Neurochem Res (2011) 36:2195–2204
DOI 10.1007/s11064-011-0544-9

ORIGINAL PAPER

The Neuroprotective Effect of Curcumin and *Nigella sativa* Oil Against Oxidative Stress in the Pilocarpine Model of Epilepsy: A Comparison with Valproate

Heba S. Aboul Ezz · Yasser A. Khadrawy · Neveen A. Noor

Accepted: 2 July 2011 / Published online: 13 July 2011

Abstract Oxidative stress has been implicated to play a role in epileptogenesis and pilocarpine-induced seizures. The present study aims to evaluate the antioxidant effects of curcumin, *Nigella sativa* oil (NSO) and valproate on the levels of malondialdehyde, nitric oxide, reduced glutathione and the activities of catalase, Na^+, K^+-ATPase and acetylcholinesterase in the hippocampus of pilocarpine-treated rats. The animal model of epilepsy was induced by pilocarpine and left for 22 days to establish the chronic phase of epilepsy. These animals were then treated with curcumin, NSO or valproate for 21 days. The data revealed evidence of oxidative stress in the hippocampus of pilocarpinized rats as indicated by the increased nitric oxide levels and the decreased glutathione levels and catalase activity. Moreover, a decrease in Na^+, K^+-ATPase activity and an increase in acetylcholinesterase activity occurred in the hippocampus after pilocarpine. Treatment with curcumin, NSO or valproate ameliorated most of the changes induced by pilocarpine and restored Na^+, K^+-ATPase activity in the hippocampus to control levels. This study reflects the promising anticonvulsant and potent antioxidant effects of curcumin and NSO in reducing oxidative stress, excitability and the induction of seizures in epileptic animals and improving some of the adverse effects of antiepileptic drugs.

H. S. Aboul Ezz (✉) · N. A. Noor
Laboratory of Neurophysiology, Zoology Department, Faculty of Science, Cairo University, Giza, Egypt
e-mail: hebasal@yahoo.com

Y. A. Khadrawy
Department of Medical Physiology, Medical Division, National Research Center, Giza, Egypt

Keywords Pilocarpine · Curcumin · *Nigella sativa* oil · Oxidative stress · Na^+ · K^+- ATPase—acetylcholinesterase

Introduction

Epilepsy and seizure disorders affect 50 million people around the world and contribute to morbidity and mortality [1]. The use of antiepileptic drugs (AEDs) is limited due to the vast array of adverse effects, such as cognitive impairment, affective disorders and recurring seizures [1, 2]. Hence, there is a need for the development of new AEDs with fewer adverse effects and higher efficacy.

Oxidative stress, defined as the excessive production of free radicals, can alter dramatically the cell function and an overproduction of these compounds has been related to seizure-induced neuronal death [3, 4]. The animal brain is often said to be especially sensitive to oxidative damage [5]. This may be attributed to its high oxygen consumption, the large quantities of oxidizable lipids and metals, and the comparatively less antioxidant mechanisms [6, 7].

Pilocarpine is a cholinergic agonist used as a model to induce epilepsy. It reproduces in rodents behavioral and electroencephalographic alterations similar to those in human temporal lobe epilepsy [8, 9]. The epilepsy model induced by pilocarpine in rats is characterized by an acute phase, characterized by seizures which progress within 1–2 h to status epilepticus (SE), by a seizure-free period (silent; 4–44 days, mean of 15 days) and by a chronic phase, characterized by spontaneous recurrent seizures (SRS) [10, 11].

Curcumin is the major active component extracted from the rhizome of the plant *Curcuma longa* Linn. (Zingiberaceae) commonly known as turmeric. Curcumin is widely

used as a food additive and also as a herbal medicine throughout Asia. Curcumin crosses the blood–brain barrier [12], and has been shown to possess neuroprotective activity [13, 14]. Previous studies have reported the efficacy of curcumin in delaying [15] or completely inhibiting the onset of convulsive seizures in kainic acid-induced epilepsy [16]. Bharal et al. [17] reported that the chronic administration of curcumin markedly elevated the seizure threshold in increasing current electroshock model and suggested that curcumin may possess anticonvulsant activity. Moreover, Jyoti et al. [18] demonstrated the potential of curcumin to inhibit spontaneous seizures in the iron-induced model of posttraumatic epilepsy.

