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Evaluation of Analgesic Activity of Thymoquinone the Major Constituent of *Nigella sativa* Seeds in Mice

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Abstract

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 cycloxygenase and 5-lipooxygenase pathways of arachidonic acid metabolism. The present study was aimed to evaluate the analgesic activity of pure compound, thymoquinone the major constituent of *Nigella sativa* seeds in mice. The analgesic activity was determined by hot plate, tail immersion, tail flick method and acetic acid induced writhing test in mice. Thymoquinone (10, 20, 30 mg/kg, body weight) and Aspirin (20mg/kg) made as suspensions prepared in 1% carboxy methyl cellulose and were fed to mice intraperitoneally. In tail flick method thymoquinone exhibited maximum analgesic effect at a dose of 30mg/kg after 120 min as compared to control and standard. Maximum analgesic effect was noted at a dose of 10 mg/kg after 120 min. the poor analgesic effect of thymoquinone at a dose of (20 and 30 mg/kg) as compared to control and standard drug by hot plate method. However with writhing test the thymoquinone showed maximum protection at a dose of 30 mg/kg (98.23%). The thymoquinone have minimum effect showed at dose of 10 mg/kg (69.72%) and 20 mg/kg (42.26%) as compared to standard and control. But in tail immersion method thymoquinone exhibited maximum activity after 120 min at a dose of 20 mg/kg as compared to control and standard drug while at a dose of 30 mg/kg the compound did not showed any further enhancement in analgesic activity. It is concluded from the present study that thymoquinone the major constituent of *Nigella sativa* possesses potent analgesic activity in mice.

Keywords: Thymoquinone, *Nigella sativa,* Hot plate, Tail immersion, Tail flick method, Acetic acid induced writhing test

INTRODUCTION

Pain is a disabling accompaniment of many medical conditions and pain control is one of the most important therapeutic priorities[1]. Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is always a warning signal and primarily protective in nature but often causes a lot of discomfort and lead to many adverse effects[2]. Analgesics are drugs used to treat or reduce pain and the classical analgesic drugs notably opiates and non-steroidal anti-inflammatory drugs have their origin in natural products but many synthetic compounds that act by the same mechanism have been developed and are associated with serious adverse effects such as ulceration, gastrointestinal bleeding, additive potential, respiratory distress, drowsiness, nausea etc[3,4]. 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[5]. Recently, some evidences suggest that *Nigella 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[6]. Thymoquinone is an active principle of *Nigella sativa*, which is a member of the *Ranunculaceae* family of plants. This plant is grown in several parts of the world, particularly Middle East, Middle Asia and Far Eastern countries; and its seeds have been used as a natural remedy for many diseases over centuries as well as flavouring agent in bakery products and pickles[7,8]. Recently many active principles have been isolated from *Nigella sativa*, including thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine N oxide, nigellicine, nigellidine and alphahedrin[9-13]. In addition, using modern scientific techniques, many pharmacological effects of

Nigella sativa and its active principles have been identified e.g. immune stimulation, analgesic, anti-inflammatory, anticancer, hypoglycemic, antihypertensive, anticestodal and antimicrobial effects[14-16]. Therefore the present study was undertaken to evaluate its analgesic activity on animal model, so as to support the local herbal drug manufacturing industry and to develop some topical preparations as an economical way to manage pain and inflammation of moderate severity.

MATERIALS AND METHODS

Chemical reagents

Aspirin and thymoquinone were purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI, USA). Carboxy methyl cellulose as a suspending agent was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), India. All the chemicals used in this study were of analytical grade.

