ORIGINAL ARTICLE

Analgesic Effect of *Nigella sativa* Seeds Extract on Experimentally Induced Pain in Albino Mice

Muhammad Usman Bashir and Hamid Javaid Qureshi

ABSTRACT

Objective: To determine the analgesic effect of ethanolic extract of *Nigella sativa* seeds on experimentally-induced pain in albino mice.

Study Design: Randomized controlled trial (RCT).

Place and Duration of Study: Physiology Department, Services Institute of Medical Sciences (SIMS), Lahore, from May to September, 2009.

Methodology: The study was carried out in 90 male albino mice using acetic acid induced writhing test as a chemical model of nociception. The mice were divided in three groups of 30 each. Group A was given normal saline (control); group B was given *Nigella sativa* seed extract in a dose of 50 mg/kg; and group C received diclofenac sodium, as a reference drug. Number of writhings in treated and control groups were compared.

Results: The ethanolic extract of *Nigella sativa* seeds given intraperitoneally caused significant ($p < 0.05$) analgesic effect on nociceptive response initiated by 0.6% acetic acid; although this analgesic effect was less than that produced by diclofenac sodium.

Conclusion: Ethanolic extract of *Nigella sativa* possessed significant analgesic effect in mice.

Key words: *Nigella sativa. Analgesic. Ethanolic extract. Acetic acid writhing test.*

INTRODUCTION

Pain is basically a protective mechanism in the human body which occurs as a response to tissue injury or damage and causes the individual to react to remove the painful stimulus. Analgesics have been one of the common therapeutic categories on which research work was done.[1] There is a wide range of medicinal plants which possess analgesic properties and have been used traditionally without any undesirable effects.[2]

Nigella sativa or *'Kalonji'* in vernacular, is one of the traditionally used medicinal plants. Its seeds are commonly used in different Pakistani foods, spices and pickles. Traditionally, these have been used as medicine for the treatment of diarrhea, indigestion, dyspepsia, anorexia, vomiting, puerperal disorders, obesity, dyspnea, and skin disorders.[3] *Kalonji* seeds contain a volatile oil, a fixed oil, proteins, amino acids, reducing sugars, mucilage, alkaloids, organic acids, tannins, resins, saponins, fats, vitamins and minerals.[4]

The effects of *Nigella sativa* on hyperglycemia, hypertension, dyslipidemia, ischemia, malaria, asthma, immunity, cancers, nervous and genito-urinary system have been widely investigated,[3] but relatively less work has been done on the analgesic properties of this plant in the world; and to our knowledge no work has been done in Pakistan as yet.

The results of using *Nigella sativa* oil and aqueous extract for relieving pain and inflammation have been encouraging.[5,6] Tanko *et al.* reported the positive effect of its ethanolic extract.[7]

Thymoquinone is the major active principle of *Nigella sativa* and most of its pharmacodynamic effects are due to thymoquinone. Al-Ali *et al.*[9] carried out a study to determine LD_{50} of thymoquinone both in mice and rats, orally as well as intraperitoneally. Autopsy and histopathology of liver, kidney, heart and lungs were also determined. The study showed that the LD_{50} in mice after intraperitoneal injection was 104.7 mg/kg and after oral ingestion was 870.9 mg/kg. Mansour *et al.*[10] reported that thymoquinone at doses of 4, 8, 12.5, 25 and 50 mg/kg intraperitoneally in mice did not alter the biochemical parameters, including serum alanine transaminase, aspartate transaminase and lactate dehydrogenase. In the same study, the LD_{50} of thymoquinone was reported to be 90.3 mg/kg when given intraperitoneally in mice.

Analgesic and anti-inflammatory drug abuse has become a major problem in our country due to over-the-counter sale of such drugs and these are causing not only gastritis, gastric ulcers, gastro-intestinal tract bleeding and renal damage, but a number of other problems too. Research on such medicinal plants and natural products like *Kalonji* may provide basis for invention of some safe, cheap and effective treatment against pain in our country.