Nigella sativa, commonly known as black cumin, belongs to the botanical family of Ranunculaceae. *Nigella sativa* seeds have been used in Middle Eastern folk medicine as a natural remedy for various diseases [19, 20]. Recently, clinical and animal studies have shown that the extracts of the black seeds have many therapeutic effects such as antioxidative [21] and neuroprotective [22] effects. Ilhan et al. [23] demonstrated a potent anticonvulsant property of *Nigella sativa* oil (NSO) against the development of kindling consequences in pentylenetetrazol (PTZ)-kindled mice.

Valproate is currently one of the major antiepileptic drugs [24, 25]. It has been proved to be active in multiple anticonvulsant tests and also has the broadest clinical utility [26].

Therefore, the aim of the present study was to evaluate some oxidative stress parameters in the hippocampus of pilocarpine-treated rats as a model of epilepsy during the spontaneous recurrent seizures phase and to investigate the antioxidant effects of curcumin and NSO, two natural herbs with reported anticonvulsant activities, on these parameters in comparison with the effects of valproate, a well established antiepileptic drug. In addition, the effects of these treatments on the activities of Na^+, K^+-ATPase and acetylcholinesterase in the hippocampus of pilocarpinized rats were also investigated.

Experimental Procedure

Experimental Animals

The experimental animals used in the present study were adult male Wistar albino rats weighing 200–250 g. The animals were purchased from the animal house of the National Research Center and were given food and water ad libitum. They were maintained under fixed appropriate conditions of housing and handling. All experiments were carried out in accordance with research protocols established by the animal care committee of the National Research Center, Egypt.

Drugs and Chemicals

Pilocarpine was obtained from Macfarlan Smith Ltd. (Edinburgh). It was dissolved in saline. Atropine sulphate was obtained from Boehringer Ingelheim (Germany). Curcumin was purchased from Sigma Chemical Company. It was suspended in 1% carboxymethyl cellulose. *Nigella sativa* oil was obtained from the seeds of Nigella sativa by hydraulic press on cold as carried out by the Department of Oils, National Research Center, Egypt. Sodium valproate was obtained from Global Napi Pharmaceuticals, Egypt.

Experimental Design

Sixty animals were subjected to chronic epilepsy induction by the intraperitoneal injection (i.p.) of a single dose of pilocarpine (380 mg/kg) according to Turski et al. [8]. Atropine sulphate was injected subcutaneously at a dose of 5 mg/kg, 30 min before the induction of epilepsy, to prevent peripheral muscarinic stimulation [27]. After about 30 min., the animals became hypoactive and then displayed oro-facial movements, salivation eye-blinking, twitching of vibrissae and yawning; generalized convulsions and limbic SE developed about 40–80 min. after the injection [4]. Mortality was recorded after 1 h. Twenty one animals (about 35%) died during SE. The animals that survived SE were left for 22 days to establish the chronic phase of the induced SRS according to Cavalheiro et al. [10]. No mortality was recorded afterwards.

These animals were then divided into four treated groups: 1. Untreated pilocarpinized animals (n = 10) were injected orally with saline till the end of the experiment. 2. The animals of the second group received a daily oral administration of curcumin (80 mg/kg) [28]. 3. The animals of the third group received a daily oral administration of NSO (4 ml/kg) [29]. 4. In the fourth group, the animals received a daily oral administration of valproate (100 mg/kg) [23].

All animals were sacrificed by sudden decapitation after 21 days of daily administration. Control animals (n = 10) received a single i.p. injection of saline and after 22 days they received a daily oral administration of saline for 21 days. They were sacrificed simultaneously with the treated groups.

After decapitation, the brain was transferred rapidly to an ice-cold Petri dish where it was dissected to remove the hippocampus. The brain samples were weighed and kept at −43° until analyzed. Each brain sample was then homogenized in 5% w/v 20 mM phosphate buffer, pH 7.6.

Determination of Nitric Oxide Level and Lipid Peroxidation

The assay of nitric oxide (NO) was carried out using Biodiagnostic kit No. NO 25 33 (Biodiagnostic Co., Egypt). This method is based on the spectrophotometric

method of Montgomery and Dymock [30] which is based on the measurement of endogenous nitrite concentration as an indicator of nitric oxide production. It depends on the addition of Griess Reagents which convert nitrite into a deep purple azo compound whose absorbance is read at 540 nm in a Helios Alpha Thermospectronic (UVA 111615, England).

