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The effect of thymoquinone on intractable pediatric seizures (pilot study)

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Summary
Introduction: Despite administration of numerous combinations of epileptic drugs, nearly 15% of childhood seizures are resistant to treatment and it is still a problem in pediatric practice. In traditional medicine, *Nigella sativa* was known to have anticonvulsant effects. Recent studies also have shown its anticonvulsant effects. Most of the properties of *N. sativa* or its extracts are mainly attributed to thymoquinone. It has been shown that thymoquinone has several therapeutic effects and no evidence of toxicity or side effects is reported.
Materials and methods: In this pilot, double-blinded crossover clinical trial study on children with refractory epilepsy, thymoquinone with dose of 1 mg/kg was administered as an adjunctive therapy and its effects on frequency of seizures were compared with those of a placebo. Twenty-two patients entered in the study. They were assigned in two groups and received either thymoquinone or placebo for a period of four weeks, and then during the two weeks of wash out period, they received only their pre-existing anti-epileptic drugs; then, after cross-overing, they received thymoquinone or placebo for a period of four weeks again. During these periods their effects on seizure frequency were investigated.
Results: The reduction of frequency of seizures at the end of first period in comparison with the same period before the study demonstrated a significant difference between two groups (thymoquinone and placebo) ($P = 0.04$). Also reduction of frequency of seizure has shown significant difference between two groups at the end of second period in comparison with end of first period ($P = 0.02$). The parental satisfaction showed significant difference between the two groups at the end of the first period ($P = 0.03$).
Conclusion: It can be concluded that thymoquinone has anti-epileptic effects in children with refractory seizures.

Introduction

Nearly 15% of childhood seizures are resistant to standard antiepileptic drugs (AEDs). In fact, these drugs cannot

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provide adequate control of epileptic seizures and do not prevent progressive epileptogenic changes (Verhelst et al., 2005; Cockerel, 1994). This situation, which is called intractable seizure, is a problem in pediatric practice and indicates a need for new anti epileptic agents to help this group of patients (Chapell et al., 2003).

Many studies showed that *Nigella sativa* has various therapeutic effects such as diuretic and hypotensive (Zaoui, 2000), anti tumor cells (Swamy, 2000), anti histaminic (Chakravarty, 1993; Mahfouz et al., 1965), anti inflammatory and analgesic (Abdel-Fattah et al., 2000), and anti epileptic effects (Ilhan et al., 2005; Akhondian et al., 2007). Also many studies have shown that most properties of *N. sativa* or its extracts are mainly attributed to thymoquinone (2-isopropyl-5-methyl-1, 4-benzoquinone, tq) (Mahfouz et al., 1960; Filippo et al., 2002). It is one of the monoterpenoid hydrocarbon compounds of *N. sativa*'s volatile oil (Nickavar et al., 2003).

Thymoquinone has chemical (nonenzymatic) metabolic activity dependent on GSH, NADPH or NADH that may represent a ''cellular switch'' able to modulate cellular antioxidant defenses (Khalife and Lupidi, 2007).

Also it has been shown that thymoquinone has anticonvulsant activity in rats; probably through an opioid receptor-mediated increase in gabaergic tone (Hosseinzadeh et al., 2005).

In a study assessing the toxicity of thymoquinone in mice after subchronic oral administration (almost 90 mg/kg/day) there were no signs of toxicity or mortality (Badary, 1998).

In this study we investigated the effect of thymoquinone on intractable seizures in children, aiming to reduce seizure frequency. We assessed the efficacy of oral administration of a thymoquinone as an adjunct to AEDs in a double-blinded clinical trial and compared the results with those of a placebo.

Materials and methods

Drugs and dosage

Thymoquinone was purchased from Sigma Chemical Co. The powder of thymoquinone was transformed to syrup by a pharmacologist experienced in industrial pharmacology. In this study, we chose the syrup form because of its easier preparation and use in children. The syrup was prepared in concentration of 25 mg/ml. The placebo was also prepared with the same specification (especially the color and taste of the solutions).

