Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

IN VIVO EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF *NIGELLA SATIVA* SEED DURING GERMINATION

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Received: 27 July 2013, Revised and Accepted: 31 Aug 2013

ABSTRACT

Objective: The medicinal values of *Nigella sativa* have been mentioned in ancient literature as useful in disorders of inflammation. The present study investigates the anti-inflammatory and analgesic activities of methanol extract of *Nigella sativa* seed during different phases of its germination on Wistar rats.

Methods: Seeds of *N. sativa* were grown *in vitro* in glass petri plates using multiple folds of damp filter paper. Complete plantlet with two leaves was obtained in 11 days. The acute anti-inflammatory activity of *N. sativa* extracts during different phases of germination was measured plethysmographically using kaolin as inflammatory agent, analgesic activity was measured by hot plate method, keeping indomethacin (10mg/kg b.w) as reference standard in both tests.

Results: All tested extracts of *N. sativa* (1gm/kg b.w) during different phases of germination showed significant reduction in paw oedema in comparison to control ($P<0.001$) in anti-inflammatory test and showed significant increased ($P<0.001$) in the reaction time on hot plate during analgesic test. Extract of 5th day germination showed significant anti-inflammatory and analgesic effect among the all tested extracts.

Conclusion: It may be concluded that extracts of *N. sativa* possess enhanced anti-inflammatory and analgesic activities during germination as compared to seed extract. High metabolic activity and higher contents of secondary metabolites expressed during germination might be responsible for this activity.

Keywords: *Nigella sativa*, Germination, NSAIDs, Anti-inflammatory Activity, Analgesic activity.

INTRODUCTION

Throughout the world inflammatory diseases are becoming common in aging society. Recent studies indicate that the mediators and cellular effectors of inflammation are important constituents of the local environment of tumors [1]. Inflammation is self-protective mechanism of the body to eliminate the injurious stimuli as well as begin the healing process for the tissue. However, if runs unchecked, lead to onset of diseases such as vasomotor rhinnorrhoea, rheumatoid arthritis, and atherosclerosis [2]. The drugs which are used presently for the management of pain and inflammatory conditions are either narcotics or non narcotics (NSAIDs), and have known toxic and lethal effects It is alleged that current drugs available such as opoids and NSAIDs drugs are not helpful in all cases of inflammatory disorders, because of their side effects, economy and effectiveness [3, 4]. On the contrary, herbal medicines with good absorption, less toxicity, and easy availability have been used since ancient times. Natural products in general and medicinal plants in particular, are believed to be a key source of new chemical substances with potential therapeutic efficacy [5].

N. sativa is a herbaceous plant which belongs to the botanical family of *Ranunculaceae,* found in the Middle East, Europe and Western and Middle Asia. Its seeds have a great medicinal importance and have been reported to exhibit many pharmacological effects that include anti-parasitic, antibacterial, antifungal, antiviral, antioxidant anti-inflammatory activity [6] and anti-stress activities [7].

Germination of seeds has been used for centuries to soften the kernel structure, to increase the nutritional value and to decrease the anti-nutritive compounds. During the recent years interest has arisen also in the secondary metabolites produced during germination which can have valuable health promoting properties and act as bioactive or functional compounds in foods [8]. The seeds of *N. sativa* in different germination stages have revealed the presence of higher amount of alkaloids, tannins and flavonoids [9, 10] as compared to seed. So, the present study was taken to investigate the higher anti-inflammatory and analgesic effects of *N. sativa* crude methanol extracts of successive germination phases.

MATERIALS AND METHODS

Collection of *N. sativa* seed

Seeds of *N. sativa* were procured from a grocery shop in Lucknow in the month of March, 2012 and authenticated by Dr. Shanthy Sundaram, Centre for Biotechnology, University of Allahabad, Allahabad (U.P.) India. A voucher specimen of the seeds was kept in the museum of the Department for future reference.

Germination of seeds

Seeds of *N. sativa* were grown in glass petri plates. Seeds were placed on four folds of damp filter paper at 25°C and incubated in the dark till the initiation of sprouting (3rd day) after which they were placed at a light intensity of 100 µmol m^{-2} s^{-1} and a 14/10 h (day/night) photoperiod till the complete plantlet with two leaves were obtained.

Preparation of distilled extracts

The samples of seed and germinated phases 5th, 7th and 11th day were shade-dried and ground to a fine powder. The powder (20 gm) was extracted by using soxhlet apparatus with 200 ml HPLC grade methanol solvent for 48 hrs in order to extract bioactive compounds. The extracts were filtered using Whatman filter paper no. 1, concentrated using rotary evaporator and oily fraction of extracts stored at 4^{0}C until use.

