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Impact of Oxidative Stress and Antiepileptic Drug Treatment on Oxidative Markers in Children with Newly Diagnosed Idiopathic Epilepsy: A Comparative Study of Valproate and Oxcarbazepine

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ABSTRACT

This study investigates the role of oxidative stress in newly diagnosed idiopathic epilepsy and the effects of antiepileptic drugs (AEDs), specifically valproate (VPA) and oxcarbazepine (OXC), on oxidative stress markers, including nitric oxide (NO), xanthine oxidase (XO), and malondialdehyde (MDA). Seventy-five children (32 girls and 43 boys, aged 5-12 years) with newly diagnosed epilepsy and 15 healthy controls were included. The patients were divided into three treatment groups: 26 treated with VPA, 26 treated with OXC, and 23 treated with other AEDs. Blood samples were collected before treatment and at 3 and 6 months after treatment to measure NO, XO, and MDA levels. At baseline, NO concentrations were significantly higher in epileptic children compared to controls ($p = 0.002$). No significant differences were observed in XO or MDA levels between groups at baseline ($p = 0.231$ and $p = 0.636$, respectively). After 3 months, MDA levels decreased significantly in both the VPA and OXC groups, with continued reduction at 6 months. In contrast, MDA levels remained unchanged in the control group. NO levels also decreased significantly in both VPA and OXC groups at 6 months. High XO concentrations were observed in both medication groups over the study period. In conclusion, this study highlights elevated oxidative stress in children with idiopathic epilepsy and suggests that both VPA and OXC reduce oxidative stress markers, with OXC showing a greater reduction in MDA. Further studies with larger sample sizes and placebo controls are needed to confirm the antioxidant effects of OXC in pediatric epilepsy.

Keywords: *Oxidative stress, Epilepsy, Nitric oxide (NO), Xanthine oxidase (XO), Malondialdehyde (MDA), Valproate (VPA), Oxcarbazepine (OXC), Antiepileptic drugs (AEDs), Pediatric epilepsy*

1. INTRODUCTION

Oxidative stress is the imbalance between the supply of the reactive oxygen species (ROS) and the antioxidant defense mechanism of the body. The situation is when there is excess-accumulation of ROS or insufficient antioxidants. Oxidative stress has been suggested to contribute to development of diverse disease processes [1].

There is emerging evidence that oxidative stress contributes to epilepsy due to aberrant modifications of cellular proteins, membrane lipids, DNA and RNA [24] [3] [4]. Different sources that yield ROS using molecular oxygen are mitochondrial and xanthine oxidation (XO) system. A key free radical in cells is the superoxide anion radical (O2(-)), which can be generated in larger quantities by the proteolytic oxidation of xanthine dehydrogenase to XO. Also, oxygen radicals have the potential to destroy several cell components and eventually can result in cell death. Free radicals have also the ability to damage lipids especially, malondialdehyde (MDA), which is formed by lipid peroxidation, can exert local and distant, deleterious consequences.

Nitric oxide (NO) is a gas, small, diffusible that can be produced by converting the amino acid l -arginine using nitric oxide synthase as an enzyme. As a neurotransmitter, NO is critical to various physiological and pathophysiological processes within the brain, most notably as the controller of neural plasticity, cerebral blood flow, cognitive and behavioral functions, and participation in neural disorders like ischemic and epileptic injury [5,6]. During endothelial injury, synthesis of NO seems to be compromised. One of the prerequisites of the development of many diseases is considered to be endothelial damage. In a number of in vivo and in vitro studies the role of NO has been investigated in epilepsy but the findings are nonsuable with pro-convulsant as well as anti-convulsant effects of NO [7].

The majority of experiments on lipid peroxidation and NO, and antioxidant status have been done in children undergoing antiepileptic drug treatment. Little research has been done in clinical exploration of the oxidative stress in epileptic children who have just been identified and who are yet to undergo treatment using drugs. A subset of these studies has reported increased lipid peroxidation and another subset reported no alterations in oxidative markers with newly enrolled epileptic patients [8-10].

