Potentiation of Valproate-induced Anticonvulsant Response by *Nigella sativa* Seed Constituents: The Role of GABA Receptors

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Abstract: This study investigated antiepileptic effects of the main constituents of *Nigella sativa* (NS) seed (i.e. aqueous extract (AE), fixed oil (FO), volatile oil (VO)) and the main components of its VO (i.e. thymoquinone, α-pinene and p-cymene) using pentylenetetrazole (PTZ) and maximal electroshock (MES)-induced convulsions. The potential of these constituents to induce minimal neurological deficit (MND) was also evaluated by using chimney test.

Except for the FO, all of the NS seed constituents protected mice effectively against PTZ-induced convulsions. The activity of the VO in this model maybe attributed mainly to its content of thymoquinone and p-cymene and to a lesser extent, α-pinene. VO and its component p-cymene effectively suppressed convulsions induced by MES. The contents of p-cymene present in the effective dose of the VO maybe partially responsible for its anti-seizure effects.

All of the NS seed constituents induced varying degrees of MND in the chimney test. MND induced by VO may pertain to its contents of thymoquinone (63%), p-cymene (23%) and α-pinene (<14%). Protective indices of p-cymene and thymoquinone were closer to one, but only in PTZ model.

Exploration on the role of receptors suggests that picrotoxin and bicuculline-sensitive GABA receptors, most probably GABAA receptors, mediate an increase in GABAergic response. In the part dealing with the interaction of valproate with thymoquinone, it can be mentioned that thymoquinone increased the potency of valproate in both PTZ and MES models.

Keywords: Anticonvulsant, Maximal electroshock seizure, *Nigella sativa*, Pentylenetetrazole, Potentiation, Valproate.

Abbreviations: *Nigella sativa* = NS; aqueous extract = AE; fixed oil = FO; volatile oil = VO; pentylenetetrazole = PTZ; maximal electroshock seizure = MES; protective indices = PIs; minimal neurological deficit = MND.

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Introduction

Nigella sativa Linn. is an annual herbaceous plant and belongs to the family Ranunculaceae. The seeds are small and black in color and possess aromatic odor and taste [1-3]. *Nigella sativa* (NS) seeds (black seeds) contain two types of oils, i.e. fixed oil (30-36% w/w) and volatile oil (0.43-0.72% w/w) [2]. Volatile oil (VO) of NS seed is composed mainly of thymoquinone (2-isopropyl-5- methyl- 1,4- benzoquinone) and monoterpenes [4]. Thymoquinone contents range from 18.4 to 24% w/w of the volatile oil [4]. The monoterpenes in the volatile oil amount to 46% w/w. The major components of these monoterpenes are p-cymene (isopropyl toluene), which comprises 31.7% of the volatile oil, and α-pinene (2,6,6-tri-methyl-bicyclo (3.1.1.) hepta-2-ene) [5], which comprises 9.3% of the volatile oil. Other components include phenols (1.7%), esters (16%), thymol, dithymoquinone and thymohydroquinone [6, 7].

Epilepsy is a very common disorder affecting 0.5-1% of the population [8, 9]. Current antiepileptic drugs (AEDs) are thought to act mainly by: a) reducing electrical excitability of cell membrane, and b) enhancing GABA mediated synaptic inhibition [9, 10]. Sodium valproate is a widely used AED and has broad spectrum of antiepileptic activity. The most serious side effects of sodium valproate are hepatotoxicity and teratogenicity [9]. Although a broad range of newer and more selective agents are currently being used, there is still a need for more selective and less toxic AEDs.