Animals

Male Wistar rats, weighing 150 - 200 gm, were purchased from Central Drug and Research Institute (CDRI), Lucknow, India and housed in a temperature controlled room (22±2°C) with a 12 hr light-12 hr dark cycle and allowed free access to a standard rat chow and filtered tap water for 7 days for acclimatization. The study received the approval of the Institutional Animal ethics Committee of Era's Lucknow medical college & hospital. Animals were cared for in accordance with the internationally accepted principles for laboratory animal use and care and the procedures followed were in accordance with the standards set forth in the Guide for the Care and Use of Laboratory Animals (published by the National Academy of Science, National Academy Press, Washington, D.C.).

Drugs

All standard chemicals used in this study were of analytical grade. Indomethacin was obtained from Sigma-Aldrich, Germany.

Acute toxicity studies

The acute toxicity test of the each extracts was carried out by using Wistar rats of either sex weighing between 150-200g. The methanol extracts of *N. sativa* from different germination phases were administered orally to overnight fasted animals at different doses (250 mg/kg, 500 mg/kg, 1000 mg/kg, 3000 mg/kg and 5000 mg/kg p.o.)[11]. After administration of the extracts, the animals were observed continuously for the first two hours, for any toxic manifestation. Thereafter, observations were made at regular intervals for 48 hours. Further the animals were under investigation up to a period of two week for mortality and general behavior.

Anti-inflammatory activity

The rats were divided into six groups containing (n=6) in each group (one control, one standard & four test groups) Paw oedema was induced by injecting 0.1 ml of 5% suspension of kaolin into the sub plantar tissues of the left hind paw of each rat [12, 13, 14]. Methanol extracts of *N. sativa* at different stages of germination (Seed, fifth day, seventh day and eleventh day germinated seed extract respectively) were administered orally in doses of 1 gm/kg body weight 1hr prior to kaolin administration in test groups. Indomethacin (10 mg/kg p.o.) was given to standard group.

Control group: 1 ml distilled water p.o.

Standard group: Indomethacin (10 mg/kg p.o.)

Test group 1: seed extract (1gm/kg p.o.)

Test group 2: 5th day extract (1gm/kg p.o.)

Test group 3: 7th day extract (1gm/kg p.o.)

Test group 4: 11th day extract (1gm/kg p.o.)

Percentage inhibition of oedema = $[V_c\text{-}V_t/V_c] \times 100$

Where, *Vc* is the inflammatory increase in paw volume in control group of animals and *Vt* is the inflammatory increase in paw volume in drug-treated animals. The paw volume was measured at 1, 3, 6, and 18 hrs after induction of inflammation using plethismometer. Anti-inflammatory activity was measured as the percentage reduction in oedema level when drug was present, relative to control [15].

Analgesic activity

Hot plate method

The analgesic activity of the extracts was measured by hot-plate method [16]. The rats were divided into six groups (n=6) in each group (one control, one standard & four test groups). Methanol extracts of *N. sativa* at different stages of germination (Seed, fifth day, seventh day and eleventh day germinated seed extract respectively) were administered orally in doses of 1gm/kg b.w. indomethacin (10 mg/kg p.o.) was given to standard group.

Control group: 1ml distilled water p.o.

Standard group: indomethacin (10 mg/kg p.o.)

Test group 1: seed extract (1gm/kg p.o.)

Test group 2: 5th day extract (1gm/kg p.o.)

Test group 3: 7th day extract (1gm/kg p.o.)

Test group 4: 11th day extract (1gm/kg p.o.)

The animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5^{0}C [17]. The reaction time was taken as the interval from the instant animal reached the hot plate until the moment animal licked its feet or jumped out. A cut off period of 15s was observed to avoid damage to the paw. The reaction time was recorded before and after 0, 30, 60 and 90 min and 120 min following administration of test or standard drug [16].

Statistical analysis

Statistical significance was determined by One Way Analysis of Variance (ANOVA) followed by Dunnet's t-test to compare group means. The level of significance was $P < 0.001$. All analysis was done using SPSS software version 16.0

RESULTS

Acute Toxicity Studies

Acute toxicity studies were carried out to evaluate the drug's toxicity of the drug extracts, using Wistar rats. No death was observed till the end of the study. The extracts were found to be safe up to the dose of 5000 mg/kg p.o., hence 1/5th of the tested dose, 1000 mg/kg dose was chosen as the experimental dose.