Recent reports have indicated that most drugs used as antiepileptics possess dissimilar actions against the oxidative stress [11-13]. Newer antiepileptic drugs are believed to exert a less negative effect on oxidative-antioxidative balance, yet there is little support of this belief [14]. The study of antiepileptic drugs (AD) with the oxidative stress has started with valproate (VPA), and many studies have been carried out on the topic of VPA effects [8-12,15]. Oxcarbazepine (OXC), an antiepileptic agent related to carbamazepine, has a similar structure as carbamazepine but has several advantages, such as improved tolerability [17,18], decreased risk of allergic skin reactions, less drug interaction, and improved metabolism. These advantages have to deal with the fact that the oxidation of OXC in the liver is different to that of carbamazepine. Hence, OXC poses less risk of epilepsy management, and its side effects are lesser in comparison.

This paper has discussed the effects of epilepsy and antiepileptic drugs (VPA, an old drug and OXC, a new drug) on the presence of MDA, NO and XO in serum belonging to children with newly diagnosed idiopathic epilepsy. We assumed that, as an emerging drug, OXC could be effective in this patient population of children due to the likely occurrence of massive endothelial injuries and ROS oversupply.

2. MATERIALS AND METHODS

A total of 75 patients (32 girls and 43 boys) ages 5 and 12 years old with newly diagnosed idiopathic epilepsy and 15 healthy children of similar age and gender were used as controls. Of the 75 epileptics with newly diagnosed epilepsy, 24 were tonic, 13 were absence epileptics, 23 were secondary generalized and the remaining had simple partial, complex partial and atonic seizures 5, 7 and 3 respectively. Amongst these 75 patients, 26 patients received valproate, 26 patients received oxcarbazepine (OXC) and 23 other kinds of antiepileptic drugs. Individuals with some form of symptomatic or syndromic epilepsy, mental motor retardation, or underlying chronic illnesses were not studied. All the patients had not done any previous medication trials prior to initiating use of OXC and none of the patients were taking any other medications throughout the study period. Moreover, no acute medical illness that could include infections, physical efforts, and traumas was present during the blood collection.

Types of seizures were determined by the International League Against Epilepsy [19]. Ample dosage of antiepileptic drugs was chosen in accordance with the recommendations of the clinical protocols [20]. VPA and OXC were used twice per day. Seizure control was good in all patients and no more than two seizures in any patient were observed during the study period, one month following treatment initiation.

Blood samples were taken to determine nanograms of nitric oxide (NO), malondialdehyde, (MDA), and xanthine oxidase (XO) prior to the commencement of antiepileptic compound therapy. Blood serum samples were collected before the therapy and within three and six months after the start of applying antiepileptic treatment after providing the informed written consent. Liver funtion tests and complete blood count were done at the same time points. The samples of blood were obtained at the interictal stage. The serum level of fasting patients and control of the patients was analyzed in terms of values of NO, XO, and MDA. The concentration of NO (mu mol/mL) was assayed according to the procedure consisted in the diazotization of sulfanilic acid with a NO at acidic pH, the subsequent linking with N-(1-naphthyl-ethylenediamine) [21,22]. XO activity (IU/mL) was assessed by monitoring uric acid generation as the result of a xanthine substrate at 293 nm [21]. MDA (nmol/mL) was measured by assaying thiobarbituric acid reactive substances (TBARS) [23,24].

Analysis of statistics was obtained through Statistical Package for Social Sciences (SPSS), Version 13.0 (SPSS Inc., Chicago, IL). Results were presented as mean SD. The variance between groups was analyzed by the Tukey test and the significant differences were further tested by the Student t -test to compare the groups. Proportion was compared with chi-square test. Repeated measure analysis was used to evaluate the data obtained at the various times in each of the groups. A p-value of <0.05 was taken as statistically significant.

3. RESULTS

The demographic features of each group (control and the antiepileptic drug groups) are presented in Table 1. The age of the control group was 8.50 year meaning that the VPA treated groups were 9.10 year old, whereas the OXC treated group is 8.20 year old. The age distribution of the groups has not shown any statistical difference ($p = 0.56$). Sex distribution was also comparable, 7/8 males to females within the control group, 12/14 in the VPA group, 14/12 in the OXC group. p-value sex distribution = 0.74 which implies that the groups were not different.

Table 2 illustrates the correlation of nitric oxide (NO), xanthine oxidase (XOD) and malondialdehyde (MDA) in comparison to the control and new cases of epilepsy patient groups. NO concentrations were 10.56 2.91 10.3 12.34 % 20.47 10.3 12.34 uM in the control and newly diagnosed epilepsy groups, respectively, and the p-value was $p = 0.002$. This is sufficient

evidence to show the presence of a marked variation in the levels of NO between control and epilepsy groups. Nevertheless, there was no significant difference in the level of the enzyme XOD (p = 0.231) and production of MDA (p = 0.636) between the two groups.