Department of Physiology, Services Institute of Medical Sciences, Lahore.

***Correspondence:** Dr. Muhammad Usman Bashir, Physiology Department, Services Institute of Medical Sciences, 6-Birdwood Road, off Jail Road, Lahore.*
E-mail: dr.gujar@yahoo.com

Received November 11, 2009; accepted April 29, 2010.

The objective of this study was to determine the analgesic effect of ethanolic extract of *Nigella sativa* seeds on experimentally-induced pain in albino mice.

METHODOLOGY

The study was conducted in the Physiology Department, Services Institute of Medical Sciences, Lahore, from May to September 2009.

Ninety adult and healthy male albino mice, each weighing 20-30 grams (mean weight 24 grams) were obtained from National Institute of Health, Islamabad. Animals were housed in groups of 30 per cage for at least one week before the start of experiments. Housing conditions were thermostatically maintained at 26±2° C and a light/dark cycle (lights on: 0900-2100). Animals were given food and water *ad libitum*.

Ethanol extract of *Nigella sativa* seeds was made and standardized using facilities available at Applied Chemistry Research Centre, PCSIR Laboratories, Lahore. *Nigella sativa* seeds obtained from local market were dried and then crushed into a coarse powder using an electric grinder. This powder was then extracted with ethanol using Soxhlet extractor. The extract was filtered and the solvent (ethanol) evaporated in vacuum with a rotatory evaporator. This yielded a blackish-brown concentrate. This concentrate was kept at 4° C prior to use. The crude extract was dissolved in sterilized distilled water and then diluted to the desired concentration.[8]

The analgesic activity was evaluated in mice by employing acetic acid induced-writhing test.[11] The adult male albino mice were randomly divided into three groups of 30 each. Group A (control) were given normal saline in a dose of 10 ml/kg of body weight, intra-peritoneally. Group B (experimental) were given ethanol extract of *Nigella sativa* seeds in a dose of 50 mg/kg of body weight intraperitoneally. Group C (reference) received diclofenac sodium, 25 mg/kg of body weight, intraperitoneally.

After the administration of the "drug" (saline/*Nigella*/ diclofenac sodium), each animal was shifted in an individual, transparent glass chamber. After 30 minutes, acetic acid (0.6%) in a dose of 10 ml/kg was injected intraperitoneally to each mouse and the number of abdominal contractions (writhings) for each mouse was counted for the next 15 minutes.[11]

The results were presented as mean±SEM and the comparisons between the experimental groups were made using ANOVA test on SPSS version 15.0. A 'p' value less than 0.05 was considered as indicative of significance. The inhibition (percent) was calculated by the following formula:

$$\text{Inhibition (\%)} = (1\text{-Wt/Wc}) \times 100$$

where Wt and Wc represented the number of writhings in treated and control groups, respectively.

RESULTS

The mean number of writhings (abdominal contractions) in each of the three groups have been shown in Figure 1. The number of writhings is highest in group A (control group). It was less in group B (*Nigella sativa* group) than group A, and the least in group C (diclofenac group) indicating maximum analgesia offered by diclofenac sodium. The percentage inhibition (pain inhibition) produced by group B and group C on the writhing (pain) response is depicted in the Table I. The number of writhings in each of three groups is shown as mean±SEM. When the mean number of writhings of group B and C were compared with group A (control) using one way ANOVA test, the p-value came to be < 0.001.

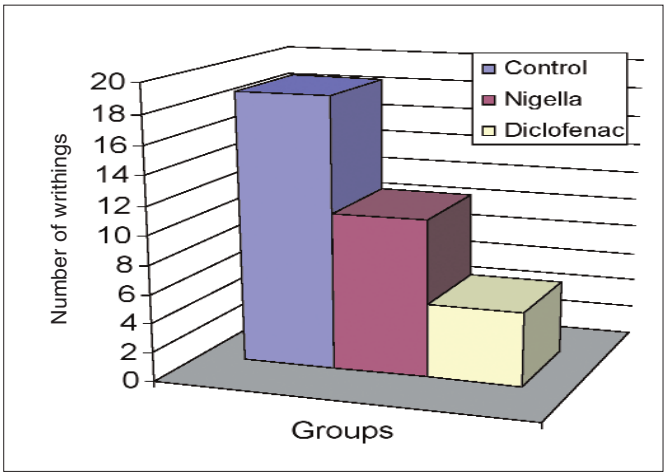

Figure 1: Mean number of writhings in the three groups.

