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Original Article

Pharmacology Section

Evaluation of Antioxidant Effects of Antiepileptic Drugs in Adult Epileptic Patients: An Open Label, Non Randomised Interventional Study

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ABSTRACT

Introduction: Oxidative stress is one of the factors implicated in the pathogenesis of epilepsy. Various antiepileptic drugs can control seizures by several mechanisms that may involve reduction of oxidative stress. Only a few studies have evaluated the antioxidant effects of Antiepileptic Drugs (AEDs).

Aim: To evaluate the antioxidant activity of the AEDs (Phenytoin, Levetiracetam, Oxcarbazepine) in adult epileptic patients.

Materials and Methods: The present open label, non randomised interventional study was conducted at the tertiary care hospital, Hyderabad, Telangana, India, from September 2016 to November 2017. Total of 45 subjects were divided into three groups, Phenytoin group, Levetiracetam group and Oxcarbazepine group with 15 patients in each group. Blood samples were collected prior to initiation of treatment and after three months of treatment for measurement of antioxidative parameters such as Malondialdehyde (MDA), Nitric Oxide (NO) and reduced Glutathione (GSH) levels. Paired t-test was used for within group analysis.

Results: Amongst the total subjects of 45,(15 in each group), 14 subjects in phenytoin group, 12 in Levetiracetam group and 13 in oxcarbazepine group were finally analysed. The mean age was 28.92±9.62 years, mean weight was 55.87±10.73 kg and mean Body Mass Index (BMI) was 21.99±3.82 kg/m^2. No statistically significant difference was seen in all oxidative parameters in phenytoin treated group. In levetiracetam group, significant decrease in MDA levels (p-value- 0.0338) and increase in GSH levels (p-value <0.0001) was observed after three months of treatment. In oxcarbazepine group, statistically significant decrease was found in MDA (p-value-0.0055) and NO levels (p-value-0.016) and increase in GSH levels (p-value-0.0004) was observed.

Conclusion: It was concluded that phenytoin has no role in reducing oxidative stress in epileptic patients, whereas levetiracetam decreases lipid peroxidation and oxcarbazepine has beneficial role in reducing oxidative stress in adult epileptic patients.

Keywords: Levetiracetam, Malondialdehyde, Nitric oxide, Oxcarbazepine, Phenytoin, Reduced glutathione

INTRODUCTION

Epilepsy is one of the common chronic neurological disorders, afflicting nearly 50 million people worldwide and 80% of this population are living in low-and middle-income countries [1]. Approximately 10-12 million persons with epilepsy are residing in India, with more prevalence in rural areas compared to urban population [1]. According to International League Against Epilepsy (ILAE), epilepsy is defined as, "Atleast two unprovoked (or reflex) seizures occurring >24 hours apart; one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (atleast 60%) after two unprovoked seizures, occurring over the next 10 years; and diagnosis of an epilepsy syndrome" [2]. Several pathophysiological processes or mechanisms that lead to the occurrence of first spontaneous seizure and subsequent epilepsy events have been proposed. The mechanisms that contribute to epileptogenesis are neurotransmission signalling pathway abnormality, imbalance in ion charges due to channelopathies, aberrant hippocampal neurogenesis and several others [3]. Current AEDs aims to modulate one of these pathological processes [3]. Thorough understanding of the pathogenesis forms the basis for development of new therapies that aim to prevent or halt the progression of epileptogenesis.

Various studies suggest the role of oxidative stress in the pathophysiology of epilepsy [4,5]. Oxidative stress is an imbalance between formation and removal of Reactive Oxygen Species (ROS) in favour of the former. Brain is particularly vulnerable to oxidative stress because it has large quantity of mitochondria, consumes highest amount of oxygen and it has limited antioxidant system. Brain is rich in polyunsaturated fatty acids which are targets for ROS, and also rich in iron which catalyses ROS formation. Catalase, which is an enzymatic antioxidant defence system, is quite low in brain compared to other organs [6].

Oxidative stress and formation of ROS leads to abnormal structural alteration in biological macromolecules namely cellular proteins, membrane lipids, carbohydrates and nucleic acids [7]. Alteration in cellular structure and functions of biomolecules, consequently lead to cellular death. Several animal models of epilepsy have demonstrated increased ROS production in epilepsy leading to mitochondrial dysfunction and apoptosis in neurons mainly in hippocampal region of brain [8,9]. Many studies have confirmed increased oxidative stress in untreated patients with epilepsy in terms of increased lipid peroxidation reflected by elevated MDA levels and increased NO levels and reduced GSH activity. Furthermore, it was reported that exogenous administration of antioxidants significantly reduced seizures [10,11].

