in rectal temperature up to 4 h interval following baker's yeast injection.

N. sativa group had not shown any significant reduction in the rectal temperature at any hour interval. The changes in the rectal temperature in *N. sativa* group were comparable with control group ($p > 0.05$).

The reduction in the rectal temperature in aspirin group was significantly greater compared with control group and *N. sativa* group ($p > 0.05$) at 3-, 4-, and 6-h intervals.

Discussion

Despite the major advances in the modern medicine, the development of new drugs from natural products is still considered important. As per WHO, about 80% of the population in the world depends on the traditional medicine for the treatment of various diseases.[9] The evaluation of new drugs, especially phytochemically obtained materials, has again opened a vast area for research and development. On the basis of this view, one such plant is *N. sativa* Linn., which is small annual herb spread throughout India with a long history of medicinal use.

Some studies have been conducted to evaluate the analgesic effect and anti-inflammatory activity of *N. sativa* oil, and results are encouraging.[10–12] But, only few studies have evaluated its antipyretic potential. So, this study was done to confirm the *N. sativa* oil as analgesic and to find out its antipyretic activity.

In this study, two widely used screening models for evaluating analgesic activity were used, i.e., tail flick method in rats and acetic acid-induced writhing in mice, and for evaluating antipyretic activity, baker's yeast-induced pyrexia method was used.

Table 1: Effects of different drugs on nociception in tail flick model of analgesia in rats

Groups (n = 6)	Basal latency (s)	At 30 min (s)	At 60 min (s)	At 90 min (s)	At 120 min (s)
Control (normal saline, 2 mL/kg p.o.)	3.50 ± 0.1732	3.65 ± 0.2306	3.867 ± 0.2716	3.967 ± 0.2565	3.667 ± 0.2871
Aspirin (300 mg/kg p.o.)	3.617 ± 0.3341	8.00 ± 0. 3337*,**	7.383 ± 0.3429*	6.967 ± 0.2171*	6.717 ± 0.2664*
Tramadol (10 mg/kg i.p.)	3.438 ± 0.01621	8.383 ± 0.3198*,***	9.183 ± 0.3198*,***,****	8.817 ± 0.2774*	7.20 ± 0.284*
Nigella sativa (10 mL/kg p.o.)	3.30 ± 0.270	6.333 ± 0.1978*	6.983 ± 0.306*	7.017 ± 0.2257*	6.483 ± 0.2587*

Values are mean ± SEM, n = 6 in each group.
*$p < 0.001$ when compared with control.
**$p < 0.01$ when compared with *Nigella sativa.*
***$p < 0.001$ when compared with *Nigella sativa*.
****$p < 0.01$ when compared with aspirin.

Table 2: Maximum possible effect of drugs in tail flick method of analgesia in rats

Drugs and doses, mg/kg (n = 6 animals)	% Maximum possible effects (s)			
	After 30 min	After 60 min	After 90 min	After 120 min
Control (normal saline, 2 mL/kg p.o.)	—	—	—	—
Aspirin (300 mg/kg p.o.)	68.18 ± 5.392***	56.24 ± 6.509	48.93 ± 5.047	47.1 ± 5.815
Tramadol (10 mg/kg i.p.)	74.28 ± 5.154****	86.93 ± 4.779**,****	80.23 ± 4.363**	55.42 ± 4.52
Nigella sativa (10 mL/kg p.o.)	41.60 ± 4.637	50.43 ± 5.123	70.78 ± 4.073*	43.58 ± 5.862

Values are mean ± SEM, n = 6 in each group.
*$p < 0.05$ when compared with aspirin.
**$p < 0.001$ when compared with aspirin.
***$p < 0.01$ when compared with *Nigella sativa*.
****$p < 0.001$ when compared with *Nigella sativa*.

The tail flick method of analgesia is highly efficient in determining the effectiveness and strength of centrally acting analgesic drugs. In our study, result of this test has shown that *N. sativa* has analgesic activity, which was comparable with aspirin.

N. sativa fixed oil showed maximum analgesic activity at 90-min interval, and then the effect diminished but was comparable with aspirin at 120-min interval. Percentage analgesic effect was 70.78% at 90-min interval, which was comparable with aspirin and tramadol groups at that interval.

