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Evaluation of the Analgesic Effects of *Nigella sativa* Ethanolic Extracts on Experimentally Induced Pain in Albino Mice

Tahani Ibrahim[1*] and Beashair AL-Rashidi[1]

[1]Faculty of Pharmacy, Qassim University, Saudi Arabia.

Authors' contributions

This work was carried out in collaboration between both authors. Author TI designed the study, wrote the protocol, analysis of the study and wrote the first draft of the manuscript. Author BAR managed the literature searches and the experimental process. Both authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Background: Analgesic and anti-inflammatory drug abuse has become a major problem in the world 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 *Nigella sativa* may provide basis for invention of some safe, cheap and effective treatment against pain in.

Objectives:

- Evaluation of the analgesic effects of *Nigella sativa* ethanolic extract.
- Assessment of synergistic effects of *Nigella sativa* ethanolic extract with non-steroidal anti-inflammatory drug.

Methodology: The analgesic activity was evaluated in mice by employing acetic acid induced-writhing test. The adult albino mice were divided into five groups of 5 each. Group I (control) was given normal saline in a dose of 10 ml/kg of body weight, intraperitoneally. Group II & III

**Corresponding author: E-mail: tahani-ibrahim@hotmail.com;*

(experimental) was given ethanolic extract of *Nigella sativa* seeds intraperitoneally in a dose of 50 mg/kg, 100 mg/kg of body weight respectively. Group IV (reference) was given diclofenac sodium, 25 mg/kg of body weight, intraperitoneally. Group V received combination of diclofenac sodium and nigella extract in dose 25 mg/kg, 50 mg/kg of body weight intraperitoneally respectively. 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 effecton nociceptive response initiated by 0.6% acetic acid; although this analgesic effect was less than that produced by diclofenac sodium. On the other hand the combination of diclofenac and *Nigella sativa* exhibit total analgesia (100% inhibition)

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

Keywords: Nigella sativa; analgesic; ethanolic extract; acetic acid writhing test.

1. 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].

Herbal medicines with good absorption, less toxicity, and easy availability have been used since ancient times. Among various medicinal plants, an annual herbaceous plant *Nigella sativa* (*N. sativa*) that belongs to the Ranunculaceae family is emerging as a miracle herb with a rich historical and religious background since many researches revealed its wide spectrum of pharmacological potential [3].

N. sativa is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia [3].

N. sativa seeds, commonly known as black seed or black cumin, and oil derived have been used for their medicinal, aromatic or flavoring properties since ancient times in different civilizations [4].

Among Muslims, it is considered as one of the greatest forms of healing medicine available due to it was mentioned that black seed is the remedy for all diseases except death in one of the Prophetic hadith. It is also recommended for use on regular basis in Prophetic Medicine [5].

The properties, composition and potential pharmacological and therapeutic activities of this species have been extensively reviewed [6,7].

Studies on *N. sativa* seeds and *N. sativa*-derived oil have provided scientific support for their traditional use as anti-diabetic activity [8], anticancer activity [9],cardiovascular-protective activity [10], gastro-protective activity [11], pulmonary protective and anti-asthmatic activity [12] , neurological activity [13], anti-inflammatory and analgesic effects [9], hepato-protective [14,15] and nephro-protective activities [16].

The seeds and oil of *N. sativa* have a broad range of activities against a number of microbes, and are thus capable of inhibiting gram-positive and gram-negative bacteria [17], coccidian [18,19] and helminthes [20].

Nearly 32 compounds have been identified, of which thymoquinone, thymohydro-quinone, dithymoquinone, p-cymene, arvacrol, 4-terpineol, t-anethol, sesquiterpene longifolene and á-pinene are some of the predominant compounds. Other derivatives found in trace amounts include carvone, limonene, citronellol. Many of these compounds are capable of inducing pharmacological effects in humans. Properties of whole seeds or their extracts are mainly attributed to quinine constituents, of which thymoquinone (TQ) is the most abundant as well as the potent pharmacologically active compound [21]. *N. sativa* oil as well as TQ have been shown to prevent oxidative injuries. It is reported that *N. sativa* and its derivative TQ inhibit eicosanoid generation in leucocytes and membrane lipid peroxidation [22].

1.1 Objectives

- Evaluation of the analgesic effects of *Nigella sativa* ethanolic extract.

- Assessment of synergistic effects of *Nigella sativa* ethanolic extract with non-steroidal anti-inflammatory drug

2. MATERIALS AND METHODS

2.1 Animals

Albino mice (weighing 25-30 g) were obtained from the animal house of Qassim University. They were housed in cages under standard environmental conditions and had free access to pellet diet and tap water. The use of animals and experimental protocol had the prior approval of the Experimental Animal Care and Use Committee of the Department of Pharmacology, College of Pharmacy, Qassim University.