Table 3 depicts the level of the NO, XOD, and MDA in the control, valproate (VPA), and OXC group before and after 3 and 6 months of treatment. The concentration of NO was comparable in VPA (n = 31.44 +/- 8.73 µmol/mL) and OXC (n = 31.44 +/- 8.73 µmol/mL) groups as compared to the control group (n = 11.83 +/- 3.23 µmol/mL) before the treatment. Following 3 months of treatment, the level of NO improved in all groups (28.93 +/- 13.06 uMol/ml in the control group, 32.83 +/- 19.76 uMol/ml in the VPA group, and 32.83 +/- 19.76 uMol/ml in the OXC group). The highest level of NO was seen in the control group (35.96 pmol/mL 11.98) compared to the reduction of NO levels both in the VPA and OXC group (19.16 pmol/mL 8.04).

In the XOD levels, the control group exhibited higher levels (113.00 32.18 IU/mL) than VPA (57.08 26.98 IU/mL) and OXC (57.08 26.98 IU/mL) groups prior to treatment. The complete positive results came after 3 months of treatment when the XOD levels of both VPA and OXCgroups showed an increase in a significant range to reach (167.00 +/-73.27 IU/mL). XOD levels in both groups were high after 6 months (159.15 41.01 IU/mL), XOD levels decreased in the control group (100.10 21.13 IU/mL).

Baseline VPA and OXC group MDA concentrations were significantly raised (2.66 V PA and OXC groups +/- 0.67 nmol/mL and the control group = 2.22 ± 0.46 nmol/mL). A 3-month treatment duration led to a reduction in MDA level in both the VPA and OXC groups to 1.91 +/- 0.77 nmol/mL, whereas no change was seen in the control group, 2.22 +/- 0.74 nmol/mL. The MDA concentration in both VPA and OXC groups further reduced at the sixth month to 1.21 +/- 0.22nmol/mL and 2.29 +/- 0.91 nmol/mL, respectively, compared to the control group, which increased to a small percentage (2.29 +/- 0.91 nmol/mL).

Table 1: Demographic features of the control and the antiepileptic drug-treated groups

Group	Control group (n: 15)	VPA treated group (n: 26)	OXC treated group (n: 26)	p-Value
Age (years)	8.50 ± 2.98	9.10 ± 2.42	8.20 ± 3.10	0.56
Sex (M/F)	7/8	12/14	14/12	0.74

Table 2: Correlation of the Levels of NO, XOD and MDA between Control and newly Diagnosed Epilepsy Groups

Groups	NO (µmol/mL)	XOD (IU/mL)	MDA (nmol/mL)
Control (n: 15)	10.56 ± 2.91	110.23 ± 28.74	2.10 ± 0.39
Newly diagnosed Epilepsy group (n: 75)	20.47 ± 12.34	119.56 ± 59.88	2.41 ± 0.72
p-Value	0.002	0.231	0.636

Table 3: Dose of NO, XO, and MDA in control, valproate, and OXC Groups Before and after 3- and 6-months treatment

Groups	Control (n: 15)	Valproate group (n: 26)	OXC group (n: 26)
	Before treatment (1)		
NO (µmol/mL)	11.83 ± 3.23	31.44 ± 8.73	31.44 ± 8.73
XO (IU/mL)	113.00 ± 32.18	57.08 ± 26.98	57.08 ± 26.98
MDA (nmol/mL)	2.22 ± 0.46	2.66 ± 0.67	2.66 ± 0.67
	Third month (2)		
NO (µmol/mL)	28.93 ± 13.06	32.83 ± 19.76	32.83 ± 19.76
XO (IU/mL)	96.41 ± 35.01	167.00 ± 73.27	167.00 ± 73.27
MDA (nmol/mL)	2.22 ± 0.74	1.91 ± 0.77	1.91 ± 0.77
	Sixth month (3)		

NO (µmol/mL)	35.96 ± 11.98	19.16 ± 8.04	19.16 ± 8.04
XO (IU/mL)	100.10 ± 21.13	159.15 ± 41.01	159.15 ± 41.01
MDA (nmol/mL)	2.29 ± 0.91	1.21 ± 0.22	1.21 ± 0.22