Table I: Effect of ethanolic extract of *Nigella sativa* and diclofenac on writhing in mice.

Treatment with	Number of writhings (mean±SEM)	Inhibition (%)
Saline (control)	18.77±0.414	-
Nigella sativa	10.90±0.268*	41.916
Diclofenac	5.10±0.251*	72.823

*$p < 0.001$ as compared to control.

DISCUSSION

This study assessed the analgesic effect of ethanolic extract of *Nigella sativa* seeds of Pakistani origin. A chemical model of nociception was employed. The extract showed a significant ($p < 0.001$) analgesic effect and produced an inhibition of 41.91% on the writhing as compared to inhibition of 72.82% caused by diclofenac sodium. Although this inhibition (i.e. 41.91%) produced by the extract is less than that reported by Tanko *et al.*[7] (i.e. 67.1%) still it is significant as compared to control and reference groups. This difference might be due to the fact that seeds of different origin were used here. Many factors can influence the percentage of active constituents in the seeds e.g. heredity, age of the plant, environment, harvesting time, fertilization and irrigation

techniques, distillation procedure etc.[13] Previously, analgesic activity has been reported in the aqueous extract, methanolic extract, ethanolic extract, *Nigella sativa* fixed oil and *Nigella sativa* essential oil.[5-7,12,13] Each of these produced inhibitory effects on writhing caused by 0.6% acetic acid; but exact mechanism of action of this analgesic effect is yet not clear. The active ingredients and components obtained in each of these extracts/oils also differ from each other. Thymoquinone has remained one of the main components in almost all of these extracts/oils.[5,12,13] But whether thymoquinone alone or some other active agents are also responsible for analgesic effect, is still unclear. Thymoquinone is reported to inhibit the generation of thromboxane A_2 and leukotriene B_4, thus suggesting an inhibitory effect on both the cyclo-oxygenase and lipo-oxygenase pathway.[14]

In addition, Abdel-Fattah *et al.*[5] suggested that supra-spinal opioid system is involved in the analgesic effects of thymoquinone but Hajhashemi *et al.* contradicted this finding and suggested that mechanisms other than the stimulation of opioid receptors are involved.[13] This difference might be due to the fact that different products were used by them. Essential oil was used by Hajhashemi *et al.* and fixed oil by Abdel-Fattah *et al.* and there is a significant difference in their chemical composition.[13] This emphasizes the need to evaluate the presence of other active agents besides thymo-quinone, and investigation of their possible mechanism of action.

It has been suggested that acetic acid acts by releasing endogenous mediators that stimulate the nociceptive neurons.[15] It was postulated that the writhing response is induced by local peritoneal receptors activation,[16] and involved prostanoids mediators. As a matter of fact, increased levels of PGE_2 and PGF_2 in peritoneal fluids as well as lipo-oxygenase production were reported.[17,18] The results of the present study showed that diclofenac sodium, which inhibits cyclo-oxygenase, causes a significant inhibition of acetic acid induced pain. This is in accordance with previous reports indicating that this test is sensitive to non-steroidal anti-inflammatory drugs.[19] Therefore, the analgesic activity of ethanolic extract of *Nigella sativa* might be due to inhibition of lipo-oxygenase and/or cyclo-oxygenases. On the other hand, Tanko *et al.* reported that the analgesic activity of ethanolic extract might be due to presence of tannins and flavanoids present in the extract as these are also believed to possess analgesic properties.[7]

CONCLUSION

Ethanolic extract of *Nigella sativa* possessed significant analgesic activity in mice. This finding supports the use of *Nigella sativa* seeds in traditional medicine for the treatment of some painful and inflammatory disorders.

