

## Effect of Long Term Treatment with Antiepileptic Drugs on Oxidant Status, Zinc and Magnesium in Epileptic Patients

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**Abstract:** Epilepsy is one of the most common neurological disorders worldwide. Long term treatment of epilepsy is a fixed strategy with little interest given to the nutritional side effects of such drugs. Aiming to study effect of drugs on antioxidant enzymes, zinc and magnesium, patients were collected from neurology clinic in Kaser Alaini, Cairo medical collage. 30 patients having specific criteria, kept for 2 years on same antiepileptic drug and 11 matched controls with recent epilepsy before starting treatment with no antioxidant supplementation for 6 months in both groups. The treated patients were divided into three groups. The first group was treated with valporic acid, the second group was treated with carbamazepine and the third group was treated with both drugs. Serum catalase, glutathione reductase, total antioxidant capacity, glutathione S Transeferase, lipid peroxidase, nitric oxide, zinc, magnesium and liver functions were estimated. Results showed that patients treated with Valporic acid have significant ( $P \leq 0.01$ ) elevation of total antioxidant capacity, catalase and glutathione S Transeferase. Significant decrease in glutathione reductase ( $P \leq 0.001$ ), nitric oxide ( $P \leq 0.01$ ), lipid peroxidase ( $P \leq 0.05$ ) versus controls was observed. Patients treated with Carbamazepine group showed significant ( $P \leq 0.01$ ) elevation of glutathione S Transeferase and decreased lipid peroxidase ( $P \leq 0.05$ ) versus control group. Patients given both drugs showed significant ( $P \leq 0.01$ ) rise of catalase versus control, glutathione S Transeferase versus valporic treated group and significant decrease in lipid peroxidase ( $P \leq 0.01$ ) and nitric oxide ( $P \leq 0.05$ ) versus control. Zinc and magnesium levels showed no change between the groups and the control. Total bilirubin was significantly high in all groups. We concluded that antiepileptic dugs affect antioxidant status which needs more investigation.

**Key words:** Antiepileptic Drugs • Antioxidant Enzymes • Zinc • Magnesium

### INTRODUCTION

Epilepsy is one of the most common and heterogeneous neurological disorders, with an estimated prevalence of 40 to 50 million patients worldwide [1]. Epilepsy or epileptic condition is defined by a state of recurrent, spontaneous (Unprovoked) seizures, which can be convulsive or non-convulsive episodes [2-5]. It was suggested that oxidative damage and consequently neuronal cell death are common pathologic processes that can contribute to epileptogenesis [6], supported by the fact that surgical removal of a damaged hippocampus improves the condition of patients suffering epilepsy [7]. Excessive reactive oxygen species (ROS) production leads to increased intracellular concentration of  $Ca^{2+}$  ions which may induce neuronal necrosis and apoptosis [8].

Moreover,  $Ca^{2+}$  can activate nitric oxide synthetase (NOS) that generate nitric oxide which inhibits complex IV that consequently leads to ROS production. Among brain cells, neurons are particularly vulnerable to oxidative insults due to low levels of antioxidant enzymes, especially catalase (CAT) and glutathione peroxidase (GPX) and of non-enzymatic antioxidants, namely vitamin E and glutathione (GSH) [9]. Lipid peroxidation (LPx) disrupts biological membranes of the neuron and is thereby deleterious to their structure and function [10]. Masella *et al.* [11] mentioned the main protective roles of glutathione against oxidative stress. Glutathione (GSH) is a cofactor of several enzymes against oxidative stress which scavenges hydroxyl radical and singlet oxygen and is able to regenerate the most important antioxidants, back to their active form. Carr and Frei [12] and Kojo [13] stated

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that there are two forms of the enzyme glutathione peroxidase, one of which is selenium-independent glutathione-S-transferase (GST) while the other is selenium-dependent (GPx). GSTs are contributing to the transformation of therapeutic drugs and products of oxidative stress [11].

Catalase (CAT) very efficiently promotes the conversion of hydrogen peroxide to water and molecular oxygen. It can convert 6 million molecules of hydrogen peroxide to water and oxygen each minute [11].