Figure 1: Demographic Features: Control vs Antiepileptic Drug Groups

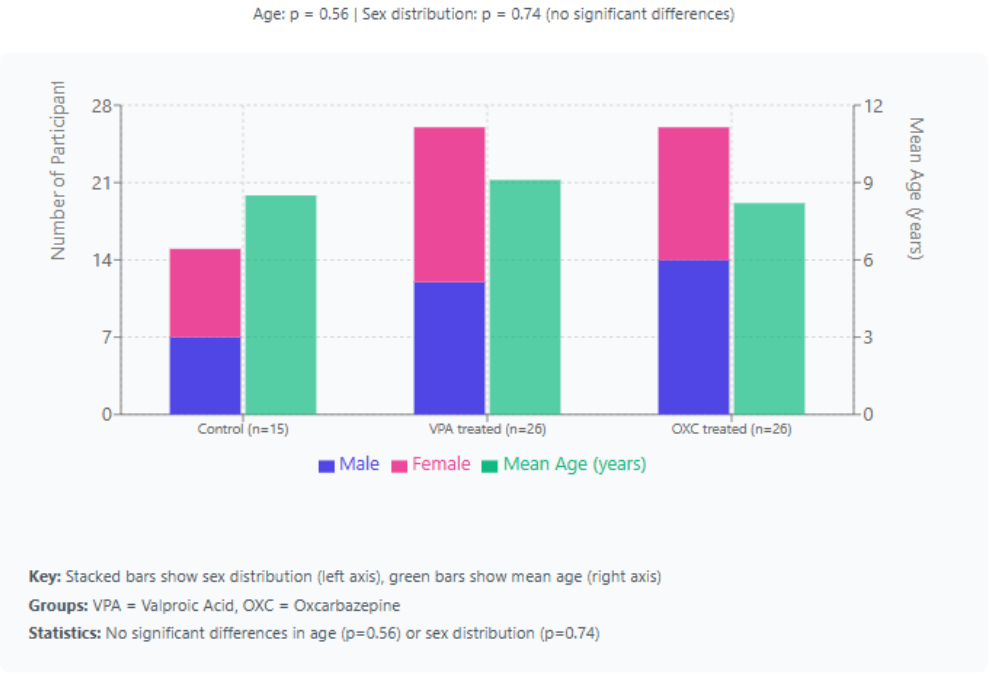


Figure 2: Biomarker Levels: Control vs Newly Diagnosed Epilepsy Groups

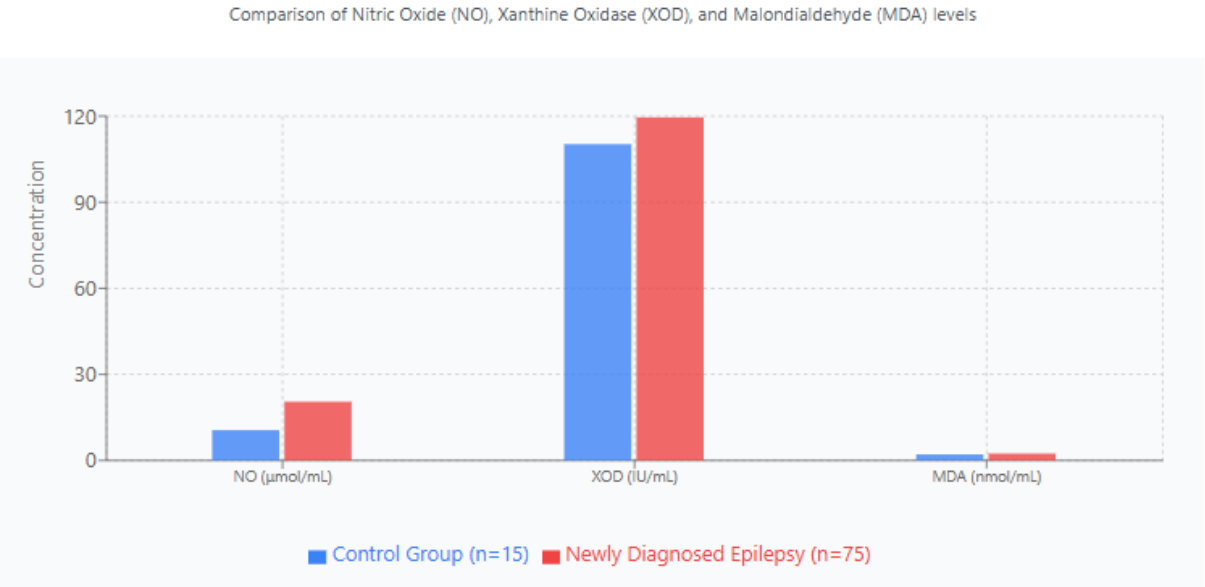
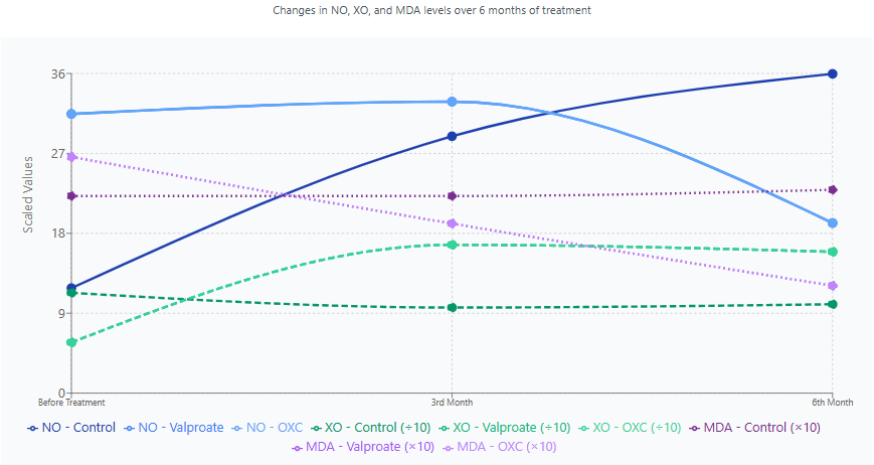