Patients

Children aged 1—14 years included in this pilot study had intractable epilepsy according to the definition (seizures that were inadequately controlled with one or more concomitant AEDs administrated at least 6 months before the time of enrollment) (Chapell et al., 2003). Inclusion criteria were at least four seizures during a four-week period preceding the screening visit and constant antiepileptic treatment at least one month before the study.

AEDs dosages remained unchanged during the study's periods. Seizure types of all patients were diagnosed according to the standard of the International Classification of Epilepsy (ILAE) (Commission on Classification and Terminology of the ILAE, 1981; Parra et al., 2001). Patients with more than two types of seizures were designated as having ''''polymorph'' seizures.

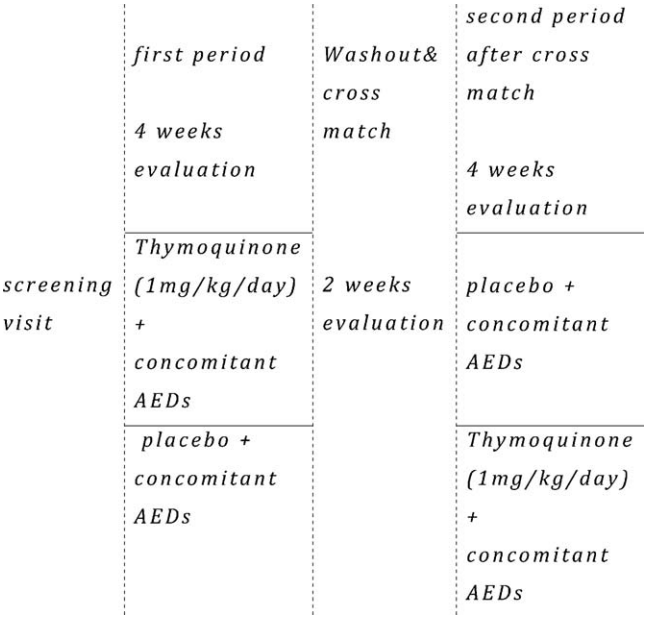

Figure 1 Trial design.

Exclusion criteria were: a treatable seizure etiology, epilepsy secondary to a progressive cerebral disease or any progressive neurodegenerative disease, a history of status epilepticus within the three months prior to the first visit, a history of pseudoseizures, seizures that could not be reliably counted due to their rapid and repetitive nature, current renal, cardiac or hepatic dysfunction, lack of cooperation (incorrect dose, irregular use of the prescribed syrup, or unreliable registration of events by parents), severe exacerbation of seizures, and withdrawal of the patients by their parents from the study.

There were 41 patients with intractable seizures but only 32 patients were available to contact. From those only 23 patients attended screening visits in the arranged time. After the first visit all patients (23 patients) satisfied inclusion criteria and were enrolled in the study. One patient dropped out due to dissatisfaction with the results. Therefore 22 patients completed the study.

This study was performed between July 2006 and September 2008 in a tertiary referral center (Ghaem Medical Center, Mashhad, Iran). Before enrollment, all study protocol aspects were reviewed with all parents and written informed consents were obtained.

Study design

We designed an add-on, double-blinded, placebo-controlled, randomized, crossover, pilot study with a two-week washout period between treatments. Crossover-designed studies do not give very valid results when using curable therapy or drugs with long-term effects, but in our study, refractory seizures are essentially almost incurable and can only be controlled. Likewise, a crossover study helps remove some confounding factors in two groups of the study. The patients were selected from dossiers which were archived by pediatric neurologists of Mashhad Ghaem Hospital (Fig. 1).

In the screening visits, investigators obtained informed consents, assessed entry criteria, and performed screening measurements. These measurements included a complete physical and neurologic history, examination and laboratory tests (CBC, AST, ALT, LDH, CPK, BUN, Cr, ALP and FBS).