Numerous studies in drug-naïve patients with epilepsy showed increased activities of super oxide dismutase (SOD) [14, 15], CAT [14, 16], decreased activities of GPX [14, 17] and glutathione reductase (GR) [18]. Other studies reported higher level of lipid peroxidase (LP) in patients with epilepsy compared to controls and increased markers of lipid peroxidation [14, 16-21]. On the other hand, there are also studies, with very weak (Unchanged SOD, CAT, GPX and GR activities) [17, 18, 21-23] or even opposite decreased lipid peroxidation markers [24].

Long-term use of certain antiepileptic drugs (AEDs) has been proposed to increase free radical formation and cause oxidative damage in neurons [22, 25-27]. The role of valproic acid (VPA) in exacerbation of oxidative stress is supported by reduced total antioxidant capacity (TAC) [30, 31] and enhanced total oxidative status (TOS) [31]. Reports for patients treated with VPA, erythrocyte GPX [26, 32], GR [33] and serum Se [34], uric acid and albumin [19], were found to be reduced in children and adults treated with VPA. Furthermore, increased levels of markers of enhanced lipid peroxidation, such as MDA [15, 26, 30, 31] and other markers [31, 35, 36] were reported. The role of carbamazepine (CBZ) in generating free radicals and consequently causing neuronal damage is not so evident. CBZ enhanced plasma LP in adolescent and adult patients [30] and in children [19]. Lower GSH [37] in plasma and GPX and CAT in erythrocytes were also observed [23]. In accordance with its pro-oxidative action, decreased SOD [32] and increased nitrite/nitrate in erythrocytes were also observed. [22, 26, 30, 35]. However, there are also studies with anti-oxidative action [33, 38, 39]. On the other hand VPA was shown to be protective against oxidative stress in both, *In vitro* and *in vivo* models of epilepsy. It was suggested that VPA increases levels of glutathione and studied confirmed the antioxidant effect of VAP [40, 41]. Thus, AEDs have been shown to contribute to both pro- and anti-oxidant effect. The objective of this study was to point out the effect of long term administration of anti epileptic drugs on

antioxidant enzymes status and minerals; raising the question that is supplementation with antioxidants and minerals (Either in diet or as supplement) are needed with antiepileptic drug treatment to decrease their effects.

## MATERIALS AND METHODS

Patients were collected from Kaser Alaini neurology clinic in medical collage, Cairo University. Patients who have been chosen in this study were having long term affection with grand mal epilepsy, kept for  $\geq 2$  years on the same antiepileptic drugs (AED) as treatment and were free of seizures for at least 6 months. 30 patients were enrolled in the study. Other 11 patients matched as control with recently diagnosed epilepsy and have not yet started any treatment. Mean age of examined patients was  $(19\pm 3.5)$  for 13 females, 17 male while in control group was  $19.3\pm 3.7$  for 6 males and 5 females. Both groups have no regular supplementation of pharmacological antioxidant for 6 months.

The thirty treated patients were classified into three groups, the first group (9 cases) were treated with valproic acid (VAP), the second group (11 cases) were treated with carbamazepine (CBZ) and the third group (10 cases) were treated with both (V+C) for at least two years. Blood samples were collected, the harvested serum was kept frozen under  $-20^{\circ}\text{C}$  for estimation of antioxidant enzymes activity using calorimetric methods Total antioxidant capacity (TAC) [42] catalase (CAT) [43], glutathione reductase (GR) [44], glutathione S Transferase (GST) [45] as well as lipid peroxidase (Malondialdehyde, LP) [46], nitric oxide (NO) [47], Serum Zinc [48] and magnesium [49], total bilirubin [50] serum glutamic oxaloacetic transeferase (SGOT) and serum glutamic pyrovic transeferase (SGPT) [51]. Results were presented as mean and standard deviation. Student T test was used for statistical evaluation.

