Original article

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The clinical outcome of adjuvant therapy with black seed oil on intractable paediatric seizures: a pilot study

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ABSTRACT – *Aim*. To evaluate the effect of black seed oil, as add-on treatment to antiepileptic drugs (AEDs), on seizure frequency and severity as well as oxidative stress in intractable epilepsy patients. *Methods*. A prospective, randomised, single-blinded, controlled, crossover pilot study. Five healthy children were included as controls. Thirty intractable epileptic children were randomly assigned to either Group I or II. Group I received placebo for four weeks, followed by a two-week washout period, and subsequently black seed oil for four weeks. Group II received the same intervention but in the reverse order. All patients received AEDs throughout the study period. Prior to allocation, all patients underwent a neurological assessment and evaluation of oxidative stress markers; total antioxidant capacity (TAC) and malondialehyde (MDA). Patients were assessed at Weeks 4 and 10 for oxidative stress markers and seizure frequency and severity. *Results*. At baseline, both groups (I, II) had significantly lower serum TAC levels relative to healthy controls (p=0.007), while MDA levels were unchanged. After the 4-week period of black seed oil administration, there was no significant difference between the two groups with regards to seizure frequency, severity, or oxidative stress markers (TAC and MDA; $p>0.05$). Eight patients had >50% reduction in seizure frequency/severity after black seed oil *versus* placebo. *Conclusion*. Children with intractable epilepsy show evidence of oxidative stress. Administration of 40-80 mg/kg/day of black seed oil as add-on therapy did not alter either oxidative stress markers or seizure frequency or severity in intractable epileptic patients.

Key words: intractable epilepsy, black seed oil, oxidative stress

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Drug resistance, or intractable epilepsy, has been defined as the failure of adequate trials of two tolerated and appropriately chosen and used AED schedules to achieve sustained seizure freedom (Kwan *et al*., 2010). Despite a wide range of effective AEDs available, up to 30%

of epileptic patients are drug-resistant and continue to have seizures (Schmidt and Loscher, 2005; Elger and Schmidt, 2008). Patients with uncontrolled seizures are subjected to many complications such as increased risk of physical injury (Appleton, 2002), cognitive impairment (Meador, 2002), psychiatric complications (Ekinci *et al*., 2009), and sudden unexpected death (Lhatoo *et al*., 1999). Innovative approaches of therapy are needed to achieve seizure control in these patients.

Oxidative stress is defined as an imbalance between the production of oxidants and antioxidant defence leading to tissue damage (Sies, 1997). Several studies have shown increased blood and CNS levels of oxidative stress markers in epileptic patients (Ben-Menachem *et al*., 2000; Sudha *et al*., 2001; Lopez *et al*., 2007; Gunes *et al*., 2009; Ercegovac *et al*., 2010) and in animals with experimentally-induced epilepsy (Singh and Pathak, 1990; Ueda *et al*., 1997; Frantseva *et al*., 2000; Gluck *et al*., 2000), thus antioxidant therapies, aimed at reducing the oxidative stress burden, have received considerable attention in epilepsy treatment.

Nigella sativa Linn., commonly known as black seed or black cumin, is an annual plant that has been traditionally used as a natural remedy for a number of illnesses and conditions (Ali and Blunden, 2003). HPLC analysis of black seed oil has demonstrated the presence of the following ingredients: thymoquinone (TQ), dithymquinone (DTQ), thymohydroquinone (THQ), and thymol (THY), which are considered to be the main active ingredients (Ghosheh *et al*., 1999).

The seeds and oil of *Nigella sativa* are reported to have strong antioxidant properties that have been documented in several *in vitro* (Kruk *et al*., 2000; Badary *et al*., 2003; Sultan *et al*., 2009) and *in vivo* studies (Houghton *et al*., 1995; Nagi *et al*., 1999; Mansour *et al*., 2001; Khan *et al*., 2003; Abdel-Wahhab and Aly, 2005; Kanter *et al*., 2006; Hosseinzadeh *et al*., 2007; Gargari *et al*., 2009). The antiepileptogenic and antioxidant effects of black seed oil have been studied against pentylenetetrazol-induced kindling in mice and it was reported that black seed oil had anticonvulsant properties that were attributed to its antioxidant activity (Ilhan *et al*., 2005).