Acknowledgements: We are very grateful to Dr. Salma Rehman of Applied Chemistry Research Centre, PCSIR Laboratories, Lahore and Mr. Muhammad Ghias, statistical officer, SIMS/SHL, Lahore.

REFERENCES

1. Elisabetsky E, Castilhos ZC. Plants used as analgesics by Amazonian Caboclos as a basis for selecting plants for investigation. *Int J Crude Drug Res* 1990; **28**:309-20.
2. Malairajan P, Gopalakrishnan G, Narasimhan S, Jessi Kala Veni K. Analgesic activity of some Indian medicinal plants. *J Ethnopharmacol* 2006; **106**:425-8. Epub 2006 Mar 27.
3. Gilani AH, Jabeen Q, Khan MA. A review of medicinal uses and pharmacological activities of *Nigella sativa*. *Pak J Biol Sci* 2004; **4**:441-51.
4. Takruri HR, Dameh MA. Study of the nutritional value of black cumin seeds *(Nigella sativa L)*. *J Sci Food Agric* 1998; **76**:404-10.
5. Abdel-Fattah AM, Matsumoto K, Watanabe H. Anti-nociceptive effects of *Nigella sativa* oil and its major component, thymoquinone, in mice. *Eur J Pharmacol* 2000; **400**:89-97.
6. Al-Ghamdi MS. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. *J Ethnopharmacol* 2001; **76**:45-8.
7. 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.
8. Abdulelah HA, Zainal-Abidin BA. *In vivo* anti-malarial tests of *Nigella sativa* (black cumin) different extracts. *Am J Pharmacol Toxicol* 2007; **2**:46-50.
9. Al-Ali A, Alkhawajah AA, Randhawa MA, Shaikh NA. Oral and intraperitoneal LD_{50} of thymoquinone, an active principle of *Nigella sativa*, in mice and rats. *J Ayub Med Coll Abbottabad* 2008; **20**:25-7.
10. Mansour MA, Ginwai OT, El-Hadiya T, El-Khatib AS, Al-Shabanah OA, Al-Sawaf HA. Effects of volatile oil constituents of *Nigella sativa* on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effects of intraperitoneal. *Res Commun Mol Pathol Pharmacol* 2001; **110**:239-51.
11. Hrabe de Angelis MM, Chambon P, Brown S. Standards of mouse model phenotyping. Weinheim: *Wiley-VCH*; 2006.
12. Al-Naggar TB, Gomez-Serranillos MP, Carretero ME, Villar AM. Neuropharmacological activity of *Nigella sativa* L. extracts. *J Ethnopharmacol* 2003; **88**:63-8.
13. Hajhashemi V, Ghannadi A, Jafarabadi H. Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug. *Phytother Res* 2004; **18**:195-9.
14. El-Dakhakhny M, Madi NJ, Lembert N, Ammon HP. *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5-lipoxygnase products in polymorphonuclear leukocytes from rats. *J Ethnopharmacol* 2002; **81**:161-4.
15. Collier HOJ, Dinneen LC, Johnson, CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br J Pharmacol* 1968; **32**:295-310.

16. Bentley GA, Newton SH, Starr J. Studies on the anti-nociceptive action of agonist drugs and their interaction with opioid mechanisms. *Br J Pharmacol* 1983; **79**:125-34.

17. Deraerdt R, Jouquey S, Delevallee F, Flahaut M. Release of prostaglandins E and F in algogenic reaction and its inhibition. *Eur J Pharmacol* 1980; **51**:17-24.

18. Dhara AK, Suba V, Sen T, Pal S, Nagchaudhuri AK. Preliminary studies on the anti-inflammatory and analgesic activity of the methanolic fraction of the root extract of Tragia involucrate. *J Ethnopharmacol* 2000; **72**:265-8.

19. Vane JR, Botting RM. The mechanism of action of aspirin. *Thromb Res* 2003; **110**:255-8.