During the study, the patients entered the study gradually and were given a number from one on, in a sequential process; then it was randomly (''toss-up'') determined that patients with even numbers take syrup A (thymoquinone) for the first four weeks, and

after the treatment was discontinued for two weeks, receive syrup B (placebo) for the second four weeks, while the other patients (with uneven numbers) take the syrups in an opposite order. The patients were provided with the syrup free of charge, and neither the pharmacologist, physicians, nor patients knew the contents of the syrups (A and B) until they were identified by the pharmacist at the end of the study. This method of randomization and blinding helped us avoid bias. Both types of syrups were prescribed at a dosage of 0.02 ml/kg/12-h (for the thymoquinone, this was equivalent to 0.5 mg/kg/12-h) throughout these periods.

The patients were visited once a week throughout the study. All information related to the type and number of seizures, possible adverse effects and changes in seizures was obtained from the parents. Because of inaccessibility of video-EEG, parents were asked to record the frequency of seizures, and the overall number of seizures was recorded. The parents were previously trained and were asked to fill out a questionnaire to better characterize what was being measured. At the end of each period (of placebo or extract), the degree of parental satisfaction was assessed and the lab testing was repeated. The clinicians were familiar with the nature and treatment of epilepsy.

The study was reviewed and approved by the Research and Ethics Committee of the Mashhad University of Medical Sciences of Iran.

Statistical analysis was done using the SPSS statistical software package (version 11.5) and a probability value of less than 0.05 was considered statistically significant.

Objectives

(1) Assessment of the efficacy of 28 days adjunctive thymoquinone treatment (1 mg/kg/day) in children with poorly controlled seizures.
(2) Assessment of the safety and tolerability of adjunctive thymoquinone therapy (1 mg/kg/day) during the study.

Assessment

The primary efficacy variable in this trial was total seizure frequency per week during treatment period. Secondary efficacy variable included reduction percentage from baseline in seizure frequency, percentage of satisfaction of parents at the end of each period by category (satisfied, ineffective, dissatisfied). The tolerability of thymoquinone was evaluated by comparing rates of spontaneously reported treatment and emergent adverse events in two treatment groups, together with the result of physical and neurological examinations and laboratory tests in each treatment groups.

Results

Patient characteristics

The patients were selected from dossiers archived by pediatric neurologists.

There was no statistical difference between the thymoquinone and placebo treatment order groups with respect to age, sex, weight, mean duration of seizures per week and type of seizures (Table 1).

All patients had polymorphic seizures (included at least two types of myoclonic, tonic, clonic, or atonic seizures). One patient discontinued the treatment in the first period because of new tics that occurred during thymoquinone treatment. Therefore he was excluded from the study.

Table 1 Demographic and baseline characteristics and concomitant AEDs[a] (intent-to-treat population).

Groups	Start with thymoquinone	Start with placebo
Gender		
Girl	5	7
Boy	7	3
Age	Mean: 5.58 ± 3.03 years	Mean: 6.15 ± 3.3 years
Weight	Mean:17.33 ± 6.7 kg	Mean: 18 ± 6.81 kg
Age of diagnosis	Mean: 10.16 ± 20.11 months	Mean: 5 ± 5.03 months
Seizure frequency	Mean: 17.41 ± 9.03 per week	Mean: 16.2 ± 9.75 per week
Type of seizure	All polymorphic	All polymorphic
Drug history	Number of patients	
Phenytoin	12	10
Clonazepam	10	5
Phenobarbital	4	5
Valporate sodium	2	4
Lamotrigine	1	3
Carbamazepine	2	0

[a] Antiepileptic drugs.