This reveals the significant increment of pain threshold at the time of recording in each of the three drug-treated groups, with maximum effect noted in tramadol group at all recording points. Tail flick method mainly evaluates the analgesic activity of centrally active drugs. Hence, tramadol, which acts by central mechanism, has shown the maximum activity. *N. sativa* showed activity comparable with tramadol in at 90- and 120-min intervals. Various authors have suggested that the site of action of *N. sativa* is at supraspinal level, and hence, they have a central mechanism of action. Hajhashemi et al.[12] reported that *N. sativa* essential oil produced a significant analgesic activity in tail flick method of analgesia. However, Ghannadi et al.[10] observed that *N. sativa* polyphenol did not produce a significant analgesia in the light tail flick test in mice. Our findings also support the observations made by Hajhashemi et al. Although aspirin has a central constituent of action, it chiefly induce analgesia through a peripheral action.

Table 3: Effect of different drugs in acetic acid-induced writhing models in mice

Groups (n = 6 animals)	Number of writhes (in 10 min)	Percentage analgesia
Control (normal saline 2 mL/kg p.o.)	23.83 ± 1.078	—
Aspirin (300 mg/kg p.o.)	4.833 ± 0.3073*	79.72
Nigella sativa (10 mL/kg p.o.)	7.167 ± 0.6009*	69.92

Values are mean ± SEM, n = 6 in each group.
*$p < 0.001$ when compared with control.

In acetic acid-induced writhing model of analgesia, the action of *N. sativa* was significantly greater than the control group, and it was comparable with aspirin. Percentage analgesia with *N. sativa* was 69.92%, and it was more than 70% with aspirin-treated animals. In this method, substances with percentage analgesia of less than 70% are regarded to exhibit minimal analgesic activity. The writhing response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. As the acetic acid-induced writhing method mainly evaluates peripherally acting analgesics, maximum analgesic activity of aspirin was observed in this model while the analgesic action of *N. sativa* was comparable with aspirin.

Bashir et al.[13] reported 41.91% inhibition in acetic acid-induced writhing method, while Tanko et al.[14] reported 67.1% inhibition with ethanol extract of *N. sativa*. The difference in activity

Table 4: Effect of different drugs on rectal temperature in baker's yeast-induced pyrexia model in rats

Group	Rectal temperature in °C at time (h)				
	-4 h	0 h	3 h	4 h	6 h
Control (normal saline 2 mL/kg p.o.)	37.22 ± 0.04773	38.38 ± 0.14	38.55 ± 0.1258	38.87 ± 0.03333	38.6 ± 0.05773
Aspirin (300 mg/kg p.o.)	37.32 ± 0.05426	38.43 ± 0.1358	37.5 ± 0.07303*,**	37.65 ± 0.07188*,**	37.47 ± 0.05578*,**
Nigella sativa (10 mL/kg p.o.)	37.25 ± 0.04282	38.40 ± 0.1317	38.43 ± 0.1054	38.60 ± 0.08563	38.28 ± 0.08388

Values are mean ± SEM, n = 6 in each group.
*$p < 0.001$ when compared with control.
**$p < 0.001$ when compared with *Nigella sativa*.

might be because seeds of different origin may be used here. Our findings are in consistent with those reported by Tanko et al.

In our study, we did not investigate the underlying mechanism by which *N. sativa* inhibit nociception. Previously, some studies have reported inhibitory effects of *N. sativa* on tail flick method and acetic acid-induced writhing method of analgesia but the exact mechanism of action of this analgesic effect is not yet clear.

Abdel-Fattah et al.[15] suggested that supraspinal opioid system is involved in the analgesic effects of thymoquinone (a major constituent of *N. sativa* oil) and that *N. sativa* oil and that thymoquinone produce antinociceptive effects through indirect activation of supraspinal μ_1-and κ opioid receptors, but Hajhashemi et al.[12] contradicted this finding and suggested that mechanisms other than the stimulation of opioid receptors are involved. This difference might be because different products were used by them. Essential oil was used by Hajhashemi et al. and fixed oil by Abdel-Fattah et al. Moreover, there is a significant difference in their chemical composition. This emphasizes the need to evaluate the presence of other active agents besides thymoquinone and investigation of their possible mechanism of action.