## RESULTS

Results in Table (1) showed the following: Total antioxidant capacity is significantly higher in patients treated with VAP group versus controls ( $2.1\pm 0.2$  mM/l versus  $1.6\pm 0.3$ ,  $p\leq 0.01$ ), other groups showed no significant difference ( $1.7\pm 0.3$  in CBZ,  $1.8\pm 0.3$  in V+B). displays that GR showed significant decrease in VAP group versus control ( $22\pm 7$  U/L versus  $43\pm 14$  U/L ( $p\leq 0.001$ ) while insignificant decrease was noted in double drug therapy ( $32\pm 11$  U/L) and CBZ group ( $42\pm 19$  U/L) versus controls, CAT was significantly

Table 1: Serum level of antioxidant enzymes, Zinc, Mg, total billirubin, SGOT and SGPT in different drug treatment groups

	VAP	CBZ	V+B	Control
TAO mM/l	2.1±.2 □	1.7±.3	1.8±.3	1.6±.3
GR u/l	22±7 □□	42± 19	32± 11	43±14
CAT u/l	678±155 □	550±200	682±154 □	524±76
GST u/l	1338±306 □	1254±284 □□	936±214	781±226
LP nmol/ml	132±12 □□□	120±30 □□□□	121±17 □	152±25
NO μmol/L	60±34 □□	93±29	77±16 □□□	113±43
Zinc μg/dL	156 ± 16	126 ± 20	136 ± 18	155 ± 11
Mg mg/dl	1.6 ± 0.4	2.2 ± 0.2	2.1 ± 0.4	2 ± 0.5
T.B μmol/L	1.55±0 .03 □ □	1.07± 0.3 □□	1.2± 0.45 □□	0.04±0 .004
SGOT U/L	26± 13	25± 11	33± 19	28± 9
SGPT U/L	15± 1.5	13± 1	15± 4	16± 4

P ≤ 0.01 □ P ≤ 0.001 □□ P ≤ 0.05 □□□

(p ≤ 0.01) high in VAP and V+C treated groups than no (678±155, 682±154 versus 524±76U/L) control difference was noted in CBZ group (551±200 U/L). While in GST level significant (P ≤ 0.01) elevation was noted in VAP (1338±307U/L) and CBZ group (1253±284U/L), non significant change in V+B group (936±214U/L) versus control (781±226 U/L) and significant change was noted between VAP drug treatment and double drug treatment (P ≤ 0.01). LP decreased significantly by treatment in mono drug groups (P ≤ 0.05), while highly significant (P ≤ 0.01) decrease was noted in double drug group compared to untreated controls (132±12, 120±30, 121±17 versus 152±25 nmol/ml in controls) NO level decreased significantly by treatment with VAP, V+C (60±3.4 μmol/L, p ≤ 0.01 and 77±16, P ≤ 0.05) respectively, insignificant decrease in CBZ (93±29) versus 113±42 μmol/L in controls.

Liver function tests showed highly significant elevation of total billirubin (P ≤ 0.001) in all groups with highest with VAP group (1.55±0.03, 1.07±0.3, 1.2±0.45 μmol/L versus 0.04±0.004 μmol/L in controls) in VAP, CBZ and V+B respectively, while liver enzymes (SGOT & SGPT) showed no change from controls. SGOT was (26±13, 25±11, 33±19 versus 28±9 U/mL in controls) in VAP, CBZ and V+B respectively. SGPT was (15±1.5, 13±1, 15±4 versus 16±4 U/mL in controls) in VAP, CBZ and V+B, respectively. Zinc and magnesium levels showed no significant change between the treated and non treated patients; zinc levels (156±16, 126±20, 136±18 μg/dL versus 155±11 μg/dL in control) in VAP, CBZ and V+B groups, respectively. Mg levels was (1.6±0.4, 2.2±0.2, 2.1±0.4 versus 2±0.5 mg/dl in controls) in VAP, CBZ and V+B groups respectively (Table 1).