Efficacy

Percentage decrease in seizure frequency

Analysis of the total seizures in the thymoquinone and placebo treatment periods, based on data from 22 patients who completed both treatment periods, showed a significant difference in percentile decrease in seizure frequencies (53.91 ± 38.75 and 9.60 ± 15.45 in thymoquinone and placebo groups, respectively, after first period) ($P = 0.002$). In the second period (after wash out period), there was 26.64 ± 41.29% decrease and 61.33 ± 83.23% increase in seizure frequencies in thymoquinone and placebo groups, respectively, which is statistically significant ($P = 0.005$). Mean reduction percent of seizures per week during the study is presented in Fig. 2.

The percentile decrease in seizure frequencies during the thymoquinone treatment in the first period was 53.91 ± 38.756 and in the second period was 26.64 ± 41.297 which *are not significantly different* (independent samples T test = 1.595, df = 20, $P = 0.126$).

With repeated measures, there was also a significant difference in the trend of changes in percentile decrease in seizure frequency between two groups ($P = 0.04$ and $P = 0.01$ in the first period and after first period, respectively).

Over ally, 54.5% of patients responded during thymoquinone period (i.e., they experienced a ≥50% reduction from baseline in weekly seizure frequency), compared with 13.6% during placebo period. This difference was statistically significant ($P = 0.007$).

In the first period an additional analysis of seizure data from the 22 patients who completed the first treatment period was also undertaken. This between-patient

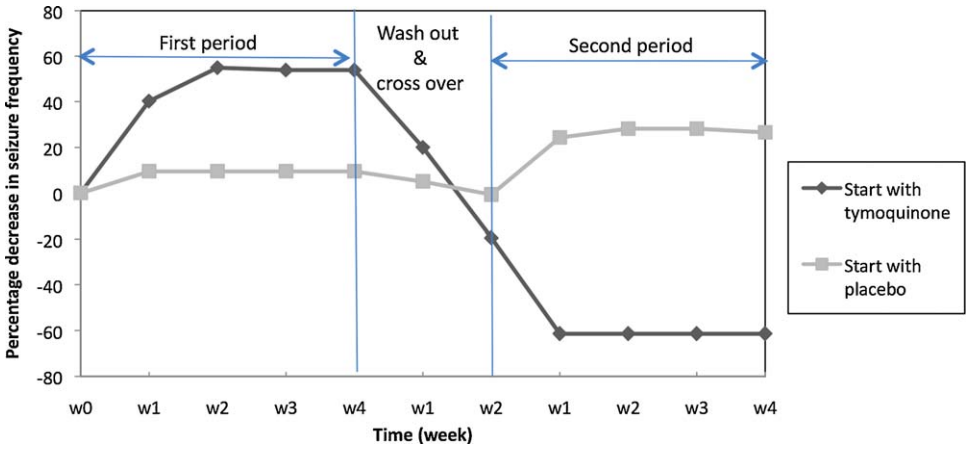

Figure 2 Mean percentage decrease in seizure frequencies per week during the study.

analysis would not be affected by any period affecting influence.

Patients treated with thymoquinone experienced a percentile reduction in mean seizure frequency of 46.16 ± 26.6 seizures from baseline, compared with a reduction of 13 ± 18.2 with placebo. This difference was statistically significant ($P = 0.002$).

Seizure free days

Two thymoquinone-treated patients in the first period (16.6%) and one patient (10%) in the second period were seizure free for 21 and 14 days, respectively, compared with no placebo patient.

Parental satisfaction

Parental satisfaction at the end of the thymoquinone period and the placebo period also showed a significant difference ($P = 0.03$) (Table 2), which indicates a better quality of life after administration of thymoquinone. 68.2% and 36.4% of patients at the end of the thymoquinone period and the placebo period, respectively, felt better. 31.8% of patients in the thymoquinone period and 63.6% in the placebo period felt no change or worse (Table 2).

Adverse events

The most common adverse event reported in the trial was nausea (9 patients during thymoquinone treatment period and 6 patients during placebo period). The base of drugs (both thymoquinone and placebo) was alcohol; so just after prescription (probably because of its bitter taste), nausea was reported in some (9 patients during thymoquinone treatment period and 6 patients during placebo treatment period) and lasted for a few minutes.