The aim of this study was to evaluate the effect of black seed oil as adjunctive treatment in order to enhance the effect of AEDs on seizure control in intractable epileptic children, by evaluating seizure frequency and severity as well as oxidative stress in these patients.

Patients and methods

The current study was a prospective, randomised, add-on, single blinded, controlled crossover pilot study carried out on 30 intractable epileptic children. The study was conducted at the Pediatric Neurology Outpatient Clinic, Ain Shams University hospitals, Cairo, Egypt. The study protocol was reviewed and approved by the Pediatric Board at the Children's Hospital, Ain shams University and the Committee of Ethics of the Faculty of Pharmacy, Ain Shams University. Written informed consent was obtained from patients' caregivers prior to inclusion. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Patients

Inclusion criteria comprised: a diagnosis of intractable epilepsy (age range: 2-16 years), a seizure frequency of $\geq$2 seizures/month, treatment with $\geq$2 AEDs, constant treatment for at least one month before inclusion in the trial, and constant treatment with AEDs during the trial period.

Exclusion criteria comprised: a history of psychiatric, renal, hepatic, thyroid, or cardiac disorders, or any systemic chronic illness or metabolic disease other than epilepsy. Five healthy children were included as controls.

Methods

At baseline, all patients underwent thorough history-taking and a neurological assessment. A full medical history was then taken from all enrolled patients with particular emphasis on the type of seizure disorder, which were classified according to the recommendations of the International League against Epilepsy (ILAE, 1981), as well as AEDs used together with the respective doses.

Eligible children were randomly assigned to either Group I, who received placebo for the first four weeks, followed by a washout period of two weeks, and then four weeks with black seed oil at a dose of 40-80 mg/kg/day (Kalus *et al*., 2003) or Group II, who received the same regimen but in the reverse order. Both black seed oil (Baraka®) and placebo were administered as add-on therapy to the existing AEDs. A computer-generated list of random numbers was used to allocate participants equally to each group. Each study group included 15 patients. Since no previous similar studies exist, a pre-specified sample size was not determined. Seizure frequency recorded in epilepsy diaries and seizure severity (using the Chalfont seizure severity scale; Duncan and Sander, 1991) were assessed at the end of the four-week period for both oil and placebo use. Blood samples were withdrawn at baseline and after periods with placebo and oil in order to evaluate serum oxidative stress markers; total antioxidant capacity (TAC) and malodialdehyde (MDA). Oxidative stress markers were measured spectrophotometrically using commercial

kits. Data management and analysis were performed using the Statistical Package for Social Sciences (SPSS) *vs*. 17. All *p* values were two-sided. *P* values <0.05 were considered significant. Categorical data were assessed using the χ^2 test. The Kruskal Wallis test was used to compare the baseline differences in oxidative stress markers in both groups, relative to healthy controls. In comparing the placebo *versus* oil period, The Wilcoxon Signed Ranked test was used to assess seizure frequency and severity, while the Friedman's test was used to assess oxidative stress markers. The Mann-Whitney test was used to compare the effect of intervention between groups over time.

Results

Thirty intractable epileptic patients fulfilled the inclusion criteria and were included in the study. Eight patients were withdrawn from the study, and only 22 completed the study, comprising 12 females and 10 males. Sixteen (72.7%) patients had generalised convulsions, 3 (13.6%) patients had partial convulsions, and 3 (13.6%) patients had partial, evolving to generalised, convulsions. The EEG evaluation was abnormal in 12 patients (54.5%). CT and MRI evaluation were abnormal in 8 patients (36.4%) and 7 patients (31.8%), respectively. Eighteen patients (81.8%) in the study were mentally impaired. Two patients had idiopathic epilepsy while 20 patients had symptomatic/cryptogenic epilepsy.