## DISCUSSION

Long term treatment of epilepsy is a fixed strategy in controlling seizures and its frequency, while this is implemented, little interest is given to the nutritional side

effects of such drugs such as Valproic acid which is intensively used in young patients and may cause increased excretion rates of many nutrients. This can especially be a major concern in pregnant women where the risk of drug-induced significant birth defects is present. These drugs nutritional deleterious side effects might be controlled with balanced diet and good amount of food and supplement rich in Antioxidant as well as minerals to those patients. Baggot *et al.* [52] have reported that during multivitamin supplementation, many previously increased excretion rate of such nutrients, decreased significantly and fetal head growth often improved. Individuals taking valporic acid might benefit from supplementation with a multivitamin formulation. Individuals taking valproic acid should consult their prescribing physician and/or a nutritionally trained healthcare professional about the potential use of a multivitamin supplement to counter the drug's adverse effects. Some nutrients affect drug toxicity as Antioxidants, especially Vitamin E (Alpha-tocopherol) and Selenium. Buchi *et al.* [53] found that the free-radical scavenging action of alpha-tocopherol (Vitamin E) protects against lipid peroxidation and hepatotoxicity caused by valproic acid (VPA) in rats.

Graf *et al.* [34] in particular, reported that selenium and zinc concentrations are lower in serious adverse experience patients than in controls and concluded that selenium dependent antioxidant activity may play a special role protecting against adverse reactions.

Valproic acid may interact with carbamazepine, as valproates inhibit microsomal epoxide hydrolase (MEH), the enzyme responsible for the breakdown of carbamazepine-10,11 epoxide (The main active metabolite of carbamazepine) leaving it as inactive metabolite [54] prolonging the effects of carbamazepine and delaying its excretion. That is why we designed our study on cases treated with either valporic acid, carbamazepine or both in combination

AEDs may alter trace element metabolism and free radical scavenging enzyme activities in humans and experimental animals. However, the results of different studies are conflicting. This can be mainly explained by the differences in the materials and methods, most studies have not been prospective, patients receiving different AEDs were classified in the same group and the information about the patients included was not satisfactory in most of the studies [55, 56]. In addition, the duration of drug treatment and the prescribed dose are important variables.

Our results showed elevated TAC in patient treated with VAP than untreated controls but this was not true in CBZ and V+B groups. This result is different from some studies that mentioned decrease in TAC [31, 33, 38, 39], while Hamed *et al.* [30] observed marked lowering of TAC in untreated versus valporic treated patients which is the same in our study, While in other studies, VAP and CBZ decreased the oxidative stress. Płonka *et al.* [57] observed that the activity of some antioxidant enzymes are higher in patients treated with VPA for a longer period (7-14 years) in comparison to controls and concluded that valporic modulate activity of antioxidant enzymes. Valporic acid regulates antioxidant enzymes and prevents ischemia/reperfusion injury in the rat retina [58]. Atmaca [59] concluded that valporate has a growing evidence of its neuroprotective and neurotrophic actions in bipolar disorder treatment and the study done by Fourcade *et al.* [60] demonstrated the antioxidant effect of valporic acid in X-linked adrenoleukodystrophy. A 6-month pilot trial of VPA in those patients resulted in reversion of the oxidative damage of proteins in peripheral blood mononuclear cells. Decrease lipid peroxidation and antioxidant effect of valporic derivatives was confirmed by study done by Rekatias *et al.* [61].

Our study results showed that valporic acid may have antioxidant modulating activity that coincides with the previous studies [57-61].

CAT showed significant elevation in treatment with valporic acid and double therapy with V+C than untreated controls with insignificant elevation in CBZ group. Some studies showed similar observation [14, 16, 21] while others showed no change with treatment [17, 18, 23].

GR showed decrease in treated patients versus untreated ones, only significant decrease was noted in VAP group, this was the same in some studies [14,16, 21, 33] and contradictory to other studies that showed no change of GR [17,18, 23].