Figure 3: All Biomarkers Over Treatment Timeline



4. DISCUSSION

One of the central determinants of development of childhood epilepsy is oxidative stress. It is notoriously difficult to detect free radicals in vivo because they are highly transient, and lipid peroxidation markers are therefore widely employed as indices of oxidative stress in clinical and experimental studies. The brain, especially, is susceptible to damage by lipid peroxidation products than other tissues and lipid peroxidation is a temptation of neuronal injury to cell membrane phospholipids [25]. Most of the research on oxidative stress among children with epilepsy have been centered on effects of antiepileptic drugs (AEDs) on the oxidative markers, however, limited studies have been individually done to find out the direct effects of epilepsy on the oxidative status. Moreover, the influence of newer AEDs on the oxidative stress in epileptic children has been shown only in a few studies [14].

The impact of epilepsy on the level of nitric oxide (NO), xanthine oxidase (XO), and MDA

Our study was aimed at evaluating the effects of epilepsy on NO, XO and lipid peroxidation markers. We have seen that there was an immense rise in serum NO levels in epileptic children. Although it has been controversial what role of NO plays in pathophysiology of epilepsy, our results imply the possible roles of NO in neuronal loss and glial proliferation that might contribute to the pathogenesis of epilepsy. It has been shown in prior studies that it is possible to prevent seizures by inhibiting NO. Indicatively, Ribeiro et al. demonstrated that on seizure induction using methylmalonate, seizures were ameliorated in NO-deficient mice [27]. The significance of l-arginine and NG-nitro-l-arginine on seizure severity was also described; de Vasconcelos et al. indicated that NG-nitro-l-arginine augmented the severity of seizures whereas, administration of l-arginine ameliorated seizure-induced injuries [28]. Nonetheless there are also studies indicating that endogenous NO can in fact have neuroprotective effect, showing a complex interaction between NO and epilepsy [30]. The evidence provided by these contradictory studies indicates that additional research has to be conducted to define the exact role of NO in epilepsy.

There have been conflicting findings with respect to lipid peroxidation. There have been reports of elevated lipid peroxidation in brains of animals that experience recurrent seizures [32,33]. Conversely, other labelled works as ours revealed no significant difference in the lipid peroxidation of the epileptic and the healthy subjects. In the same manner, Michoulas et al. did not find any fluctuation in the degree of urinary oxidative stress measures (15-F2T-isoprostane) when comparing new epileptic individuals and healthy controls [9]. We are in agreement with Verrotti et al., in that they also did not find a difference in the oxidative markers between the epileptic patients and healthy controls [10]. Although some studies such as Yuksel et al. have reported elevated lipid peroxidation in epileptic children prior to treatment [noitem dont CV1625909299olv raIL dea sphere century hardly watched brick schooled ancestral fished jocular scattering chivalrous hasp lick pickle yodels teatimes anaesthetizing dazzles kingfishers conscribed snarled crackling imprinting magician jovial message cajoles icicles mischievaking

In our experiment, XO levels have not significantly differed between the control and epileptic children groups so that it can be implied that the detected changes in the lipid peroxidation could be associated with changes in antioxidant enzyme activity. XO is one of the primary sources of free radicals, and its tissue activation peak happens in the case of a significant proliferation of substrate following the degradation of adenine-nucleotides.