Lipid peroxidation (LP) was determined by measuring the level of thiobarbituric reactive species (TBARs) using the method of Ruiz-Larrea et al. [31] in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532 nm.

Determination of Reduced Glutathione Level

The assay of reduced glutathione (GSH) levels was performed using Biodiagnostic kit No. GR 25 11 (Biodiagnostic Co., Egypt) which is based on the spectrophotometric method of Beutler et al. [32]. It depends on the reduction of 5,5′-dithiobis 2-nitrobenzoic acid with glutathione to produce a yellow color whose absorbance is measured at 405 nm.

Determination of Enzyme Activities

Catalase activity was measured using Biodiagnostic Kit No. CA 25 17 (Biodiagnostic Co., Egypt) which is based on the spectrophotometric method described by Aebi [33]. Catalase reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample.

The procedure used for the determination of acetylcholinesterase (AchE) activity in the hippocampus and cortex was a modification of the method of Ellman et al. [34] as described by Gorun et al. [35]. The principle of the method is the measurement of the thiocholine produced as acetylthiocholine is hydrolyzed. The colour was read immediately at 412 nm.

Na^+, K^+- ATPase activity was measured spectrophotometrically according to Bowler and Tirri [36] as described by Tsakiris et al. [37].

Statistical Analysis

The data were expressed as means ± S.E.M. Data were analyzed by analysis of variance (ANOVA) followed by the Duncan multiple range test when the *F*-test was significant ($P < 0.05$). All analyses were performed using the Statistical Package for Social Sciences (SPSS) software in a PC-compatible computer.

Results

The behavior of the animals was monitored visually during the diurnal period since it has been reported that seizure frequency was higher during this period [38]. All pilocarpine-treated animals developed SRS (3–4 seizures/rat/week) which ranged from facial automatisms to forelimb clonus and rearing and falling as described previously [39]. No seizure manifestations were observed after treatment of epileptic animals with curcumin, NSO or valproate. Moderate excitation and aggression were observed in pilocarpinized animals treated with NSO during handling.

Lipid Peroxidation

A single injection of pilocarpine resulted in a non significant decrease in the lipid peroxidation marker malondialdehyde (MDA) in the hippocampus after 6 weeks i.e. during SRS. However, treatment of pilocarpinized animals with curcumin decreased MDA levels by 14.82% ($P < 0.05$) when compared to control values. Both NSO and valproate induced non significant changes in MDA levels in pilocarpine-treated animals in comparison with control levels (Table 1; Fig. 1).

Nitric Oxide Levels

ANOVA revealed significant differences in NO levels between groups. Pilocarpine injection increased NO levels in the hippocampus by 16.67%. Curcumin administration to pilocarpine-treated animals restored the levels of NO to control values. NSO treatment slightly attenuated the increased NO levels resulting from pilocarpine, recording a percentage difference of 11.11% above the control level (in comparison with 16.67% in pilocarpinized rats). Meanwhile, treatment of pilocarpinized rats with valproate reduced NO levels to 5.56% in comparison with control values (Table 1; Fig. 1).

Reduced Glutathione Levels

Significant differences in GSH levels were obtained between groups after ANOVA analysis. After a single injection of pilocarpine, GSH levels were decreased by 25.69% below the control levels ($P < 0.05$). Treatment of pilocarpinized animals with curcumin restored GSH levels (2.75%) to nearly control values. On the contrary, NSO administration to pilocarpine-treated animals decreased GSH levels by 22.02% as compared to control values. A

Table 1 Effect of curcumin (80 mg/kg), NSO (4 ml/kg), and valproate (100 mg/kg) on the levels of MDA, NO and GSH and activities of catalase, ATPase and AchE in the hippocampus of pilocarpinized rats