The 8 patients who were withdrawn did not complete the study for the following reasons: non-compliance (3), changing AEDs during the study (2), exacerbation of seizures when receiving black seed oil, (2) and suffering nausea and vomiting after two days of black seed oil use (1).

Baseline evaluation

The demographic data, baseline characteristics, and oxidative stress markers are summarised in *table 1*. There was no significant difference between the two groups regarding gender, age, weight, age at onset of epilepsy or AEDs used ($p>0.05$). The number of AEDs used ranged from 2-4 drugs.

Oxidative stress markers

There was no significant difference in baseline serum for TAC or MDA between Group I and Group II. However, the control group had significantly higher levels of serum TAC, compared to both Groups I and II (*figure 1*).

Table 1. Baseline evaluation of children with epilepsy.

Parameter	Group I	Group II	*p* value
Gender:			
Male: *n* (%)	7 (53.8)	3 (33.3)	0.45
Female: *n* (%)	6 (46.2)	6 (66.7)	
Age:			
Median (range)	11 (2.5-16)	13 (2.5-15.5)	0.845
Weight:			
Median (range)	34 (9-70)	32 (12-65)	0.695
Age at onset of epilepsy:			
Median (range)	0.5 (0-7)	3 (0-10)	0.209
Antiepileptic drugs			
Valproate (VPA)	8	6	1
Levetiracetam (LEV)	6	5	1
Phenobarbital (PB)	1	1	1
Carbamazepine (CBZ)	5	4	1
Topiramate (TPA)	4	3	1
Lamotrigine (LTG)	3	2	1
Clonazepam (CNZ)	4	1	0.36
Phenytoin (PHT)	1	0	1

Oxidative stress parameters				
	Group I	**Group II**	**Control**	**p-value**
Serum TAC (mmol/L) Mean ± SD	1.19 ± 0.49	1.26 ±0 .41	1.36 ± 0.49	0.007#
Serum MDA (nmol/mL) Mean ± SD	6.3± 2.37	6.23 ± 1.94	6.34 ± 2.04	0.776#

Statistical test: Kruskal Wallis test, $p>0.05$: non-significant. Multiple pairwise comparisons were performed using the Bonferroni adjustment test.

After treatment evaluation

Effect on seizure frequency and severity

Comparing the seizure frequency and severity between the placebo and black seed oil period, a significant difference was found in Group I, while no significant difference was found in Group II between the two periods (*table 2*). However, there was no significant

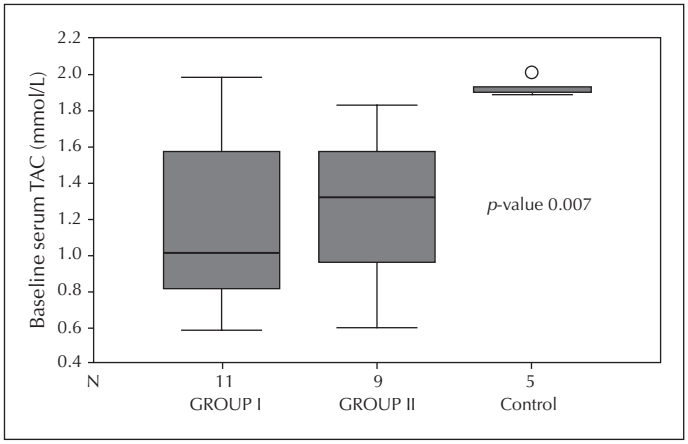

Figure 1. Baseline serum TAC in the study groups and control group.

difference between the two groups over time, indicating the non-significance of the treatment sequence on seizure frequency and severity (p=0.552 and p=0.601, respectively). Six patients showed >50% reduction in seizure frequency and 2 patients showed >50% reduction in seizure severity after the black seed oil period, compared to the placebo period.