2.2 Chemicals

The following chemicals were used in the experiments; acetic acid, diclofenac sodium.

2.3 Methods

2.3.1 Plant materials and extracts preparation

N. sativa seeds was obtained from local market and crushed into a coarse powder using an electric grinder. Forty grams from the powder was accurately weighed. This powder was extracted with 100 ml ethanol using Soxhlet extractor for 24 hours. 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 40 C prior to use. The crude extract was dissolved in sterilized distilled water and then diluted to the desired concentration.

2.3.2 Evaluation of analgesic activity

2.3.2.1 Acetic acid test method

The analgesic activity was evaluated in mice by employing acetic acid induced-writhing test as a chemical model of nociception.

The adult albino mice were divided into five groups of 5 each. Group I (control) was given normal saline in a dose of 10 ml/kg of body weight (IP). Group II &III (experimental) was given ethanolic extract of *N. sativa* seeds (IP) in a dose of 50 mg/kg, 100mg/kg of body weight respectively. Group IV (reference) was given diclofenac sodium, 25 mg/kg of body weight (IP). Group V was received combination of diclofenac sodium and nigella extract in dose 25 mg/kg, 50 mg/kg of body weight (IP) respectively. After the administration of the "drug" (saline/Nigella/ diclofenac sodium, diclofenac sodium and Nigella combination), 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. The inhibition (percent) was calculated by the following formula:

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

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

2.4 Statistical Analysis

The results presented as Mean±SEM and the comparisons between the experimental groups performed using ANOVA test on SPSS version (21). A 'p' value <0.05 was considered significant.

3. RESULTS

The result of analgesic study was presented as Mean ± SEM. Intraperitoneal administration of *N. sativa* ethanolic extract at doses 50 mg/kg and 100 mg/kg decreased the number of abdominal constrictions by 45.18%, and 59.64%, respectively .These changes were significant (P >0.05). On the other hand the reference (diclofenac Na) showed 82.53% inhibition. The mean number of writhings (abdominal contractions) in the four groups has been shown in Table 1. All tested extracts of *N. sativa* (50 &100 mg/kg) as well as diclofenac Na (reference drug) decreased the number of writhings in comparison to that of the control.

The administration of ethanolic extracts of *N. sativa* and diclofenac Na, abolished the writhing effect i.e.100% inhibition (zero writhing) in comparison to that of the reference (diclofenac Na) 82.53% which mean there is synergistic effect between diclofenac Na and *N. sativa*.

The synergistic effect of *N. sativa* and Diclofenac is shown in Table 2.

Table 1. Effect of ethanolic extract of *N. sativa* and diclofenacon writhing in Mice

Treatment	Number of writhings (Mean±SEM)	Inhibition (%)
Normal Saline (control)	33.2±1.98	-
N. sativa (50 mg/ kg)	18.2±1.39	45 .18%
N. sativa (100 mg/kg)	13.4±0.81	59.64%
Diclofenac injection (25 mg /kg)	5.8±0.58	82.53%

Table 2. Synergistic effect of ethanolic extract of *N. sativa* and diclofenac Na

Treatment	Number of writhings (Mean±SEM)	Inhibition (%)
Normal Saline (control)	33.2±1.98	-
Diclofenac injection (25 mg /kg)	5.8±0.58	82.53%
N. sativa (50 mg/ kg)	18.2±1.39	45 .18%
N. sativa (50 mg/ kg) + Diclofenac Na (25 mg /kg)	0	100.0%

4. DISCUSSION

The present study compared the analgesic effect of ethanolic extract of *N. sativa* seeds and diclofenac sodium. Our results showed that the ethanolic extracts of *N. sativa* (50 mg/kg & 100 mg/kg) produced significant (P>0.05) analgesic effect. The extracts produce 45.18% and 59.64% inhibition on writhing as compared to inhibition of 82.53% produced by diclofenac sodium.

The inhibition produced by the extracts is more than that reported by Bashir MU and Qureshi HJ (41.91%) [23] and less than that reported by Tanko et al. (67.1%) [24]. These differences might be due to different origin seeds in addition to many factors that can influence the percentage of active constituents in the seeds e.g. heredity, age of the plant, environment, harvesting time, fertilization and irrigation techniques, extraction procedure etc [25].

The analgesic effect of aqueous extract, methanolic extract, and ethanolic extract of *N. sativa* fixed oil and *N. sativa* essential oil was studied by [24,26,27,28,25]. Each of these produced inhibitory effects on writhing caused by 6% acetic acid. Evaluation of analgesic and anti-inflammatory effects of *N. sativa* seeds has been the subject of several studies in recent years.