	Control	Pilocarpinized animals	% diff	Curcumin	% diff	NSO	% diff	Valproate	% diff
MDA	31.52 ± 0.90^{a} [6]	30.02 ± 0.83^{ab} [6]	−4.76	26.85 ± 1.22^{b} [5]	−14.82	32.27 ± 1.82^{a} [5]	2.38	30.29 ± 1.02^{ab} [5]	−3.9
NO	0.18 ± 0.00^{ac} [6]	0.21 ± 0.01^{b} [6]	16.67	0.18 ± 0.01^{a} [6]	0.00	0.20 ± 0.01^{bc} [6]	11.11	0.17 ± 0.01^{a} [6]	5.56
GSH	1.09 ± 0.05^{ac} [7]	0.81 ± 0.03^{b} [6]	−25.69	1.12 ± 0.05^{a} [5]	2.75	0.85 ± 0.03^{bd} [6]	-22.02	0.97 ± 0.06^{cd} [6]	−11.01
Catalase	6.78 ± 0.57^{a} [5]	5.26 ± 0.22^{b} [6]	−22.42	3.41 ± 0.21^{c} [6]	−49.71	5.81 ± 0.63^{ab} [5]	-14.31	5.33 ± 0.36^{b} [6]	−21.39
ATPase	1.02 ± 0.05^{a} [6]	0.76 ± 0.04^{b} [5]	−25.49	1.28 ± 0.10^{c} [5]	25.49	0.88 ± 0.07^{ab} [6]	-13.73	0.97 ± 0.10^{ab} [5]	−4.90
AchE	2.68 ± 0.18^{a} [6]	3.38 ± 0.23^{b} [5]	26.12	3.32 ± 0.21^{b} [5]	23.88	3.41 ± 0.20^{b} [5]	27.24	1.99 ± 0.12^{c} [6]	−25.75

The values represent the means ± S.E. with the number of animals between parentheses

The same letters represent statistically non significant values while different letters represent statistically significant values

The % diff.: the percentage differences as compared to control levels

non significant decrease in GSH levels was obtained after treatment of pilocarpinized animals with valproate, recording—11.01% below the control levels (Table 1; Fig. 1).

Catalase Activity

Pilocarpine injection resulted in a significant decrease in catalase activity by 22.42% when compared to control group. This decrease was exaggerated after curcumin treatment, recording −49.71% below the control level. The decrease in catalase activity obtained in the pilocarpine-treated animals also continued non significantly (−14.31%) and significantly (−21.39%) after NSO and valproate treatments, respectively (Table 1; Fig. 1).

Na^+, K^+- ATPase Activity

ANOVA revealed significant differences in Na^+, K^+-ATPase activity in the hippocampus between groups. A significant decrease in the enzyme activity by 25.49% was recorded in the hippocampus of animals treated with pilocarpine. This was reversed to a significant increase after treatment with curcumin (25.49% above the control levels). Both NSO and valproate slightly attenuated the decrease in hippocampal Na^+, K^+- ATPase activity induced by pilocarpine, the values were −13.73 and −4.90% below the control, respectively, and were non significant with respect to those of pilocarpine-treated animals (Table 1; Fig. 2).

Acetylcholinesterase Activity

Similarly, significant differences in AchE activity were obtained in the hippocampus between groups. A significant increase in AchE activity by 26.12% was recorded in pilocarpine-treated animals. Both curcumin and NSO failed to restore the enzyme activity to control values, an increase of the enzyme by 23.88 and 27.24% was recorded after the two treatments, respectively. Valproate reversed the increase in AchE activity to a significant decrease, recording—25.75% in comparison with control animals (Table 1; Fig. 2).

Discussion

The present study revealed a significant increase in nitric oxide levels accompanied by a significant decrease in GSH levels and catalase activity in the hippocampus of pilocarpine-treated rats providing evidence of oxidative stress in this area during SRS. This was associated with a decrease in Na^+, K^+—ATPase activity and an increase in AchE activity.