Whole oil from NS seeds has been shown to have analgesic, antidepressant and central nervous system (CNS) sedative activity in experimental animals [11]. Literature has indicated that the whole oil from black seeds is effective against PTZ-induced kindling in mice [12]. Some other studies have pointed out that the treatment of mice with thymoquinone reduced the duration of myoclonic seizures and effectively protected the mice from mortality [13]. However, in all of these studies, most of the work has been done with thymoquinone and other constituents have yet to be explored. A study on the hepatoprotective role of thymoquinone against free radical induced oxidative damage by valproate has already been published from this laboratory [14]. Our continuing interest in the pharmacological properties of *Nigella sativa* constituents and considering the vast use of valproate in the treatment of epilepsy prompted us and is one of the avenues that may help to understand its utility in protecting against free radical load produced in the treatment of epilepsy by valproate. The present study is, therefore, designed to investigate the anticonvulsant potential of NS seed constituents, alone and in combination with valproate, a widely used AED. The objectives of the study were achieved by determining:

- Anticonvulsant activity using pentylenetetrazole (PTZ) and maximal electroshock seizure (MES) models in mice.
- The possible mechanisms of action by exploring the role of GABA receptors.
- Their interaction with the known anticonvulsant sodium valproate.
- Minimal neurological deficit by using chimney test as a measure of neurotoxicity.

Methods

Material

Isolation of Nigella sativa seed constituents:

The aqueous extract (AE) of Nigella sativa seed: The authenticated *Nigella sativa* seeds of Ethiopian origin were procured from local markets of Riyadh, Saudi Arabia. The identity of the seeds was verified by the Center of Medicinal, Aromatic and Poisonous Plants and a voucher specimen was kept on record in the herbarium of the College of Pharmacy. These seeds were finely crushed to powder, washed with n-hexane and macerated in water for 48 hours with occasional shaking. After filtration, the solvent (water) was evaporated under vacuum according to the procedure described in literature [15]. The yield of the AE was 116 g/kg of crushed NS seeds.

The fixed oil (FO): Whole oil was extracted from the finely powdered black seeds using hexane extraction overnight at room temperature 22-23ºC. The yield was 240 ml/kg crushed black seeds. The accompanying volatile oil was removed by steam-distillation. The remaining fixed oil was then purified using silica gel column chromatography [16]. The yield of purified fixed oil was 152 ml per 240 ml whole oil, i.e. 152 ml fixed oil/kg crushed NS seeds.

The volatile oil (VO): *Nigella sativa* seeds were crushed and their volatile oil was extracted using steam distillation. The oil was separated

from water using diethylether. The latter was completely removed from oil via distillation under reduced pressure (400 mbar) at 40ºC [17]. The yield of the volatile oil was 6.2 ml/kg crushed NS seeds.

Chemicals and drugs:

Thymoquinone, pentylenetetrazole, bicuculline methiodide and sodium valproate (Sigma Chemical Co., St. Louis, MO, USA), α-pinene (Hopkins and Williams, England), p-cymene (Riedel De Haens, Germany), picrotoxin (Fluka Biochemika-Buchs, Switzerland), sodium carboxymethylcellulose (Winlab, Edgware, Middlesex, UK) and corn oil (Afia®, Savola Edible Oils, Saudi Arabia) were used. All the other chemicals used in this study were of analytical reagent grade procured from commercial sources.

The drug solutions were prepared fresh. Sodium valproate was dissolved in distilled water. α-pinene and p–cymene are oily in nature and were dissolved in corn oil by gentle heating at 45ºC for 5 minutes with occasional stirring. All of the solutions were used within 1 hour of their preparation, except bicuculline that was prepared just before use. Bicuculline was dissolved in 0.3 ml 0.1N HCl using ultrasonic bath and the final volume was made up with 0.9% w/v aqueous NaCl. Picrotoxin was dissolved in distilled water and diluted in normal saline. Thymoquinone was suspended in 0.5% carboxymethylcellulose.

Animals and treatment:

Swiss albino (SWR) male mice, 5-6 weeks old and weighing 25-30 g each, were used in the present study. Experimental animals were obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The animals were housed in groups, under controlled conditions of temperature (22 ± 1°C) and relative humidity (~50 %) as well as a 12-hour light/dark cycle, with light between 7 a.m. to 7 p.m. The mice were allowed food (a standard laboratory rodent's chow) and water ad libitum. 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, King Saud University, Riyadh.