Table 2 Parental satisfaction in the study.

Satisfaction	Satisfied	Ineffective	Dissatisfied
Thymoquinone	68.3%	27.2%	4.5%
Placebo	36.4%	50%	13.6%

Somnolence was an adverse event which appeared in 4 and 6 patients of thymoquinone and placebo treatment period, respectively. Vomiting (one patient during placebo period) and pharyngitis (one patient during both thymoquinone and placebo periods) were other adverse events that may not be related to this trial. One patient developed a maculopapular rash on the trunk in the thymoquinone period. One patient had tics during thymoquinone treatment; therefore he was excluded from the study.

Lab data

There were no clinically significant changes in mean values for laboratory variables (ALT, AST, ALP, CPK, LDH, BUN, Cr, CBC) between two groups (thymoquinone and placebo) in each period and also no significant difference was shown between the two treatments and baseline periods.

Discussion

This pilot is the first double-blind placebo controlled randomized cross over trial of thymoquinone, the major component of *N. sativa*, in human population.

In a study, Abdel-Fattah et al. examined antinociceptive effects of *N. sativa* oil and its major component, thymoquinone, in mice. Results suggested that *N. sativa* oil and thymoquinone produce antinociceptive effects through indirect activation of the supraspinal mu(1)- and kappa-opioid receptor subtypes (Hosseinzadeh et al., 2005), and no toxicity was reported in subchronic administration of thymoquinone in rats with doses of 90 mg/kg/day (Badary, 1998).

In another study, the mean frequency of seizures decreased significantly in children with intractable seizures during treatment with *N. sativa* (Akhondian et al., 2007).

This study demonstrated that adjunctive thymoquinone, administered as 0.5 mg/kg bid for 4 weeks, was effective to a statistically and clinically significant extent in a small population of children with epilepsy refractory to long-term treatment with conventional AEDs. Although treatment duration was short and the study population was small,

the response rate to thymoquinone was encouraging with median seizure frequency reduced by approximately one third in the study population. Three patients who completed both treatment periods were seizure free in thymoquinone period (of course, only for 2—3 weeks), whereas no patient was seizure free during placebo treatment. Because all patients had polymorphic seizures, and the overall number of seizures was reported, we cannot conclude that seizure freedom in some patients was more syndrome-related rather than being a direct effect of the drug; although, of the 3 patients, 2 patients had got thymoquinone in the first period and after discontinuing the drug, seizures recurred. So we think that seizure freedom in these patients must not be syndrome-related.

We had no access to AED blood level monitoring, and as most patients were taking phenytoine, carbamazepine or phenobarbital, an influence of thymoquinone on blood levels of these drugs cannot be ruled out.

Adjunctive thymoquinone administered at 1 mg/kg/day was generally well tolerated. This level of reported adverse events is not unusual in a study of this duration involving patients with refractory epilepsy taking long-standing concomitant medications. The adverse effects most commonly attributed to thymoquinone treatment involved CNS (somnolence) and gastrointestinal tract (nausea) effects. The base of drugs (both thymoquinone and placebo) was alcohol; so just after prescription (probably because of its bitter taste), nausea was reported in some patients (9 patients during thymoquinone treatment period and 6 patients during placebo treatment period) and lasted for a few minutes.

Thymoquinone was not associated with any clinically significant changes in neurological function, laboratory variables or vital signs compared with placebo. We were unable to measure levels of thymoquinone and/or antiepileptic drugs in blood during the study.

Conclusions

This trial demonstrated that thymoquinone is effective and tolerable in children with intractable epilepsy. However, more trials with greater sample sizes should be conducted in such patients to confirm these preliminary findings. The result of this trial provides clinicians additional options for improving the overall management of epilepsy in children.

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