Regardless of abundance of drugs for epilepsy, 20-30% patients still continue to have seizures [10]. Antioxidant activity of phenytoin has been evaluated in several animal models but the reports were inconclusive [12,13]. Oliveira AA et al., demonstrated that levetiracetam pre-treatment in pilocarpine induced seizures significantly decreased lipid peroxidation and nitrite-nitrate levels and prevented the loss of GSH in hippocampal region of mice brain and it was postulated that levetiracetam ability to block the calcium influx into cells might contributing to its activity against oxidative stress [14]. In children with idiopathic epilepsy oxcarbazepine treatment resulted in decrease in MDA levels after three and six months of treatment and reduced NO levels after three months of treatment [15].

Limited number of studies have evaluated antioxidant effects of AEDs [14,15]. But most of these studies were done in animal models and children with epilepsy using NO, lipid peroxidation and xanthine oxidase system parameters for evaluating antioxidant status [14,15]. Some of these studies [13] concluded increase in oxidative stress, while others [15] decrease in oxidative stress. Hence the present study was aimed at evaluating the antioxidant activity of the AEDs (phenytoin, levetiracetam, oxcarbazepine) in adult epileptic patients.

MATERIALS AND METHODS

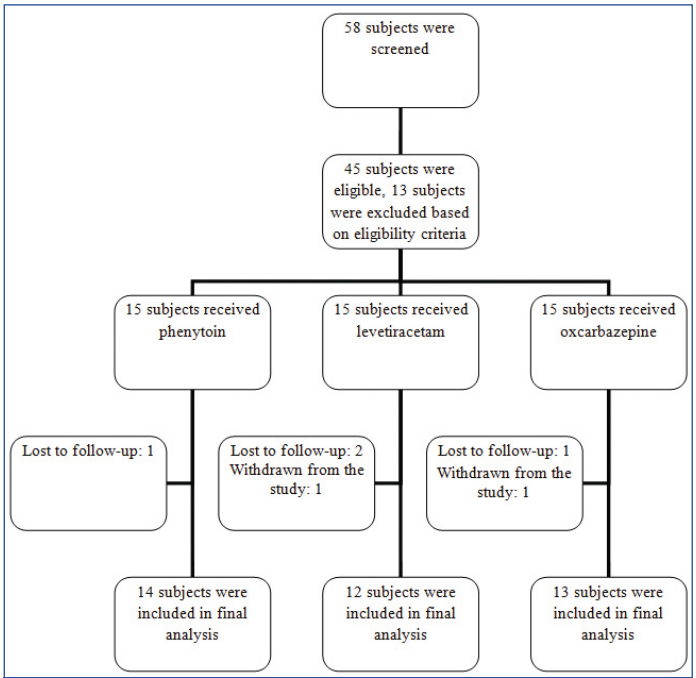
The present open label, non randomised interventional study was conducted at the tertiary care hospital, Hyderabad, Telangana, India, from September 2016 to November 2017. The study was registered with clinical trial registry-India (CTRI/2017/12/010840). Approval of Institutional Ethics Committee (IEC) (EC/NIMS/1792/2016) was taken prior to initiation of the study. Written informed consent was obtained from all the eligible patients prior to the study.

Inclusion criteria: Newly diagnosed drug naive epileptic patients aged between 18-60 years of either gender, who were initiated on monotherapy with phenytoin or levetiracetam or oxcarbazepine, and willing to give informed consent were included in the study.

Exclusion criteria: Patients with history of psychiatric or progressive neurological disorders, other co-morbid conditions like ischaemic heart disease, kidney disease, liver disease, thyroid disorders and other endocrinopathies, history of diseases that could influence the level of oxidative stress, such as diabetes mellitus, arterial hypertension, malignancies, recent surgery and trauma, and patients on drugs that influence the level of oxidative stress such as vitamins, herbal medicines for the last three months, pregnant and lactating women were excluded from the study.

Classification of seizures was based on International League Against Epilepsy (ILAE) 2017 classification of seizure types [16], it includes focal onset with impaired awareness, focal onset with awareness, generalised onset, combined generalised and focal epilepsy, and unknown category. Temporal lobe epilepsy is the most common type of focal onset seizures.

Sample size estimation: A total sample size of 45 was required based on the study by Arhan E et al., [15], with predicted decrease in MDA levels after three months of treatment for oxcarbazepine compared to baseline of 0.7 nmol/mL with Standard Deviation (SD) of 0.80 nmol/ml with 85% power, type 1α error of 5% and dropout rate of 20% [Table/Fig-1].