For the determination of ED50, the mice were randomly assigned to treatment and control groups. Selected doses of black seed constituents were given by intraperitoneal route to different groups of animals. Separate groups were used for PTZ and MES models and the animals were used once only. The dose volume was kept constant at 10 ml/kg of body weight. The same treatment groups were used for chimney test 15 minutes before they were subjected to MES or PTZ test. To determine ED50, four dose levels were used for each constituent and the animals were tested 1 hour post-treatment for the anticonvulsant activity.

Additional experiments were conducted to determine the role of GABA receptors. The selected doses of constituents were administered to two sets of mice. Every set consisted of 10 groups of 8 mice each. After the pretreatment with the selected constituents, one set was challenged with the least convulsive dose of picrotoxin and the other with the least convulsive dose of bicuculline. Similarly, a control group was injected intraperitoneally with valproate for comparison and was challenged with either picrotoxin or bicuculline and the convulsive response of the animals was recorded.

To study the interaction of thymoquinone with valproate in chimney test, another set of 7 groups was treated. Different groups of mice consisted of the following: Groups 1 and 2, valproate (75 and 100 mg/kg, respectively); Groups 3 and 4, thymoquinone (50 and 100 mg/kg, respectively); Groups 5 and 6, thymoquinone (50 mg/kg) followed by valproate (75 or 100 mg/kg); Group 7, thymoquinone (100 mg/kg) followed by valproate (75 mg/kg). The animals were exposed to chimney test 15 minutes after valproate administration. Thymoquinone was administered 45 minutes before valproate in the combined treatment groups and the animals were tested for their neurological integrity 60 minutes after thymoquinone.

Methods

Minimal neurological deficit test (MND; chimney test):

MND was assessed using chimney test introduced by Boissier and colleagues as a simple test for the assessment of tranquilizing and muscle relaxant activity in mice [18]. A 30-cm high Pyrex® glass cylinder that was drilled at the base for ventilation and a possible target for the mice to escape through was used. The internal

diameter varies with the animal's weight. For mice weighing 25-30 g, a cylinder with 30 mm internal diameter was used.

Initially the tube was held in a horizontal position. A mouse was introduced with the head forward. When it reached the other end of the tube, the tube was moved to a vertical position. Immediately the mouse tries to climb backwards. The time required by the mouse to climb backwards out at the top of the cylinder was recorded. Before going for the real experiment, the naive animals were tested for their neurological integrity to the chimney model as a criterion for their selection. This results in prior training of test animals and helps to minimize the variability in the response of animals. Only those mice that succeeded to climb up within 30 seconds were selected to be used in the evaluation of test agents. After treatment, the inability of mice to climb up backwards in the tube within 30 seconds was taken as a measure of neurological deficits induced by the test agent. At the end of each experiment, the treated animals were humanely killed by using large dose of anesthesia in a special chamber.

Methods for the determination of anticonvulsant activity:

The anticonvulsant activity was evaluated by PTZ- and MES threshold tests.

PTZ seizure threshold test: Pentylenetetrazole (PTZ) seizure threshold test is a well-known chemical test used to evaluate anticonvulsant activity [19]. PTZ threshold seizure was utilized as a model for petit-mal epilepsy [20]. Before going for the actual protocol, preliminary experiments were conducted to select the dose of PTZ. The dose of PTZ at 83 mg/kg subcutaneously was found to be the approximate minimal dose (CD100) that induced convulsion in 100% male SWR mice (i.e. control group) in this study. The mice that received subcutaneous dose of PTZ were observed for 30 minutes. A single 5-second episode of clonic spasms was taken as a threshold seizure. Any agent that caused inhibition of PTZ-induced seizures was considered protective against convulsions.