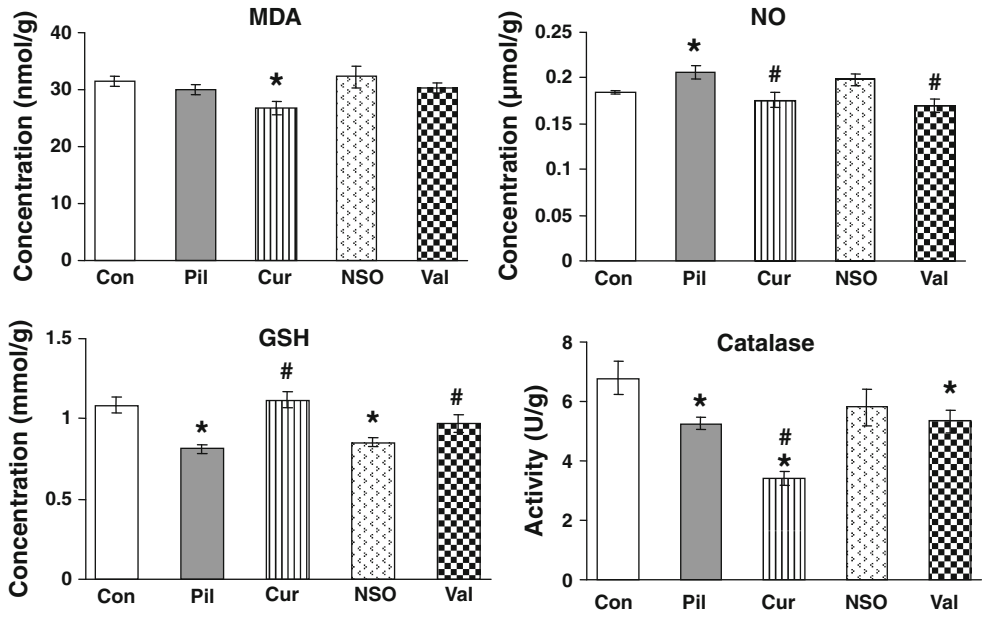

Fig. 1 Effect of curcumin (80 mg/kg), NSO (4 ml/kg), and valproate (100 mg/kg) on the levels of MDA, NO and GSH and catalase activity in the hippocampus of pilocarpinized rats. The sign * indicates that there is a significance at $P < 0.05$ in comparison with the control value. The sign # indicates that there is a significance at $P < 0.05$ in comparison with the pilocarpine-treated group. *Con* Control group, *Pil* pilocarpine-treated group, *Cur* curcumin-treated group, *NSO Nigella sativa* oil-treated group, *Val* valproate-treated group

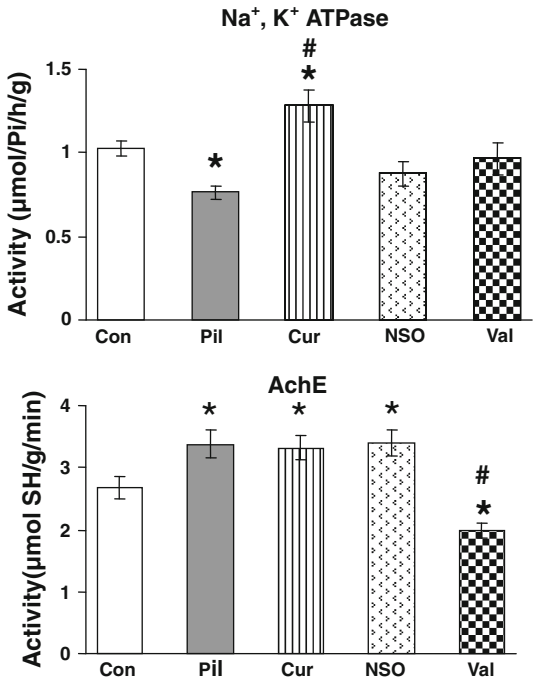

Fig. 2 Effect of curcumin (80 mg/kg), NSO (4 ml/kg), and valproate (100 mg/kg) on the activities of Na^+, K^+ ATPase and acetylcholinesterase in the hippocampus of pilocarpinized rats. The sign * indicates that there is a significance at $P < 0.05$ in comparison with the control value. The sign # indicates that there is a significance at $P < 0.05$ in comparison with the pilocarpine-treated group. *Con* Control group, *Pil* pilocarpine-treated group, *Cur* curcumin-treated group, *NSO Nigella sativa* oil-treated group, *Val* valproate-treated group

The pilocarpine model of epilepsy mimics several phenomenological features of temporal lobe epilepsy including a particular resistance to anticonvulsant medication [8]. Oxidative stress has already been demonstrated to play a role in epileptogenesis and has been related to the neurochemical changes observed during SE and SRS induced by pilocarpine [40, 41]. For these reasons and owing to the vast array of adverse effects accompanying the use of antiepileptic drugs, it seemed plausible to investigate the effects of two antioxidant medicinal herbs with reported anticonvulsant activities on the pilocarpine-induced changes in several oxidative stress parameters and related enzymes during SRS. It was also of interest to compare these effects with those of valproate, an established and widely used anticonvulsant with conflicting reports about its oxidant [42, 43] and antioxidant activities [44, 45].