Experimental animals

Swiss albino male mice (20-25 gm) were group housed (n= 6) under a standard 12 hr light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65%). Mice received standard rodent chow and water *ad libitum*. Animas were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00hr. Separate group (n=6) of mice was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity studies

Acute toxicity studies were conducted on Swiss albino mice as per the Organization for Economic Co-operation and Development (OECD) 423 guidelines[17]. Four different doses of thymoquinone, i.e. 200, 150, 100 and 50 mg/kg body weight were administered intraperitoneally to the mice of each tested group. The mice in both test and control groups were allowed for free access to water and feed. The animals were observed continuously just after drug administration at 30 min, 60 min and 120 min interval of time for changes in general behaviors and physiological activities. Furthermore the occurrence of mortality was noted up to 72 h. The maximum non lethal dose was found to be 50mg/kg body weight; hence 10, 20, 30mg/kg dose was taken as effective dose for thymoquinone to evaluate analgesic activity.

Animal grouping

Animals were divided into three major groups. Each group contain six animals (n=6).

Group-1: drug treated group (Aspirin)

Group-2: treated with saline water served as control group.

Group-3: further divided in three sub groups

Analgesic activity

Analgesic activity was investigated using following methods: (1) tail immersion method based on thermal radiant heat as a source of pain[18]; (2) hot plate method based on jumping from hot plate at 55°C[19]; (3) acetic acid induced writhing based on chemical radiant as a source of pain[20]; (4) tail flick method using an analgesiometer[21].

Tail immersion method

After administration of designed drugs as above mentioned, the base line latency was measured before and after drug treatment in a regular interval of 30 min, 60 min and 120 min by immersing the tail tips (1-2 cm) of the mice in water bath thermostatically maintained at temperature of (45±1) °C. The actual flick response of mice was measured by stop watch and results were compared with control and standard group. The maximum cutoff time for immersion was 180 seconds to avoid the injury of the tissues of tails.

Hot plate

Animals in all groups were individually exposed to the hot plate. The time taken in seconds for fore paw licking or jumping was taken as reaction time and was measured in a regular time interval and the reaction strength of each rat was determined before and after drug treatment in a regular interval of 30 min, 60 min and 120 min. A cutoff period of 15 seconds was set up to avoid damage to the paws. The groups administered with tested extracts and pure compound were compared to control and standard drug groups.

Acetic acid induced writhing

Tested extracts and pure compound were administered intraperitoneally using different doses as defined in study design, 30 min prior to administration of 0.1 ml acetic acid (1%). Animals were observed individually and the number of writhes was counted for 20 min commencing 5 min after injection of acetic acid. The significant reduction in number of writhes of treated groups was compared to that of the control and standard groups. The percentage inhibition of abdominal constrictions was calculated using the following formula.

Inhibition (%) =Mean No. of writhes (Control) - Mean No. of writhes (Test)/ Mean No. of writhes (Control) ×100

Tail-flick method

Analgesia was measured using modified method of D Amour and Smith[21] called as tail flick method using an analgesiometer. Reaction time in seconds was used as the unit for measurement of pain and an increase in reaction time was indicative of analgesia. Time between placing the tail of the rat on the radiant heat source and sharp withdrawal of the tail was recorded as “reaction time”. Cut off time of ten seconds was imposed in all sets of experiments taken as maximum latency so as to rule out thermal injury while noting down the reaction time. Animals that showed a mean reaction time outside the range of five-six seconds, were discarded. In all the groups, tail-flick test was performed prior to drug administration, and at 30, 60, 90 and 120 minutes after drug administration, and the reaction time at each time interval (test latency) was calculated.

Statistical analysis

Data are given as mean ± standard error of mean. Data were analyzed with ANOVA where control group was compared with rest group.