Anti-inflammatory activity

The increase of paw volume after 1st, 3rd, 6th and, 18th hr was observed and compared with volume measured immediately after the injection of Kaoline in each rat. The result of anti-inflammatory studies was presented as mean ± SEM. The values obtained showed a significant reduction in the growth of oedema in the hind paw of the rats. The inhibition was higher in 5th day germination extract (1gm/kg p.o.) followed by 7th day and indomethacin (10 mg/kg p.o.) which was used as a standard drug in the studies. Extract of 5th day germination caused 72.5% inhibition at 18 hrs while this was 51.25%, 46.25% in indomethacin group and seed extract group respectively at the same time. So, extracts of germination stages showed higher inhibition of inflammation than seed extracts, as the result showed in Table 1.

Table 1: Evaluation of anti-inflammatory effect of *N. sativa* extracts from different germination phases on kaolin induced paw oedema in rats.

Groups	Volume of paw oedema after drug administration (ml)					% inhibition in paw oedema after drug administration			
	0 hrs	1 hrs	3 hrs	6 hrs	18 hrs	1 hrs	3 hrs	6 hrs	18 hrs
Control	0.20±0.0	0.59±0.06	0.71±0.03	0.78±0.04	0.80±0.02	-	-	-	-
Indomethacin (10 mg/kg p.o.)	0.18±0.02	0.29±0.02^a	0.33±0.03^a	0.37±0.03^a	0.39±0.03^a	50.84	53.52	52.56	51.25
Test group 1	0.20±0.02	0.35±0.03^a	0.40±0.01^a	0.42±0.02^a	0.43±0.04^a	40.67	43.66	46.15	46.25
Test group 2	0.20±0.02	0.20±0.01$^{a\,b}$	0.22±0.02^{ab}	0.23±0.02^{ab}	0.22±0.02^{ab}	66.10	69.01	70.51	72.5
Test group 3	0.19±0.01	0.21±0.01^{ab}	0.23±0.03^{ab}	0.25±0.01^{ab}	0.27±0.01^{ab}	64.4	67.60	67.94	66.25
Test group 4	0.20±0.02	0.30±0.01^a	0.34±0.01^a	0.38±0.01^a	0.40±0.01^a	49.15	52.11	51.28	50.00

*Data are expressed as Mean ± S.E.M of n=6. **Values significantly differ from the control, ^{a}P<0.001 vs Control and ^{b}P<0.001 vs indomethacin group. Test group 1: seed extract, Test group 2: 5th day extract, Test group 3: 7th day extract, Test group 4: 11th day extract.

Analgesic activity

The result of analgesic studies was presented as mean ± SEM. The values obtained showed a significant increase in reaction time. All the rats showed a reaction time of 2-3 seconds on hot plate before administration of any drug. The reaction time was increased in the groups receiving *N. sativa* extracts from different germination phases and indomethacin. However, compared to control group, significant increase was seen at 60 and 90 minutes in the entire test group. All tested extracts of *N. sativa* from different germination phases as well as indomethacin (standard drug) showed increase in reaction time in hot plate test for rats. The reaction time was higher in 5th day germination

extract (1gm/kg p.o.) followed by 7th day and indomethacin (10 mg/kg p.o.) group. Extract of 5th day germination increased reaction time of rats on hot plate up to 14.99 sec at 90 min this was 12.98 sec, 10.89 sec in indomethacin group and seed extract group respectively at the same time. So, extracts of germination stages showed higher analgesic effect than seed extracts, as the result showed in Table 2.

Table 2: Analgesic effect of *N. sativa* extracts of different germination phases by hotplate test in wistar rats.

Groups	Mean latency (s) before and after drug administration				
	0 min	30min	60min	90min	120min
Control	2.62±0.41	2.75±0.42	2.68±0.40	2.68±0.42	2.63±0.46
Indomethacin (10 mg/kg)	2.62±0.30	3.50±0.41[a]	7.71±0.51[a]	12.98±0.50[a]	10.89±0.55[a]
Test group 1	2.61±0.36	3.0±0.50	6.19±0.46[a]	10.89±0.56[a]	9.89±0.52[ab]
Test group 2	2.62±0.36	3.3±0.44[ab]	7.49±0.49[ab]	14.99±0.51[ab]	13.16±0.51[ab]
Test group 3	2.59±0.40	3.2±0.40[a]	7.38±0.50[ab]	14.01±0.55[ab]	12.41±0.53[ab]
Test group 4	2.60±0.44	3.2±0.47[a]	6.90±0.41[a]	12.97±0.42[a]	11.93±0.45[ab]

*Data are expressed as Mean ± S.E.M of n=6. **Values significantly differ from the control, [a]P<0.001 vs Control and [b]P<0.001 vs indomethacin group. Test group 1: seed extract, Test group 2: 5th day extract, Test group 3: 7th day extract, Test group 4: 11th day extract.