SRSs have been reclassified by Veliskova [39] according to the following criteria: staring and mouth clonus; automatisms; monolateral forelimb clonus; bilateral forelimb clonus; bilateral forelimb clonus with rearing and falling and tonic-clonic seizure. The present incidence of seizures was consistent with other studies which reported that adult male Wistar rats given 300–320 mg/kg of pilocarpine showed a mean latent period of 14 days and a frequency of 2.8 seizures per week by continuous video monitoring [10, 38]. Seizure frequency was also reported to be higher during the diurnal period [38].

The present study showed a non significant decrease in LP in pilocarpine-treated animals during SRS which may

be due to long-term compensatory mechanisms that may attenuate LP. Supporting our finding, Dal-Pizzol et al. [4] found a decrease in TBARs levels in the hippocampus in the pilocarpine model during SRS and explained it by the neuronal loss [10, 46] and hypometabolism observed in this structure [47]. Curcumin treatment resulted in a significant decrease in LP below control and pilocarpine-treated levels. This may be attributed to its redox metal-binding activity [12], free radical scavenging properties [48], and antioxidative potential [49].

Several reports have suggested the involvement of NO in various models of epilepsy [50, 51]. However, the role exerted by NO during seizures has never been clearly understood. While some authors believe that NO may be an endogenous anticonvulsant [52], others suggest a proconvulsant role for NO [53]. However, several lines of evidence suggest that NO produced by the activation of neuronal nitric oxide synthase (nNOS) triggers seizures [54].

The present data revealed a significant increase in NO in the hippocampus of pilocarpine-treated rats. Kovács et al. [55] suggested that enhancement of NO formation might provide a general mechanism for seizure initiation. Consequently, the increased NO levels in the hippocampus of the present pilocarpine model may underlie the neurotoxicity and initiation of seizures during SRS induced by pilocarpine. The oral administration of curcumin restored the increased NO levels resulting from pilocarpine treatment to control values which could be explained by the potential of curcumin to inhibit the expression of inducible NOS [56] and its potent NO scavenging effects [57, 58]. Although the aqueous extract of *Nigella sativa* seeds exhibited an inhibitory effect on NO production by murine macrophages [59], NSO slightly attenuated the elevated levels of NO induced by the present pilocarpine model (from 16.67 to 11.11%). Valproate administration to epileptic rats reduced NO levels, a finding that was confirmed in other epileptic models [60]. Similar to the well established antiepileptic drug valproate, it may be suggested that the anticonvulsant effect of curcumin may be mediated by its ability to restore NO level in the hippocampus to the control level and this may suppress the occurrence of seizures observed after both treatments.

The present significant decrease in catalase activity and GSH levels in the hippocampus of pilocarpine-treated rats may be due to the overproduction of free radicals and the consumption of both antioxidants in scavenging the rapidly generating free radicals. The present study showed that only NSO attenuated the decrease in catalase activity induced by pilocarpine. However, treatment with curcumin exaggerated the decrease in the enzyme activity whereas valproate had no effect on this enzyme activity. Brannan et al. [61] measured regional catalase activity in 11 areas of adult rat brain and found that the frontal cortex and hippocampus had the lowest activity. In addition, it has been reported that catalase activity in rat brain is too subtle to elicit significant changes in response to oxidative stress [62].

Glutathione plays a key role as an essential cellular antioxidant in the defense of brain cells against oxidative damage induced by ROS [63]. GSH reacts directly with free radicals in nonenzymatic reactions and is the electron donor in the reduction of peroxides catalyzed by glutathione peroxidase. The product of oxidation is glutathione disulfide (GSSG) which is reduced back to GSH by glutathione reductase [64]. GSH is also consumed in the detoxification of electrophilic compounds via glutathione-S-transferases, thereby providing cells with multiple defenses against both ROS and their by-products [65].