[Table/Fig-1]: Flowchart of study protocol.

Procedure

Patient demographic details, present history, drug history were noted in case record form. Ten mL of venous blood was collected from each subject prior to initiation of treatment for baseline antioxidant status. All the subjects were divided into three groups:

Phenytoin group: 100 mg tablets or capsules were prescribed, orally, one tablet or capsule in the morning and two tablets or capsules in the evening for three months.

Levetiracetam group: 500 mg tablets, orally, twice daily for three months.

Oxcarbazepine group: 300 mg tablets, orally, twice daily for three months.

Patients were started on AEDs treatment according to physician discretion. After three months of treatment, oxidative parameters and adverse drug reactions were assessed and noted in case record form.

Ten ml of venous blood was collected for antioxidant assessment after three months of treatment. Blood samples were obtained in the interictal period. Oxidative stress was assessed by estimation of MDA, NO and GSH levels using ultraviolet visible spectrophotometer (UV-1601, Schimadzu) (Ellman GL. 1959). MDA, the secondary product of lipid peroxidation, was estimated in the serum samples by thiobarbituric acid reactive acid substance test [17]. NO level (µM/L) was estimated by a test that involves reduction of nitrate by vanadium (III) and detection with Griess reagents [18]. GSH was estimated by 5, 5-Dithiobis 2-Nitrobenzoic acid (DTNB) method [19].

STATISTICAL ANALYSIS

Data were statistically analysed using GraphPad Prism 7. Data was expressed as mean±standard deviation. Paired t-test was used for within group analysis. One-way Analysis of Variance (ANOVA) followed by post-hoc Tukey's test was performed for between group comparisons. The p-value <0.05 was considered to be statistically significant.

RESULTS

A total of 58 subjects were screened, 45 subjects were enrolled and remaining 13 subjects were excluded based on the exclusion criteria. Out of 45 subjects, four subjects were lost to follow-up and two withdrew from the study. Total 39 subjects were included in final analysis. The mean age was 28.92±9.62 years. The mean weight was 55.87±10.73 kg. The mean BMI was 21.99±3.82 kg/m^2. Among the 39 subjects, 14 were in Phenytoin group, 12 were in Levetiracetam group and 13 were in Oxcarbazepine group. Seizure frequency was assessed before treatment in all the groups. Among the total subjects <3 seizures were observed in 25 subjects, 3-5 seizures in 10 subjects and >5 seizures were observed in four subjects [Table/Fig-2].

Characteristic		Phenytoin group (n=14)	Levetiracetam group (n=12)	Oxcarbazepine group (n=13)	p-value
Age (years)		29.57±10.26	27.75±9.57	29.31±9.65	0.88
Gender (M/F)		6/8	5/7	8/5	0.53
Weight (Kg)		53.57±12.13	55.75±7.63	58.46±11.78	0.51
BMI (Kg/m^2)		21.64±4.28	21.79±2.91	22.58±4.25	0.80
Number of patients with seizure frequency	<3	8	9	8	0.786
	3-5	5	2	3	
	>5	1	1	2	

[Table/Fig-2]: Demographic characteristics of three groups at baseline.
Analysis of variance; p-value <0.05 was considered to be significant; BMI: Body mass index

The types of seizures observed in total number of subjects were generalised tonic clonic seizures in 29 (74.36%) subjects, focal onset with impaired awareness in 6 (15.39%) subjects, focal onset

with awareness in 1 (2.56%) subject and temporal lobe epilepsy in 3 (7.69%) subjects.

All oxidative parameters before treatment in all the three groups were similar and there was no statistically significant difference observed. The oxidative parameters in three groups before treatment and after three months of treatment are shown in [Table/Fig-3,4]. No statistically significant difference was observed for all the oxidative parameters after three months among all the three groups.

Oxidative parameter (before treatment)	Phenytoin group (n=14)	Levetiracetam group (n=12)	Oxcarbazepine group (n=13)	p-value
MDA (nmol/mL)	4.2±1.21	4.51±1.61	4.51±1.46	0.8072
NO (µmol/L)	52.28±14.78	49.24±13.03	52.23±13.78	0.8241
GSH (umol/L)	531.79±63.03	447.52±105.1	491.21±97.97	0.07

[Table/Fig-3]: Oxidative parameters before treatment in all the three groups.
Analysis of Variance; p-value <0.05 was considered to be significant; MDA: Malondialdehyde; NO: Nitric oxide; GSH: Glutathione

Oxidative parameter (after three months of treatment)	Phenytoin group (n=14)	Levetiracetam group (n=12)	Oxcarbazepine group (n=13)	p-value
MDA	4.37±1.23	4.02±1.34	3.87±1.26	0.5745
NO	53.23±15.67	42.37±12.11	42.72±9.21	0.0542
GSH	555.66±80.3	522.96±121.2	550.02±100.73	0.6886

[Table/Fig-4]: Oxidative parameters after three months of treatment in all the three groups.
Analysis of variance; p-value <0.05 was considered to be significant

In phenytoin group, there was no statistically significant difference seen after three months of treatment when compared to before treatment values in all oxidative stress parameters. Oxidative parameters before treatment and after three months of treatment in phenytoin group are shown in [Table/Fig-5].