DISCUSSION

The preliminary phytochemical screening of methanolic extracts of *N. sativa* from different germination phases showed the presence of alkaloids, flavonoids, terpenes, saponins, and tannins in our previous study which showed higher content of these metabolites during germination phases [9, 10]. Thus, the activity of *N. sativa* during germination could be due to flavonoids, terpenes and other metabolites. The methanolic extracts of *N. sativa* from different germination phases did not show any toxicity and behavioral changes in rats up to 5000 mg/kg hence doses of (1000 mg/kg, p.o.) were selected for the present study.

The extracts of different germination phases of *N. sativa* showed inhibition of the kaolin-induced rat paw oedema in a time-dependent manner throughout the duration of the study. The extracts of germination stages significantly ($P < 0.001$) inhibited formation of oedema in rat paw than indomethacin throughout the duration of the study. The extracts of germination stages significantly ($P < 0.001$) increased in reaction time than indomethacin and seed extract throughout the duration of the study. The onset of action was seen at 60-90 minutes in all the tested groups. This shows that the drug takes around 1 hr time in being absorbed passage through the liver and reaching the systemic circulation and CNS. The peak effect occurs at 90 minutes .the analgesic effect decreased at 120 minutes probably because of metabolism and elimination of the drug.

The methanolic extract of *N. sativa* in different phases of its germination exhibited a significant anti inflammatory activity. After the administration of Kaoline plasma leukotriene (LT) C_4-like and prostaglandin (PG) E2-like activities were increased [18]. Kaolin oedema appears to have a significant prostaglandin component since large amounts of prostaglandin-like materials production in kaolin blebs and indomethacin reduced the kaolin induced paw oedema [19]. indomethacin is a nonselective inhibitor of cyclooxygenase (COX) 1 and 2, enzymes that participate in prostaglandin synthesis from arachidonic acid. Prostaglandins are hormone-like molecules normally found in the body, where they have a wide variety of effects, some of which lead to pain, fever, and inflammation. In the study, injection of commonly used NSAIDs (indomethacin) was observed to significantly reduce inflammatory indices while histamine and 5- hydeoxytryp-tamine (5-HT) were not effective. This observation is consistent with those of others [20, 21] that PGs are the major mediators of kaolin-induced inflammation. The researchers suggested that kaolin-induced inflammation should be used as a model of inflammation for assessing the efficacy of NSAIDs and other drugs acting via the same mechanism. The advantages of kaolin compared to other models of inflammation like carrageenan, are its longer duration of inflammation and being a clay mineral, it is unlikely to have anti-genicity or to cause hypersensitivity reactions.

It may therefore be suggested that the extracts of different germination phases of *N. sativa* remission of kaolin-induced rat paw oedema observed through inhibition of prostaglandins biosynthesis. The anti-inflammatory effects of thymoquinone (active constituent of *N. sativa*) was supported by its ability to attenuate allergic airway inflammation by inhibiting Th2 cytokines and eosinophil infiltration into the airways and goblet cell hyperplasia Attenuation of airway inflammation occurred concomitant to inhibition of COX-2 (cyclogenase) protein expression and prostaglandin D2 production in a mouse model of allergic airway inflammation induced with ovalbumin [22].

Several investigations have been directed towards *N. sativa* anti-inflammatory and analgesic activity [23, 24]. The anti-inflammatory activity of black cumin seed oil has been evaluated using carrageenan-induced paw edema in rats and croton oil-induced ear edema in mice by Hajhashemi and colleagues in 2004. The aqueous and methanolic extracts of *N. sativa* shown analgesic effect in mice as it produced significant increases in reaction times in the hot plate and pressure tests [25]. *N. sativa* demonstrated analgesic activity through peripheral mechanism including inhibition of Cyclooxygenase enzyme. Since, *N. sativa* acts through both central and peripheral mechanism to reduce pain; it may be develop into a drug which provides better control of pain then the currently available analgesic. Further experiments are needed to clarify the mechanisms underlying the antinociceptive action of *N. sativa* oil and thymoquinone.

CONCLUSION

This is the first anti-inflammatory and analgesic study of *N. sativa* seed in germination phases. The methanol extracts of germinative phases of *N. sativa* showed significant anti-inflammatory and analgesic activity as compare to seed extract and standard drug indomethacin, the extracts showed highest anti-inflammatory activity from 5th day to 11th day of germination. Both activities were higher in 5th day germination extract. High metabolic activity and higher contents of secondary metabolites during germination might also be responsible for the anti-inflammatory and analgesic activity. Although existence of anti-inflammatory effect in extracts suggest a NSAID-like mechanism for it. This might be due to the presence of metabolites in methanol extracts in these days of germination. Phytochemical studies indicate that alkaloids, flavonoids, sponins, tannins and phenols are the major component in the *N. sativa* during germination [10].

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