Thymoquinone has been identified one of the chief constituent in almost all of these extracts/oils and considered to be in charge of analgesic activity. However, is it only thymoquinone or some other active components are also accountable for analgesic effect is yet to be elucidated. Thymoquinone is noted to hinder the synthesis of thromboxane A2 and leukotriene B4, thus signifying an inhibitory effect on both the COX and LOX pathways.[4] The analgesic activity of *N. sativa* in this study might be owing to inhibitory effect on both COX and LOX pathways.

The action of *N. sativa* was comparable with aspirin in the tail flick model of antinociception and in acetic acid-induced writhing method. This suggests that, besides central mechanism of action, other pain mechanisms are also important.

Very few studies have been carried out to evaluate the antipyretic activity of *N. sativa*.

Al-Ghamdi[16] evaluated antipyretic activity of aqueous extract of *N. sativa* and concluded that it had no effect on yeast-induced pyrexia method.

The absence of antipyretic effect of *N. sativa* in yeast-induced pyrexia method suggests that it may not inhibit the synthesis of interleukins.

The result of our study supported by other reports suggests that *N. sativa* fixed oil may play a role as an analgesic agent. However, further pharmacological studies are required to assess therapeutic benefits.

Conclusion

In conclusion, *N. sativa* fixed oil has shown significant analgesic activity in both tail flick and acetic acid-induced method of analgesia suggesting that, besides central mechanism of action, other pain mechanisms are also important. But, it does not have any significant antipyretic activity in baker's yeast-induced pyrexia method.

References

1. Pain terms: a list with definitions and notes on usage. Recommended by the IASP Subcommittee on Taxonomy. Pain. 1979; 6(3):249.
2. Dalal S, Zhukovsky DS. Pathophysiology and management of fever. J Support Oncol. 2006;4:9–16.
3. Paarakh PM. *Nigella sativa* Linn.—A comprehensive review. Indian J Nat Prod Resourc. 2010;1(4):409–29.
4. Houghton P, Zarka R, delas HB, Hoult RS. Fixed oil of *Nigella Sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med. 1995;61:33–6.
5. Abdel-Sater KA. Gastroprotective effects of *Nigella sativa* oil on the formation of stress gastritis in hypothyroidal rats. Int J Physiol Pathophysiol Pharmacol. 2009;1(2):1–8.
6. D'Armour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther. 1941;72:74–9.
7. Parmar NS, Prakash S. Evaluation of analgesics, anti-inflammatory and anti-pyretic activity In:(Ed.) Screening Methods in Pharmacology. New Delhi, India: Narosa Publishing House, 2006. pp. 225–6.
8. Dewan S, Kumar S, Kumar VL. Antipyretic effect of latex of *Calotropis procera*. Indian J Pharmacol. 2000;32:252.
9. World Health Organisation. Fact Sheet N0134 December 2008. Available at: www.who.int/mediacentre/factsheets/fs134/en/ (assessed February 16, 2013).
10. Ghannadi A, Hajhashemi V, Jafarabadi H. An investigation of analgesic and inflammatory effects of *Nigella sativa* seed polyphenols. J Med Food. 2005;8(4):488–93.
11. Tekeoglu I, Dogan A, Ediz L, Budancamanak M, Demirel A. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. Phytother Res. 2007;21(9):895–7.
12. Hajhashemi V, Ghannadi A, Jafarabadi H. Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug. Phytother Res. 2004;18(3):195–9.
13. Bashir MU, Qureshi HJ. Analgesic effect of *Nigella sativa* seeds extract on experimentally induced pain in albino mice. J Coll Physicians Surg Pak. 2010;20(7):464–7.
14. Tanko Y, Mohammad A, Okasha MA, Shuaibu A, Magaji MG, Yaro AH. Analgesic and anti-inflammatory activities of ethanol seed extract of *Nigella sativa* (black cumin) in mice and rats. Eur J Sci Res. 2007;18:277–81.
15. Abdel-Fattah AM, Matsumoto K, Watanabe H. Antinociceptive effects of *Nigella sativa* oil and its major component, thymoquinone, in mice. Eur J Pharmacol. 2000;400(1):89–97.
16. Al-Ghamdi MS. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. J Ethnopharmacol. 2001;76:45–8.

How to cite this article: Pise HN, Jadhav SS. Evaluation of analgesic and antipyretic activity of *Nigella sativa*: an experimental study. Natl J Physiol Pharm Pharmacol 2016;6:291–295

Source of Support: Nil, **Conflict of Interest:** None declared.