MES-seizure test: The maximal electroshock seizure (MES) test is one of the electrical tests used to evaluate anticonvulsant activity [19]. In this test, alternating current with certain adjustable intensity (milliAmperes; mA), frequency (Hz) and duration (seconds) was delivered through ear electrodes (moistened with normal saline), using ECT UNIT (Model, 7801; UGO Basile, Varese, Italy). The mice were restrained by hand and subjected to electric shock by using pinna electrode, and released immediately following electrical stimulation, to permit the observation of the maximal seizure. Typically, the maximal seizure consists of a short period of initial tonic flexion and a prolonged period of tonic extension (especially the hind limb) [19]. Various frequencies, current intensities and the duration of stimulation were tried on male SWR mice (control group). The minimal electroshock that induced 100% maximal seizures was found to be 50 mA alternating a current of 100 Hz frequency for 0.2 seconds duration. Protection was defined as complete absence of hind limb tonic extension. MES-induced seizure was employed as a model for grand-mal epilepsy [21].

To know if the anticonvulsant activity of a certain compound is selective, the value of the protective index (PI) for that compound was calculated [19]. The protective index (PI) is the ratio of median sedative dose (TD50) to the median effective dose (ED50) and is calculated as follows:

Where TD50 is the median dose, which caused decline in motor performance (i.e. minimal neurological deficit) in 50% of animals in the chimney test, ED50 is the median effective dose (i.e. anticonvulsant dose), which caused anticonvulsant effect in 50% of the test animals. PI of unity or less means that the agent is not a specifically effective anticonvulsant and the effect shown is probably due to general CNS sedation. Proper anticonvulsants show PIs higher than unity [19].

Picrotoxin and strychnine-induced convulsions:

In the experiments dealing with the role of GABA receptors in the anticonvulsant activity of NS constituents, convulsive doses of bicuculline (1.5 mg/kg, subcutaneously) and picrotoxin (3.15 mg/kg, subcutaneously) were administered into the nab region (the skin on the back of the neck) of the mice 60 minutes after valproate or NS constituents. The animals were placed individually into transparent Perspex® boxes and observed immediately for the induction of convulsions up to 30 minutes post-administration. The percentage

of protection against the incidence of seizure was recorded.

Statistical analysis

From dose-response analysis of MND (in chimney test) and anticonvulsant activities, median MND-inducing doses causing decline in motor performance (TD50), as well as median anticonvulsant dose (ED50) were calculated. A computer program (Pharmacological Calculation System, version 3.2, Medical College of Georgia, Augusta, Georgia, USA) based on Litchfield and Wilcoxon method was used to calculate this parameter. To calculate the effect of thymoquinone on doses of valproate, comparison was done using Student's t-test.

Results

Effect of Nigella sativa constituents on PTZ-induced convulsions

The results presented in Table 1 show that NS constituents, except FO and AE, exhibited appreciable anticonvulsant activities against seizures induced by PTZ (83 mg/kg, subcutaneously) in SWR mice. Thymoquinone (in 0.5%CMC) was the most potent compound (93.2 mg/kg) followed by VO (376 mg/kg), p-cymene (394 mg/kg) and α-pinene (440 mg/kg). AE was the least effective (2000 mg/kg) as an anticonvulsant agent against PTZ. Fixed oil was practically ineffective as an anticonvulsant (Table 1).

For the comparison of anticonvulsant activities of black seed constituents in PTZ model, protective indices (PI) were calculated (Table 2). The standard antiepileptic drug valproate shows PI more than one (i.e. 1.13). The highest PI values of black seed constituents were achieved by thymoquinone (0.95), followed by p-cymene (0.93), VO (0.8) and α-pinene (<0.39).

Anticonvulsant effect of Nigella sativa

Table 1. Determination of ED50 for N. sativa constituents and sodium valproate in PTZ- and MES-induced convulsion models

Model	Treatment	SVP	AE	FO	VO	TQ (CMC)	AP	PC
ED50 (PTZ model) (95% C.L.) mg/kg		161 (140-185) –	2000 (1000-3900) –	>3244 – –	375.8 (236-611.8) –	93.2 (59-147.7) [0.57 mmoles/kg]	440 (103.6-1899) [3.2 mmoles /kg]	393.8 (175-875) [2.9 mmoles/kg]
ED50 (MES model) (95% C.L.) mg/kg		170.1 (139.2-207.91)	Up to 2000 No protection	Up to 3244 No protection	716.7 (428.3-1188.6)	Up to 300 No protection	Up to 1380.8 No protection	971.3 (805-1137.5) [7.2 mmoles/kg]