Influence of Antiepileptic Medications on the NO, XO and MDA Level

These lines of evidence have led to the speculation that AED therapy may enhance markers of oxidative stress [3638]. VPA has been the best studied AED in its effects on oxidative status on epileptic children. We have discovered that VPA did not have any significant effect on the oxidative status in epileptic children and this shows they agree with Verrotti et al., who reported that VPA therapy did not influence oxidative markers in epileptic children who were not obese at the time of

treatment [10]. Likewise, Sobaniec et al. did not find significant elevation of the MDA level in patients receiving VPA [11]. Conversely, Yuksel et al. and Cengiz et al., studies have found significantly higher concentrations of MDA in children with VPA dose and could be exposed to VPA-induced oxidative stress [8,12]. Some studies have postulated that VPA can cause oxidative stress especially concerning hepatotoxicity [15], however, our study indicates that there was no pathological level of oxidation of fats and NO levels in children undergoing VPA therapy. This means that even though VPA could increase the formation of oxidative markers, there is no compromise caused by VPA on the parameters of lipid peroxidation and the levels of NO during the treatment period.

Rapport angle point, concerning NO levels, we noticed no remarkable variations in children with VPA treatment. In-spite of the findings of others that have shown increased NO level in children using VPA, we could not confirm such a finding in our study. To illustrate, Peker et al. and Karabiber et al. observed elevated serum NO and nitrite + nitrate levels, respectively, in epileptic children with and without VPA treatment [45,41]. It should be mentioned, though, that their control groups were consisted of children previously treated with AEDs, in contrast to our healthy controls. On balance, our data indicate that VPA treatment is not associated with significant alterations in the levels of NO and/or markers of lipid peroxidation.

It is not clear how newer AEDs, including OXC, may impact oxidative stress, and only a few studies have examined it [14]. In our practice, within 3 months of administration of OXC, the level of MDA decreased significantly in children who were treated with OXC, which indicated a potentially beneficial impact of OXC on the level of lipid peroxidation. The decrease in the levels of MDA and NO was highly important after 6 months of treatment compared to the initial levels. These findings are favourable with regard to the potential antioxidant of the OXC effect in epileptic children even though our study design was not issued with placebo group, and thus does not permit a conclusive exclusion of epoxysuccinate effects. Our findings, despite the above mentioned limitations, are the first data to indicate that OXC might possess antioxidant effects in management of childhood epilepsy. Further research particularly large-scale prospective randomized controlled trial (including double-blind approaches) with extended follow-up is required to elucidate the possible benefit of antioxidant activity of OXC in pediatric epilepsy.

5. CONCLUSION

To conclude, the article under discussion emphasizes the important role of oxidative stress in children with newly detected idiopathic epilepsy when the level of nitric oxide (NO) is increased in patients. Nevertheless, there was no significant difference between epileptic children and healthy control markers of xanthine oxidase (XO) as well as malondialdehyde (MDA), supporting the utility that the very presence of epilepsy does not lead to significant changes in the lipid peroxidation state. Antiepileptic medication, namely valproate (VPA) and oxcarbazepine (OXC) were also tested on oxidative stress markers. Although VPA did not caused significant change in the levels of the oxidative markers, OXC treatment lowered the MDA level indicating a possible antioxidant effect.

The results of the present research provide evidence on the complexity of oxidative stress in epilepsy, and the divergent influence of antiepileptic drugs on oxidative status. Although OXC seems to be effective in decreasing markers of lipid peroxidation, the effect is inconclusive and requires quantitative studies with more sample sizes, placebo-controlled research, and extended observational time before antioxidant potential of OXC in pediatric epilepsy can be concluded. These findings add to an emerging body of literature which suggests that newer agents like OXC could provide benefit in the treatment of complications of oxidative stress in epileptic children.

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