Oxidative parameter	Phenytoin group		
	Before treatment	After 3 months of treatment	p-value
MDA	4.2±1.21	4.37±1.23	0.0758
NO	52.28±14.78	53.23±15.67	0.7484
GSH	531.79±63.03	555.66±80.3	0.1846

[Table/Fig-5]: Oxidative parameters in phenytoin group.
Paired t-test; p-value <0.05 was considered to be significant

In levetiracetam group, statistically significant decrease (p=0.0338) in MDA levels and significant increase (p<0.0001) in GSH levels was observed after three months of treatment compared to pretreatment levels. There was no statistically significant difference seen in NO levels after three months of treatment when compared to before treatment [Table/Fig-6].

Oxidative parameter	Levetiracetam group		
	Before treatment	After 3 months of treatment	p-value
MDA	4.51±1.61	4.02±1.34	**0.0338**
NO	49.24±13.03	42.37±12.11	0.0791
GSH	447.52±105.1	522.96±121.2	**<0.0001**

[Table/Fig-6]: Oxidative parameters in levetiracetam group.
Paired t-test; p-value <0.05 was considered to be significant; bold p-values are significant

In oxcarbazepine group, there was statistically significant decrease found in MDA (p<0.01) and NO (p=0.016) levels and significant increase (p<0.001) in GSH levels after three months of treatment compared to pretreatment. Oxidative parameters before treatment and after three months of treatment in oxcarbazepine group are shown in [Table/Fig-7].

Among the three groups, number of subjects with seizure free during three months of treatment was 7 (50%) in phenytoin group, 7 (58.33%) in levetiracetam group and 9 (69.23%) in oxcarbazepine group. Out of 13 subjects in oxcarbazepine group, weight gain was observed in 8 (61.54%) subjects. In levetiracetam group, withdrawal seizures were observed in 2 (16.67%) subjects, and ataxia in one (7.14%) subject in phenytoin group.

Oxidative parameter	Oxcarbazepine group		
	Before treatment	After 3 months of treatment	p-value
MDA	4.51±1.46	3.87±1.26	**0.0055**
NO	52.23±13.78	42.72±9.21	**0.016**
GSH	491.21±97.97	550.02±100.73	**0.0004**

[Table/Fig-7]: Oxidative parameters in oxcarbazepine group.
Paired t-test; p-value <0.05 was considered to be significant

DISCUSSION

Oxidative stress has been implicated as one of the several pathophysiological mechanisms causing epilepsy [4,5]. Brain is particularly susceptible to lipid peroxidation because it is rich in polyunsaturated fatty acids and mitochondria. ROS have short life span and it is difficult to measure them in-vivo [20]. So lipid, protein and DNA peroxidation products are used as indicators of oxidative stress in experimental and clinical studies [4,5]. In the present study, NO level was towards upper limit, whereas GSH level was towards lower limit of normal level at pretreatment in all the three groups. There was no significant difference in MDA, NO and GSH levels in three groups before treatment. Many authors studied [13,21,22] the effect of phenytoin on oxidative parameters in experimental animals and in epileptic patients. Present study showed no significant difference in MDA, NO and GSH levels after three months of treatment compared to pretreatment levels in adult epileptic patients treated with phenytoin, but MDA, NO and GSH levels were increased compared to pre-treatment levels.

Present study results were in accordance with a study done by Menon B et al., they evaluated the oxidative parameters in 100 patients with epilepsy and 100 healthy controls. Among the epileptic patients, 66% were on monotherapy, out of which 20% were on phenytoin [21]. In this study, there was no significant difference in MDA and NO levels in patients with epilepsy who were on treatment versus who were not on treatment. Present study findings varied from studies done in epileptic patients. A study done by Liu CS et al., in which 20 female epileptics treated with phenytoin monotherapy, there was significant increase in MDA and significant decrease in GSH levels when compared to 12 female epileptics without anticonvulsant therapy and healthy controls [13]. In another study done by the Liu CS et al., MDA concentrations were elevated and GSH concentrations decreased in 30 epileptic patients treated with phenytoin monotherapy compared to 35 healthy controls [22].