SVP = Sodium valproate, AE = Aqueous extract, FO = Fixed oil, VO = Volatile oil, TQ =Thymoquinone, AP = α-pinene, PC = p-cymene. Eight dose levels were used to calculate ED50 (in mg/kg). Eight male mice were used at each dose level. Litchfield and Wilcoxon (1949) test.

Table 2. Protective index (PI) values of sodium valproate and Nigella sativa seed constituents against MES- PTZ-induced convulsions

Treatment	SVP	AE	FO	VO	TQ (CMC)	AP	PC
PTZ model PI = TD50/ED50	1.13	0.48	< 0.22	0.81	0.95	< 0.39	0.93
MES model PI = TD50/ED50	1.07	–	–	0.43	–	–	0.34

SVP = Sodium valproate, AE = Aqueous extract, FO = Fixed oil, VO = Volatile oil, TQ = Thymoquinone, AP = α-pinene, PC = p-cymene.

constituents in MES-induced seizures:

Table 1 also shows that the AE, FO, thymoquinone and α-pinene exhibited no protective activities against MES-induced seizures. However, the other NS constituents (VO and p-cymene) were found to afford complete protection. VO (717 mg/kg) was found to be more potent than p-cymene (971 mg/kg).

The anticonvulsant potentials of valproate, VO and p-cymene are indicated in Table 2. PI of valproate (1.07) in MES model was less than its value in PTZ model (1.13), but it was more than volatile oil and p-cymene values. However, VO had higher PI (0.43) was compared to p-cymene (0.34).

Effect of Nigella sativa constituents on MND in chimney test

Table 3 shows that NS constituents induce varying degrees of MND when the chimney test was used. Thymoquinone was most potent (as indicated by low TD50; 88.1 mg/kg), followed by α-pinene (<173 mg/kg), VO (306 mg/kg) and p-cymene (368 mg/kg). FO and AE were the least effective in inducing decline in motor performance. However, AE (950 mg/kg) was more potent than fixed oil (1403 mg/kg).

Role of GABA receptors in the anticonvulsant activity of Nigella sativa constituents:

Table 4 shows that valproate antagonizes picrotoxin (a non-competitive GABAA receptor antagonist) induced convulsions in a dose-dependant manner. Thymoquinone at 150 mg/kg dose was the only component that successfully prevented (80%) picrotoxin (3.15 mg/kg; subcutaneously)-induced convulsions. The protection offered by thymoquinone at 150

Table 3. Determination of TD50 for Nigella sativa seed constituents and sodium valproate in the motor performance test (chimney test) in mice

Sl No.	Test compound	TD50 Average, mg/kg	TD50 (95% C.I.) Range, mg/kg
1	SVP	182.2	146.4-226.8
2	AE	950	430-2100
3	FO	1403	584-3374
4	VO	306	192-463
5	TQ (CMC)	88.1 [0.5 mmoles /kg]	54.3- 143.1
6	AP	<173 [<1.3 mmoles/kg]	--
7	PC	368 [2.7 mmoles/kg]	271-508

SVP = Sodium valproate, AE = Aqueous extract, FO = Fixed oil, VO = Volatile oil, TQ = Thymoquinone, AP = α-pinene, PC = p-cymene. 3 to 5 dose levels were used to calculate TD50 (in mg/kg). Eight male mice were used at each dose level. Litchfield and Wilcoxon (1949) test.