RESULTS

Result of analgesic activity of pure compound thymoquinone measured by tail flick method was shown in Table 1. Data are presented as mean ± SEM significant with correspond time of normal saline group. The thymoquinone exhibited good analgesic effect at a dose of 10 and 20 mg/kg as compared to control and standard. Maximum analgesic effect was noted at a dose of 30 mg/kg after 120 min. Reaction time is a licking time in seconds of animals for front paw. Results of analgesic activity of pure compound thymoquinone, measured by hot plate method were shown in Table 2. The thymoquinone exhibited good analgesic effect at a dose of 10 and 30 mg/kg as compared to control and standard. Maximum analgesic effect was noted at a dose of 10 mg/kg after 120 min. the poor analgesic effect of thymoquinone at a dose of (20 and 30 mg/kg) as compared to control and standard drug. Results of

the analgesic activity of pure thymoquinone analyzed by writhing test were shown in Table 3. Thymoquinone exhibited no analgesic effect at dose of 10 and 20 mg/kg as compared to control and standard. The drug showed maximum protection at a dose of 30 mg/kg (98.23%). The thymoquinone drug has minimum effect showed at dose of 10 mg/kg (69.72%) and 20 mg/kg (42.26%) as compared to standard and control. Reaction time is taken by mice to withdraw the tail, recorded by stop watch in seconds. Results of the analgesic activity of pure compound thymoquinone measured by tail immersion method were shown in Table 4. At a dose of 10mg/kg and 20mg/kg, the thymoquinone showed good analgesic effect as to control and standard drug. Thymoquinone exhibited maximum activity after 120 min at a dose of 20 mg/kg as compared to control and standard drug while at a dose of 30 mg/kg the compound did not showed any further enhancement in analgesic activity.

Table 1 Analgesic activity of thymoquinone using tail flick method in mice

Group(n=6)	Dose	Test latency at various time point				
		30min	60min	90min	120min	Mean
Standard (Aspirin)	20mg/kg	8.73±0.29	7.78±0.13	6.79±0.12	6.77±0.23	7.51±0.19
Control	-	2.44±0.24	2.40±0.17	2.33±0.12	2.30±0.13	2.36±0.16
Thymoquinone	10mg/kg	4.22±0.12*	3.24±0.13	4.16±0.23*	5.22±0.22*	4.21±0.17*
	20mg/kg	4.08±0.24*	4.60±0.32*	5.28±0.02*	5.24±0.10*	4.08±0.17*
	30mg/kg	4.23±0.20*	4.26±0.16*	5.21±0.12*	6.32±0.03*	5.00±0.12*

Effect of thymoquinone on basal reaction time in tail flick test n=6 in each group, values expressed in mean ±S.E p<0.001. *= p<0.001 significant difference from vehicle treated group.

Table 2 Analgesic activity of thymoquinone using hot plate method in mice

Group (n=6)	DOSE	Time latency at various time points				
		30min	60min	90min	120min	MEAN
Standard (Aspirin)	20mg/kg	14.2±0.01	12.2±0.02	11.26±0.03	11.53±0.02	12.29±0.02
Control	–	6.22±0.13	5.32±0.11	5.01±0.18	4.20±0.02	5.18±0.11
Thymoquinone	10mg/kg	11.68±0.79*	11.38±0.80*	10.24±0.54*	10.1±0.75*	8.29±0.72*
	20mg/kg	11.43±0.86*	11.12±0.76*	10.02±0.89*	10.02±0.69*	6.08±0.8*
	30mg/kg	11.42±0.95*	11.06±0.98*	10.41±0.79*	10.00±0.88*	6.42±0.9*

Effect of thymoquinone in hot plate test n=6 in each group, values expressed in mean ±S.E p<0.001. *= p<0.001 significant difference from vehicle treated group.

Table 3 Analgesic activity of thymoquinone using writhing test in mice

Groups (n= 6 animals)	DOSE(mg/kg)	Number of writhes	Percentage analgesia
Standard drug (Aspirin)	20	23.00±2.48	54.90%
Control (normal saline2 mL/kg p.o.)	0.1	20.83 ± 1.078	–
Thymoquinone	10	7.149 ± 0.6008*	69.72%
	20	3.73 ± 0.3063*	42.26%
	30	1.21±0.20*	98.23%

Effect of thymoquinone in writhing test n=6 in each group, values expressed in mean ±S.E p<0.001. *= p<0.001 significant difference from vehicle treated group.