Several studies reported low GSH levels in chronic epilepsy models [66, 67]. In line with the present findings, Freitas [68] reported that seizure episodes induced by pilocarpine were accompanied by a decrease in brain GSH levels and a lack of effects in glutathione peroxidase and glutathione reductase activities. Accordingly, the decrease in GSH induced by pilocarpine, in the present study, may reflect a state of oxidative stress resulting from accelerated degradation or decreased de novo synthesis. It is known that intracellular concentrations of GSH are an important factor in dictating cellular susceptibility to nitric oxide and its derivatives [69]. Furthermore, in vivo studies support that GSH depletion in the brain can cause mitochondrial dysfunction, and contribute to neuronal damage [70].

Treatment with curcumin or valproate ameliorated the decrease in GSH induced by pilocarpine whereas NSO failed to improve GSH levels. The free radical scavenging ability of curcumin may be attributed to the H-donating phenolic groups [71, 72]. In addition, curcumin contains two electrophilic α, β-unsaturated carbonyl groups, which can react with nucleophilic compounds such as GSH and form glutathionated products of curcumin [73]. Moreover, curcumin induces GSH synthesis in cells by activation of glutamyl cysteine ligase (GCL) activity in vivo [74]. Similarly, it has been reported that valproate increased the synthesis of GSH and restored total antioxidant capacity in the brain [60, 75]. Thus, the restoration of GSH levels in the hippocampus of curcumin- and valproate-treated epileptic animals may reflect the potent antioxidant activity of these treatments.

The black seed of *Nigella sativa* contains 36–38% fixed oils, proteins, alkaloids, saponin and 0.4–2.5% essential oil. The fixed oil is composed mainly of unsaturated fatty acids [76]. The biological activity of *Nigella sativa* seeds is attributed to its essential oil components [77]. The main compounds contained are thymoquinone (30–48%), p-cymene (7–15%), carvacrol (6–12%), 4-terpineol (2–7%), t-anethole (1–4%) and longifolene (1–8%) [78].

Some reports showed that high doses of thymoquinone, the main active ingredient in NSO, cause depletion of cellular glutathione in vital organs [79–81]. This may explain the failure of NSO to restore GSH levels in pilocarpine-treated rats in this study.

Na^+, K^+-ATPase enzyme plays a pivotal role in maintaining cellular ionic gradients across plasma membranes and it is particularly sensitive to ROS [82]. Failure of Na^+, K^+-ATPase activity may increase cellular excitability and facilitate the appearance or propagation of convulsions [68]. In the present study, a significant decrease in Na^+, K^+-ATPase was evident in the hippocampus after pilocarpine treatment. Contrary to the present findings, Fernandes et al. [83] found an increase in Na^+, K^+-ATPase activity during the chronic period. The longer period after induction of epilepsy (120 days) in their study may account for this discrepancy. The current significant decrease in Na^+, K^+-ATPase observed during SRS may be due to neuronal damage [46], inhibition of protein synthesis [84] and deficiency in ATP concentration [85]. Moreover, recent studies have demonstrated that reactive nitrogen species inhibit the activity of Na^+, K^+-ATPase by oxidation of SH groups [86, 87]. Curcumin treatment to pilocarpinized animals reversed the decrease in Na^+, K^+-ATPase activity in the hippocampus of pilocarpinized rats. Consistent with the present study, Kaul and Krishankanth [88] showed a 148% increase in Na^+, K^+-ATPase activity in brain microsomes from curcumin-fed rats. Moreover, curcumin treatment significantly activated Na^+, K^+-ATPase activity in iron-induced posttraumatic epileptic rats. Na^+, K^+-ATPase activity is also known to be sensitive to lipid peroxidation [89] which is negatively correlated with this enzyme activity [90]. Therefore, the observed reduction in lipid peroxidation in curcumin-treated epileptic rats may also underlie the ability of curcumin to reverse the disruption of Na^+, K^+-ATPase activity in the present pilocarpine-treated rats. Thus, curcumin may help in the suppression of excitability in the hippocampus in the present pilocarpine model of epilepsy and this may underlie its reported anticonvulsant activity. On the other hand, a slight improvement in hippocampal Na^+, K^+-ATPase activity was evident, in the present study, after treatment of pilocarpinized rats with valproate and NSO, being more prominent in the case of valproate (from −25.49 to −4.9%).