Table 4. Involvement of picrotoxin- and bicuculline-sensitive GABA receptors in anticonvulsant activity of valproate, AE, VO, thymoquinone, α-pinene and p-cymene

Treatments	SVP		AE		VO	TQ	AP		PC	
Doses, mg/kg	300	400	1500	2000	699.2	150	517.8	1035.6	700	1400
% Antagonism to picrotoxin @	60%	100%	40%	40%	20%	80%	0.0%	20%	0.0%	0.0%
% Antagonism to bicuculline $	80%	100%	0.0 %	0.0%	33.3%	50%	0.0%	33.3%	0.0%	40%

SVP = Sodium valproate, AE = Aqueous extract, VO = Volatile oil, TQ = Thymoquinone, AP = α-pinene, PC = p-cymene. Eight male mice were used at each dose level. @= The least convulsive dose of picrotoxin = 3.15 mg/kg, s.c.
$= The least convulsive dose of bicuculline = 1.5 mg/kg, s.c.

mg/kg dose was more than the protection offered by 300 mg/kg dose of sodium valproate (60%). Other NS constituents failed to protect animals against clonic convulsions induced by picrotoxin.

The administration of the NS constituents, except thymoquinone 1 hour prior to bicuculline which is a competitive GABAA antagonist (1.5 mg/kg, subcutaneously), produced no protection against the expected incidence of clonic convulsions. However, thymoquinone (150 mg/kg) was the only component, which partially protected (50%) against bicuculline-induced seizures. However, the protection offered by thymoquinone against picrotoxin-induced convulsions (80%) was more pronounced than that against bicuculline (50%) (Table 4).

Comparison of valproate treatment with thymoquinone and valproate combined treatment:

Thymoquinone at 50 and 100 mg/kg doses reduced the ED50 value of valproate from 161 to 112 and 35 mg/kg, respectively, in PTZ model that was significantly ($p<0.001$) less at 100 mg/kg. The reduction in valproate dose was more pronounced at 100 mg/kg and was significantly less ($p<0.01$) than 50 mg/kg (Table 5).

Thymoquinone at a dose of 50 mg/kg had no significant additional protective effect ($p>0.05$) on the ED50 value of valproate against MES-induced seizures. But, thymoquinone

Table 5. Effect of thymoquinone on the dose of sodium valproate in MES- and PTZ-induced convulsion tests in mice

Group	Treatment/Dose (mg/kg)	ED50 of valproate (MES test; mg/kg)	ED50 of valproate (PTZ test; mg/kg)
1	Vehicle (CMC; 10ml/kg)	170.1±9.5 (139.2-207.91)	161±7.5 (140-185)
2	TQ 50	152± 7.7(112.5- 206.2)	112±6.3 (85-153)
3	TQ 100	124.5±12.2* (114.2-136.4)	35±21.5***@ (18.8-65)

TQ = Thymoquinone
ED50 value of valproate is from Table 1 in both the models (Group 1).
*Groups 2 and 3 were statistically compared to Group 1. *p < 0.05; ***p < 0.001*
Group 3 was statistically compared to Group 2 in its respective column. @p < 0.01

Table 6. Effect of thymoquinone treatment and sodium valproate on motor performance in chimney test

Sl. No.	Treatment	Dose (mg/kg)	Failure in chimney test (%)
1	SVP, n=10	75	0.0
2	SVP, n=8	100	25
3	TQ, n=10	50	60
4	TQ, n=10	100	80
5	TQ + SVP, n=10	TQ 50 + SVP 100	100
6	TQ + SVP, n=10	TQ 50 + SVP 75	80
7	TQ + SVP, n=10	TQ 100 + SVP 75	100

SVP = Sodium valproate, TQ = Thymoquinone
n = Number of animals in each group.

at a higher dose of (100 mg/kg) significantly ($p<0.05$) reduced the ED50 of valproate from 170.1 to 124.5 mg/kg (Table 5) resulting in its potentiation.

In the MND test, thymoquinone (50 and 100 mg/kg), when combined with valproate even at a low and ineffective doses against PTZ and MES-induced seizure models, increased the incidence of MND, indicating an increase in its potency (Table 6).