Effect on oxidative stress

There was no significant difference in serum MDA or TAC between the different periods for either Group I or Group II (*table 3*), neither was there a difference between the two groups over time indicating the non-significance of the treatment sequence (TAC [p=0.602], MDA [p=0.069]).

Discussion

Several studies have suggested that the decrease in free radical scavenging enzyme activity and the increase in membrane lipid peroxidation may be involved in some forms of epilepsies and are believed to cause an increased risk of seizure recurrence (Singh and Pathak, 1990; Ueda *et al*., 1997; Ben-Menachem *et al*., 2000; Frantseva *et al*., 2000; Gluck *et al*., 2000; Sudha *et al*., 2001; Lopez *et al*., 2007; Gunes *et al*., 2009; Ercegovac *et al*., 2010). Two oxidative stress markers were selected for evaluation of oxidative stress in intractable epileptic children in the current study: TAC and lipid peroxides (MDA).

TAC is an integrated parameter for assessing the cumulative action of all antioxidants present in body fluids, giving an insight into the balance between oxidants and antioxidants (Ghiselli *et al*., 2000). MDA is a marker of lipid peroxidation. The brain is particularly susceptible to injury by lipid peroxidation and this could be attributed to the high content of polyunsaturated phospholipids. Lipid peroxidation is a free radical-mediated pathway and is used as an index for irreversible neuronal damage of cell membrane phospholipids, and is suggested to be a possible mechanism involved in epileptic activity (Waldbaum and Patel, 2010).

Table 2. Effect of black seed oil on seizure frequency and severity.

Seizure frequency			
	During 4 weeks of placebo	**During 4 weeks of black seed oil**	***p* value**
Group I; Median (Range)	33 (2-608)	12 (0-461)	0.034*
Group II; Median (Range)	28 (4-516)	17 (5-200)	0.44
Seizure severity			
	After placebo	**After black seed**	***p* value**
Group I; Median (Range)	28 (6-123)	24 (0-93)	0.038*
Group II; Median (Range)	35 (8-127)	31 (7-84)	0.225

Statistical test: Wilcoxon Rank Signed test.

Table 3. Comparison of TAC and MDA serum level at baseline, after placebo and after black seed oil administration.

	Serum TAC at baseline (mmol/L)	**Serum TAC after placebo (mmol/L)**	**Serum TAC after black seed oil period (mmol/L)**	***p* value**
Group I; Mean ± SD	1.19 ± 0.49	1.34 ± 0.46	1.32 ± 0.50	0.905
Group II; Mean ± SD	1.26 ± 0.41	1.38 ± 0.37	1.25 ± 0.55	0.717
Total; Mean ± SD	1.22 ± 0.44	1.35 ± 0.41	1.29 ± 0.51	0.949
	Baseline Serum MDA (nmol/mL)	**Serum MDA after placebo period (nmol/mL)**	**Serum MDA after black seed oil period (nmol/mL)**	***p* value**
Group I; Mean ± SD	6.30 ± 2.37	7.38 ± 2.08	5.17 ± 2.94	0.407
Group II; Mean ± SD	6.23 ± 1.94	6.70 ± 1.67	7.37 ± 2.53	0.236
Total; Mean ± SD	6.26 ± 2.13	7.10 ± 1.91	6.11 ± 2.93	0.692

Statistical test; Friedman's test.