Those studies were focused on pure thymoquinone, fixed oil and aqueous extracts of the seeds [22,29,30]. Thymoquinone has remained one of the main components in almost all of these extracts/oils [26,28,25]. But whether thymoquinone alone or some other active agents are also responsible for analgesic effect, is still unclear.

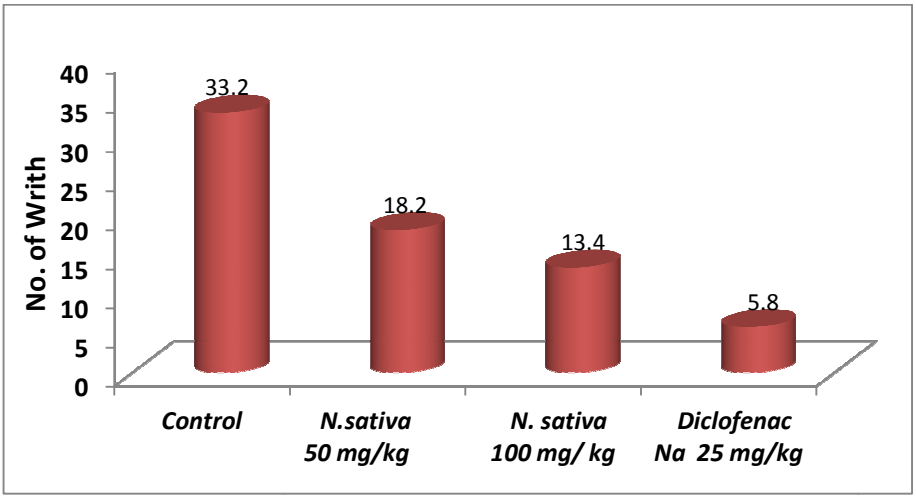

Fig. 1. Mean number of writhing in the four groups

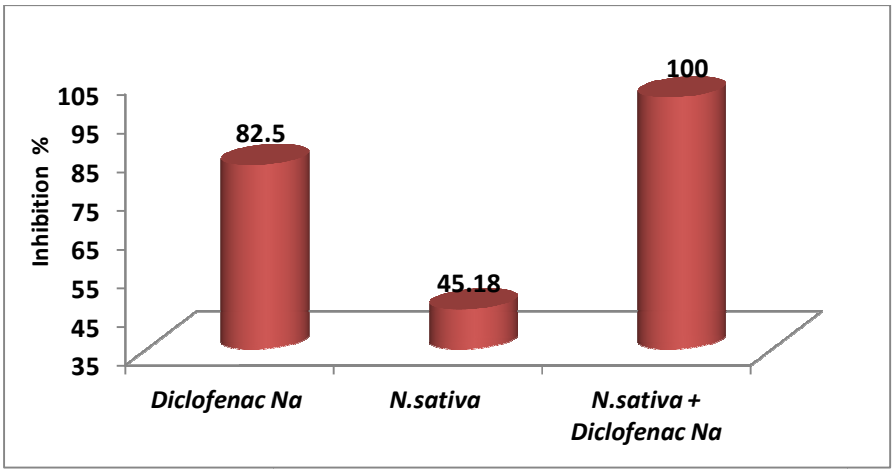

Fig. 2. Inhibition percentage in the three groups

Thymoquinone is reported to inhibit the generation of thromboxane A2 and leukotriene B4, thus suggesting an inhibitory effect on both the cyclo-oxygenase and lipo-oxygenase pathway [29]

It has been suggested that acetic acid acts by releasing endogenous mediators that stimulate the nociceptive neurons [31]. It was assumed that the writhing response is induced by local peritoneal receptors activation [32], and involved prostanoids mediators. It was reported that there were increased levels of PGE2 and PGF2 in peritoneal fluids as well as lipo-oxygenase production [33,34].

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 consistent with previous reports indicating that this test is sensitive to non-steroidal anti-inflammatory drugs [35]. Therefore, the analgesic activity of ethanolic extract of *N. sativa* might be due to inhibition of lipo- oxygenase and/or cyclo-oxygenases. On the other hand, Tanko et al. [24] 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. This emphasizes the need to evaluate the presence of other active agents besides thymoquinone and investigation of their possible mechanism of action.

5. CONCLUSION

Natural products in general and medicinal plants in particular, are believed to be a key source of new chemical substances with potential therapeutic efficacy.

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 disorders.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The use of animals and experimental protocol had the prior approval of the Experimental Animal Care and Use Committee of the Department of Pharmacology, College of Pharmacy, Qassim University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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