Table 4 Analgesic activity of thymoquinone using tail immersion method in mice

Groups	DOSE (mg/kg) BW	RREACTION TIME (seconds)				
		0min	30min	60min	120min	Mean
Standard drug (Aspirin)	20	3.63±0.20	6.16±0.04	7.14±0.06	7.75±0.13	6.17±0.09
Control (saline water)	_	2.34±0.10	2.32±0.23	2.23±0.21	2.20±0.22	2.20±0.22
Thymoquinone	10	3.02±0.03	5.26±0.25*	4.89±0.01*	4.55±0.1	5.33±0.10*
	20	2.56±0.00	4.45±0.00*	10.1±0.00*	3.00±0.09	6.04±0.02*
	30	2.33±0.28	3.61±0.00	1.28±0.02	5.02±0.47*	2.81±0.19

Effect of thymoquinone in tail immersion test n=6 in each group, values expressed in mean ±S.E p<0.001. *= p<0.001 significant difference from vehicle treated group.

DISCUSSION

Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness. Centrally acting analgesics act by raising the threshold for pain and also altering the physiological response to pain. On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain. The animal models employed for screening of analgesic activity in this study are pain-state models using thermal stimuli which include tail-flick and hot plate methods. Both methods are useful in illustrating centrally mediated antinociceptive responses which focus generally on changes above the spinal cord level. While the tail-flick method mediates a spinal reflex to a nociceptive stimulus, hot plate method involves higher brain functions and is regarded a supraspinally organized response. In the present study analgesic activity of pure compound thymoquinone was analyzed by four different methods (hot plate, tail immersion, tail-flick and acetic acid induced writhing method). The activities were determined at a dose of 10, 20 and 30 mg/kg. Aspirin was used as standard reference drugs. Tail immersion method which was used for evaluating centrally acting analgesic effects of drugs showed no increase in latency. Analgesic effect against thermal noxious stimuli may be obtained through opioid receptors or through modulation of several neurotransmitters involved in relevant phenomena. The overall analgesic effect of thymoquinone by thermal parameter was increased with passage of time and maximum effect was achieved after 60 and 120 min. The Intraperitoneal administration of thymoquinone test involved the increased nociceptive threshold in mice and it had increased up to 60 min and then reduced with passage of time. Significant reduction in the animal sensitivity to pain caused by the pressure showed central protecting effect of thymoquinone was comparable to aspirin. The analgesic effect of thymoquinone was investigated by using eddy's hot plate method. This test involved marked central analgesic effect as evidenced by significant increase in reaction time. The control group has no analgesic at all doses while pure compound thymoquinone showed maximum activity at a dose of 10 mg /kg after 120 min by increase in the reaction time (increase threshold potential of pain) may be due to the inhibition of prostaglandins synthesis. Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles like phenylquinone or acetic acid in mice. Analgesic activity of the test compound is inferred from decrease in the frequency of writhes. The writhing response is considered as a reflexive test. Signals transmitted to central nervous system in response to pain due to irritation, cause release of mediators such as prostaglandins which contributes to the increased sensitivity to nociceptors. Thymoquinone decreased the number of writhes significantly at all doses compared to reference drug aspirin and control. Decreases in writhes are generally considered as an important parameter of analgesic activity in acetic acid induced writhing test.

CONCLUSION

In conclusion, it is evident that thymoquinone, the major constituent of *Nigella sativa* seeds has a wide spectrum of favorable effects. In our review we concentrated on properties of thymoquinone, analgesic effects, which are supported by evidence-based research elaborating the molecular mechanisms. In the present study thymoquinone possess potent analgesic activity. The administration of thymoquinone showed zero percent quantal incidence of mortality in rats, indicating excellent safety profile. These results indicate that TQ might be used for the treatment of animal analgesic under field conditions.

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