The current study demonstrates that intractable epileptic children were subjected to increased oxidative stress which was evidenced by the significant lower levels of TAC as compared to control, in agreement with the study of Hamed *et al*. (2004) which reported significant low serum TAC levels in untreated epileptic patients and patients treated with valproate, carbamazepine, and polytherapy. Similar results were documented by Aycicek and Iscan (2007) with untreated epileptic children. However, the current study does not show a significant difference in lipid peroxidation marker in epileptic children, when compared to controls. Similarly, Arhan *et al*. (2011) did not show any significant difference in lipid peroxidation between children with newly diagnosed idiopathic epilepsy and controls. Verrotti *et al*. (2008) have found that serum MDA levels were normal in epileptic children before starting therapy. On the other hand, other studies have documented an increase in lipid peroxidation markers in adults with drug-resistant temporal lobe epilepsy (Lopez *et al*., 2007), adult epileptic patients (Surekha and Melinkeri, 2010), untreated epileptic patients, patients on polytherapy (Hamed *et al*., 2004), and epileptic children with structural abnormality (Turkdogan *et al*., 2002). Hence, studies evaluating the effect of epilepsy on lipid peroxidation are conflicting which may be attributed to multiple factors including the differences in study methodology undertaken and the heterogeneity in epileptic patient variables regarding the age of epileptic patients, age at onset of epilepsy, classification and aetiology of seizures, duration of drug treatment, and AEDs used.

In order to evaluate the efficacy of black seed oil on intractable paediatric seizures, a crossover design was chosen. A crossover design is useful to assess a new AED as add-on treatment for intractable epileptic patients, compared to add-on placebo, because the patient inter-variation is perhaps two to three orders of magnitude greater than patient intra-variation (Richens, 2001).

Despite the absence of any previous clinical trial evaluating the effect of black seed oil on epilepsy in humans, the preliminary results of animal studies of black seed oil (Ilhan *et al*., 2005) and the clinical trials of the aqueous extract of *Nigella sativa* (Akhondian *et al*., 2007) and TQ (Akhondian *et al*., 2011) on intractable epileptic children suggest that *Nigella sativa* possesses an antiepileptogenic activity, reflected by reduced seizure frequency. In the current study, although there was a significant difference in seizure severity and frequency between the two periods in Group I, a non-significant difference was found in Group II, and the effect of treatment sequence was excluded and significance attributed to chance. Hence, the overall analysis of the current crossover study showed that the administration of black seed oil, at a dose of 40-80 mg/kg/day, did not significantly affect seizure frequency or severity. However, six patients showed >50% reduction in seizure frequency and two patients showed >50% reduction in seizure severity after the black seed oil period, compared to the placebo period. Similarly, in a study by Noor *et al*. (2012), although no seizure manifestations were observed after the treatment of epileptic animals with either curcumin or black seed oil, moderate excitation and aggression were observed in the pilocarbinized animals treated with black seed oil which were attributed to the failure of black seed oil to restore the biochemical changes of the hippocampal or cortical excitatory amino acids induced by

pilocarpine. These findings could partially explain the lack of anticonvulsant activity observed in our study.

Moreover, in the current study, black seed oil administration did not significantly affect serum levels of oxidative stress parameters, namely TAC and MDA. In accordance with our results, a study by Ezz *et al*. (2011) showed a non-significant change in the hippocampal MDA levels of pilocarpine-treated animals in a rat model of epilepsy, however, the authors proposed a potential neuroprotective effect of black seed oil that was attributed to mechanisms other than MDA alteration.

The results of the current study could be attributed to the small sample size included due to the limited number of intractable epileptic patients, the heterogeneity of patient characteristics, the lack of a double-blinded design, and the limitations of epilepsy diary recording.

The current study reported that add-on treatment of black seed oil, administered at a dose of 40-80 mg/kg, was well tolerated during the study period, with the exception of one patient who suffered from nausea and vomiting after receiving the oil. Similarly, kalus *et al*. (2003) had reported mild gastrointestinal symptoms upon administering black seed oil in children with an empty stomach. No other adverse effects were reported during the study period, in agreement with other studies which have shown that the oil was well tolerated (Boskabady *et al*., 2007; Najmi *et al*., 2008; Salem *et al*., 2010; Sabzghabaee *et al*., 2012).

Conclusion

Oxidative stress was evident in intractable epileptic children. The administration of black seed oil at a dose of 40-80 mg/kg/day in intractable epileptic children had no effect on seizure frequency or severity. Moreover, black seed oil administration had no effect on oxidative stress parameters in intractable epileptic children. □

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