Discussion

In the first part of the present study, the anticonvulsant activities of NS constituents were studied against PTZ- and MES-induced convulsions. Their effects on motor performance were also investigated using the chimney test. All NS seed constituents tested were found to be effective against the PTZ model. Our results confirm the findings of a previous study where *Nigella sativa* seed oil effectively protected against PTZ-induced kindling in mice [12]. In the present study, the doses of the volatile oil, thymoquinone, α-pinene and p-cymene per 25 g mouse needed to elicit anticonvulsant activity in PTZ model were 9.4, 2.3, 11 and 9.85 mg, respectively. The amounts of thymoquinone, α-pinene and p-cymene in the ED50 of VO per mouse (i.e. 9.4 mg) were calculated as their percentage in the VO and they were 2.0 mg, 0.87 mg and 2.98 mg per mouse, respectively. The VO anticonvulsant activity can be attributed mainly (i.e. 70%) to thymoquinone, then to p-cymene (24%) and α-pinene (6%).

The volatile oil and p-cymene were found to be effective against MES induced seizures in the present study. No protection was observed in this model by other black seed constituents (i.e. AE, FO, thymoquinone and α-pinene). ED50 of the VO and p-cymene per 25 g mouse were 17.9 mg and 24.3 mg, respectively. The amount of p-cymene present in the median effective dose of VO was found to be 5.7 mg. This finding indicates that p-cymene, partially, might have participated in the anticonvulsant activity of the VO against MES induced seizures. The components in the VO, other than thymoquinone and α-pinene, might be responsible for the remaining anticonvulsant activity.

Concerning the effects of black seed constituents on motor performance in the chimney test, all constituents showed varying degrees of MND. The median MND-inducing doses of AE, FO, VO, thymoquinone (suspended in 0.5%CMC), α-pinene and p-cymene were 950, 1403, 306, 88.1, <173 and 368 mg/kg, respectively. Thymoquinone was the potent and FO was the least effective constituent in the iduction of MND. TD50 of VO, thymoquinone, α-pinene and p-cymene per mouse were 7.65 mg, 2.2 mg, 4.3 mg and 9.2 mg, respectively. Sedation induced by the VO can be referred mainly to its content of thymoquinone (63%) and to some extent to p-cymene (23%) and α-pinene (14 %). Literature has indicated that the treatment of mice with aqueous and methanolic extract on *Nigella sativa* seeds induced a significant reduction of spontaneous motility, a decreased exploratory conduct with a concomitant decreased motor coordination [22].

NS constituents that were effective against PTZ-induced seizures showed PI less than unity. The volatile oil and p-cymene, which were effective against MES-induced convulsions, also scored PI less than one. It is important here to compare PI of tested NS constituents, with established AEDs. In this study, valproate produced PI of 1.13 and 1.07 using PTZ and MES models respectively, compared to 2.3 and 1.7 observed by White and his colleagues [18]. This difference in valproate PI values may be due to the use of different mice strains. In the present study, SWR mice were used, compared to NMRI mice used by others, which are known to be more sensitive. For example, in the present study, 83 mg/kg PTZ was needed to induce 100% convulsions in the SWR mice, whereas 65 mg/kg were needed for the same purpose, in the NMRI mice strain [23]. Using the MES model, 100 Hz and 50 mA were needed to elicit tonic convulsion in SWR mice, whereas 60 Hz and 50 mA were enough for NMRI mice as reported in the literature [23].

It was clear that thymoquinone, volatile oil and p-cymene had lower PIs (0.95, 0.93 and 0.81, respectively) in PTZ model compared to valproate (1.13). Similarly, in the MES model, the PIs of volatile oil (0.43) and p-cymene (0.34) were less than that of valproate (1.07). These findings indicate that the anticonvulsant activities of NS seed constituents might be due to the general CNS depression at the tested doses.