Acetylcholinesterase has a crucial role in cholinergic neurotransmission as it causes the rapid hydrolysis of acetylcholine released into the synapse [91]. In the nervous system, epilepsy has been related to overproduction or release of acetylcholine by cholinergic neurons, due to a neuronal hyperactivity and/or an excitotoxicity, that might induce a neuronal damage during pilocarpine-induced seizure and SE [92, 93]. It has been described that the impairments in learning, memory and behavior observed in patients with epilepsy are caused, at least in part, by changes in cholinergic system function [94] since there is consistent evidence that high levels of acetylcholine in the brain are associated with cognitive dysfunction [95].

Neurochemical as well as functional studies suggest that pilocarpine alters acetylcholine metabolism in rat brain. Acetylcholine synthesis is increased in the cortex, hippocampus and striatum of epileptic adult rats [96]. Santos et al. [97] concluded that the constant inhibition of choline acetyltransferase and AchE by seizures during SE induced by pilocarpine might increase acetylcholine levels which could be associated with the memory deficit observed in seized rats. The present study revealed a significant increase in AchE activity in the hippocampus of pilocarpine-treated rats during SRS. This may reflect a compensatory mechanism by which the brain attempts to terminate the increase in acetylcholine. Supporting this notion is the inhibition of Na^+, K^+-ATPase activity which has been related to the enhancement of acetylcholine release [98]. It has been demonstrated that AchE has a fundamental role in learning and memory [99, 100].

The present data revealed that treatment with curcumin or NSO had no effect on the increased AchE activity induced by pilocarpine in the hippocampus during SRS. Reports on the effect of curcumin on AchE activity have yielded conflicting results. Sharma et al. [101] reported that curcumin lowered AchE level in the cerebral cortex and hippocampus of rat brain. Ahmed and Gilani [102] reported that curcumin was relatively weak in its AchE inhibitory effect in scopolamine-induced amnesia but showed memory enhancing effect in the Morris water maze test. The authors suggested that curcumin enhanced memory in this model possibly through mechanism(s) independent of AchE inhibition. Jyoti et al. [18] reported that curcumin was effective in preventing the cognitive deficits associated with epileptogenesis. However, no literature has been reported on the effect of NSO on AchE or cholinergic activity. The increased AchE activity that continued after treatment of pilocarpinized rats with curcumin or NSO may also help in terminating the increased acetylcholine content resulting from pilocarpine.

However, a significant decrease in the AchE activity occurred after treatment of pilocarpinized animals with valproate. It has been reported that divaloproex which is related to valproate slightly but significantly increased acetylcholine efflux in the hippocampus [103]. This finding together with the increased acetylcholine levels after pilocarpine may result in an increase in cholinergic activity. Thus, the inhibition of AchE activity after valproate may also lead to an augmentation of the increased cholinergic activity. Valproate and other anticonvulsant mood stabilizers have generally been found to have some adverse

effects on cognition in patients with epilepsy [104, 105]. They have also been reported to induce cognitive impairment in healthy individuals [106]. Sgobio et al. [107] concluded that the demonstration that valproate induces morphologic alterations and impairment in specific hippocampal-dependent memory task might explain the detrimental effects of antiepileptic treatment on cognition in human subjects. It may thus be suggested that the inhibition of AchE after valproate treatment in the present model together with the enhanced cholinergic activity may participate in the cognitive side effects of valproate.

The present study revealed that curcumin has potent antioxidant and anticonvulsant effects in reducing oxidative stress, excitability and the induction of seizures in epileptic animals. The slight improvement observed after treatment of epileptic rats with NSO suggests that further studies are needed to adjust the dose used.

In conclusion, the ability of antioxidants to reduce the seizure manifestations and the accompanying biochemical changes in several markers of oxidative stress further supports a role of free radicals in seizures. It also highlights a possible role of antioxidants as adjuncts to antiepileptic drugs for better seizure control and fewer side effects. These medicinal herbs may also give new insights into the development of new therapies for the treatment of chronic epilepsy.

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