GST in this study showed significant elevation in VAP and CBZ than controls which was not significant in double drug treatment, this was controversial to catalase results which was elevated in double drug treatment which emphasize the theory that both enzymes compete to neutralize the oxidant stress of hydrogen peroxide that increased by double drug treatment. Graf *et al.* [34] have demonstrated that GPx can be depressed in VPA-treated patients with clinically defined toxicity of the drug, while Yüskel *et al.* [26] believed that VPA affects the antioxidant system and added that a decrease level of GPx is not an indicator of the risk of drug toxicity. In contrast to our study, Verrotti *et al.* [24] found unaltered GPx levels among their epileptics. The significantly increased levels of GST in our VPA-treated group could be attributed to induction of hepatic synthesis of GST and transport to blood [62]. While Hamed *et al.* [30] demonstrated elevated Gpx in valporate group.

In our study LP decreased by treatment but it was highly significant in double drug treatment this goes with some studies [24]. On the other hand Arhan *et al.* [63] showed that LP decrease with treatment with insignificant effect with VAP.

The demonstrated decrease of LP with the elevation of catalase in double drug treatment patients can be explained that catalase neutralize the elevated product of lipid peroxidation causing LP level to decrease.

While another studies showed elevation of LP by treatment [15, 19, 26, 30, 31], this conflict may be due to different group character and duration of treatment, where modulation of enzyme activity can happen. NO was decreased in treatment group versus untreated controls but this was significant only in VAP and V+C groups, with lowest level in VAP group, this is similar to results observed by Arhan *et al.* [63]. As elevation of Ca<sup>2+</sup> can activate nitric oxide synthase (NOS) and generate nitric oxide. Nitric oxide has been shown to inhibit complex IV, which can in turn lead to ROS production [15].

Treatment in our study and other studies leading to decrease in NO level which mean decrease in the oxidant stress and break NO effect in increasing ROS which in turn leave antioxidant enzymes free and lead to elevation of their levels versus that in untreated patients. Many studies showed that patients with epilepsy have lower levels of antioxidant enzymes when compared to normal controls without epilepsy, so it might be that long term decrease in NO by treatment may cause return of some antioxidant enzymes level to near normal, as Oxidative stress is defined as an imbalance between the

production of ROS on one side and endogenous antioxidant and repair capacity on the other, in favor of the former [20]. But in fact, in spite that some studies went to criticize AED to elevate oxidant stress [30, 31] our study showed decrease of NO and LP with period of 2 years treatment and the successful control of seizures for at least 6 months, LP decreased significantly in all treatment groups which goes with the study done by Verrotti *et al.* [24] while NO was lowest in VAP treatment which emphasized the antioxidant effect of VAP [57-61, 63].

Zinc was estimated showing no significant difference between treated and untreated patients, this is similar to results in some studies of children with epilepsy being treated with valproic acid. Serum zinc levels remained unchanged relative to control groups [64]. On the other hand Sozuer *et al.* [65] found that patients treated with valproic acid, alone or in combination with other drugs, have significantly lower levels of serum zinc than do normal controls however no definitive evidence has emerged as to the prevalence or clinical significance of zinc depletion or deficiency associated with valproic acid. Dietary sources of zinc are often inadequate, supplementation with zinc, at levels of 20-40 mg three times per day, may also be beneficial. Anyone adding zinc to their therapeutic regime will also need supplement with copper to prevent zinc-induced deficiencies of that mineral [65]. Mg showed similar results as zinc with no difference among the groups with studies confirming the observation. In our study, Mg was unaltered in all groups of epileptics and this is consistent with many studies [12, 24, 30, 63]. In contrast, some studies reported low Mg levels in epileptics [66]. In literature VAP treatment caused hepatic toxicity in both animal and human studied, with jaundice as one of its side effects, in this study highly significant elevation in total bilirubin was observed in all treated groups versus untreated patients with the highest with VAP and while liver enzymes showed no significant elevation between the groups [67].

It is important to emphasize that in all our treated patients, the drugs were prescribed at the usual dosage and all serum levels of AEDs were within the therapeutic ranges and none of our patients reported significant side effects from the AEDs, which is consistent with many studies. The study concluded that antiepileptic drugs affect antioxidant status. Supplementation with antioxidants from food or drug sources should be provided to patients to protect patients from the nutritional side effect of the drugs. Further investigation in this respect is needed.

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