In another part of this study concerning mechanisms involved in NS seed constituents' anticonvulsant activities, picrotoxin and bicuculline were used to explore GABA-

mediation. GABA receptors within the CNS are known to have two distinct subtypes; GABAA and GABAB receptors. Picrotoxin is a blocker for GABA-activated chloride-ion channels [24] and a non-competitive GABAA receptor antagonist, whereas bicuculline is a competitive antagonist to these types of receptors [25, 26]. The activation of GABA receptors by GABA leads to the opening of the chloride ion (Cl-) selective channels resulting in an increase in the influx of the negatively charged extracellular Cl- ions into the cells with the concomitant increase in electronegativity and hyperpolarization or inhibition of the cells [23]. It seems that picrotoxin-sensitive GABA receptors (most probably GABAA receptors) and bicuculline-sensitive GABA receptors (GABAA receptors) were involved in the anticonvulsant activity of thymoquinone. But, the anticonvulsant activity of the AE, VO, α-pinene and p-cymene appear not to be mediated via this mechanism.

In one study Hosseinzadeh and Parvardeh [13] have demonstrated that thymoquinone was effective in petit mal epilepsy in mice most probably through an opioid receptor mediated increase in GABAergic tone and as such our results support their findings [28]. Furthermore, the involvement of GABAA receptors in thymoquinone effects seems to be in agreement with the findings of Mohamadi and colleagues [29]. They found thymol (a compound structurally related to thymoquinone and both are phenol derivatives) directly activated chloride currents via GABAA receptors in rat α1β2γ2GABAA receptors heterologously expressed in HEK 293 cells. Agel reported that the VO from NS seeds inhibits spontaneous contractions of rabbit jejunum, probably through calcium antagonism [30]. This finding can give a clue to the mechanism of this oil as an anticonvulsant agent. Another proposed mechanism of thymoquinone's action may be through the depression of intracellular calcium release from its stores, as this effect is claimed for its structurally related compound, thymol [31].

In these experiments, the effects of two doses (50 and 100 mg/kg) of thymoquinone on the dose-response curve of valproate were investigated on the anticonvulsant (using PTZ and MES models) and sedative effects. Thymoquinone, at 50 and 100 mg/kg doses, significantly reduced the ED50 values of valproate in PTZ model from 161 mg/kg to 112 and 35 mg/kg respectively. Against MES-induced seizures, 50 mg/kg of thymoquinone had no significant additive effect on the ED50 value of valproate. However, the high dose of thymoquinone (100 mg/kg) significantly reduced the ED50 of valproate from 170.1 mg/kg to 124.5 mg/kg.

Literature has suggested that NS oil attenuated the PTZ-induced injury in the brain tissue and behaved as an antioxidant when administered as pretreatment prior to PTZ injection during kindling acquisition [12]. In this study, black seed oil showed antiepileptogenic properties by reducing the sensitivity of kindled mice to the convulsive and lethal effects of PTZ in comparison to valproate which was ineffective in preventing such effects. However, thymoquinone, the major constituent of NS oil, has already been proved to be an effective antioxidant and scavenger, protecting against free radical load [32, 33].

The combined treatment (i.e. thymoquinone 50 or 100 mg/kg with valproate) increased MND-inducing potential of valproate. It can be mentioned that thymoquinone (in both PTZ and MES models) increased valproate potency and apparently decreased its PI. In other words, the increase in valproate potency when combined with thymoquinone was masked by the resultant decrease in motor performance.

Conclusion

The results of the present study reveal that all of the NS constituents (except FO) protected against PTZ or MES-induced convulsions and this effect is attributed mainly to thymoquinone contents present in it. Correspondingly, all of these constituents induced varying degrees of MND and thymoquinone being the major constituent of volatile oil may be held responsible for this act. GABAergic transmission is indicated as a pathway for its antiepileptic effects most probably through GABAA receptor mediation. The combination of subtherapeutic doses of valproate with thymoquinone resulted in the potentiation of valproate and a subsequent reduction in its ED50. However, the reduction in the valproate dose required for anticonvulsant activity may be valuable in suppressing its unwanted effects like hepatotoxicity and teratogenic implications. On the basis of the known antioxidant and antiepileptic potential of thymoquinone as observed in the present study, its use alone or as an adjunct with valproate in seizure therapy seems to be appropriate and further investigations are suggested.

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