Ono H et al., enrolled 45 epileptic patients and 53 healthy controls without seizures into the study [23]. In this study, they reported that plasma GSH concentrations were significantly lower in epileptic patients treated with carbamazepine or phenytoin monotherapy when compared to healthy controls. Mahle C and Dasgupta A, showed significantly increased concentrations of lipid hydroperoxide and decreased antioxidant capacity in serum samples of phenytoin treated patients when compared to sera of control samples [24]. Very few studies [25-27] evaluated the effect of levetiracetam on oxidative stress in epileptic patients. Present study showed significant decrease in MDA levels and significant increase in GSH levels, but there was no significant difference in NO levels after three months of treatment compared to pretreatment levels. The present study results were in concordance with the two studies conducted by Oliveira AA et al., [14,25] in mice. As previously stated, Oliveira AA et al., [14] evaluated the effect of levetiracetam on lipid peroxidation level, nitrite-nitrate formation and antioxidant enzymatic activity in mice brain after pilocarpine induced seizures. They found that administration of pilocarpine alone produced significant increase in lipid peroxidation level,

nitrite and nitrate formation, catalase activity and decrease in GSH levels. But administration of levetiracetam 60 minutes before pilocarpine counteracted alterations in these levels, preserving the levels in normal range. In another study done by Oliveira ADA et al., [25] it was demonstrated that the effect of levetiracetam on mice brain homogenates after in-vitro induced oxidative stress. The heating-induced oxidative stress showed an increase in lipid peroxidation, nitrite-nitrate content, and catalase activity in mice brain homogenates. Previous incubation with levetiracetam reduced the lipid peroxidation, nitrite-nitrate contents and catalase activity, and increased the GSH levels.

In contrast to these findings Sarangi SC et al., [26] reported a marked increase in MDA levels and decrease in GSH levels in levetiracetam group compared to control group in male wistar rats. The results of the study done by Varoglu AO et al., in epileptic patients were in contrast to present study findings. They studied the effect of valproate, carbamazepine and levetiracetam on antioxidant and oxidant systems in epileptic patients. They observed higher serum 8-hydroxyguanine (8-OHG) level in the patients taking antiepileptic drugs for the first two months than in the controls [27]. In another study done in epileptic patients by Ozden H et al., they determined the urinary 15f-2t-isoprostane levels, which is a marker of oxidative stress. After three months of treatment with levetiracetam, urinary 15f-2t-isoprostane levels were elevated compared to pre-treatment levels indicating increase in oxidative stress [28].

The present study showed significantly low levels of MDA and high levels of GSH, but no significant change in NO after three months of treatment compared to initial values, suggesting beneficial role in lipid peroxidation and antioxidant enzyme system. In present study, significant decrease in MDA and NO levels and significant increase in GSH levels after three months of treatment compared to pre-treatment levels were observed. Present study results were consistent with a study done by Arhan E et al., [15]. In their study among 49 epileptic children 21, were treated with oxcarbazepine. They observed significant decrease in MDA levels after third and six months compared to pre-treatment values. They also found significant decrease in NO levels after three months however the change was insignificant after six months compared to pre-treatment values.

In contrast to present study, oxcarbazepine effect on MDA level was not significant in the study done by Bolayir E et al., they studied the effect of oxcarbazepine on MDA, catalase, glutathione peroxidase and on superoxide dismutase in epileptic patients. There was no significant difference in MDA and catalase levels after one year of treatment compared to pre-treatment values. But a significant difference in GSH peroxidase and superoxide dismutase activities after one year of treatment compared to pre-treatment values [29]. The above study results were in contrast to the present study, the reason could be owing to the shorter duration of present study. Further randomised double blind investigations with long-term use of AEDs are required.

Limitation(s)

Study limitations were that there was no healthy control group, small sample size and study duration was shorter, thus to confirm the results, more advanced prospective long-term clinical studies are warranted.

CONCLUSION(S)

Phenytoin has no role in reducing oxidative stress in epileptic patients, whereas levetiracetam decreases lipid peroxidation. Oxcarbazepine has beneficial role in reducing oxidative stress in adult epileptic patients, because there was reduction in MDA, NO and increase in GSH levels compared to initial values after three months. But thorough analysis will be needed to deduce whether oxcarbazepine has truly favourable action in oxidative stress and whether it is contributing to its antiseizure activity in these patients.

Author's contribution: Dr. D. Aruna has finalised the draft and guarantor, Dr. B. Swathi has prepared the conceptual framework, designing of draft, and data analysis.

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