Effects of Omega-3 Fatty Acids on Pentylenetetrazole-induced Kindling, Cognitive Impairment and Oxidative Stress

Basel A. Abdel-Wahab[1], Samy M. Abd El-Aziz [2], and Jobran M. Al-Qahtani[3]

[1]Department of Pharmacology, College of Medicine, Assiut University, Assiut, Egypt.
[2]Department of Physiology, College of Medicine, Najran University, Najran, Saudi Arabia.
[3]Department of Pediatrics, College of Medicine, Najran University, Najran, Saudi Arabia.
jobrancv@yahoo.com samoalsafty@gmail.com, basel_post@msn.com

Abstract: Epilepsy as well as chronic use of most antiepileptic drugs, particularly in children, predisposes to cognitive impairment. The brain is considered abnormally sensitive to oxidative stress and damage which observed during seizure activity. Omega-3 (OM3) fatty acids are known antioxidants that play a role in nervous system activity, cognitive development, memory-related learning, and synaptic transmission. **Aim**: The aim of the present study was to investigate the protective effects of OM3 on cognitive impairment after development of seizures and corresponding oxidative stress and neuronal DNA damage in young pentylenetetrazole (PTZ)-kindled rats. **Methods**: The effect of pretreatment with OM3 (200, 300 and 500 mg/kg, orally) on PTZ-induced kindling, corresponding cognitive impairment, using passive avoidance test, hippocampal oxidative stress. Furthermore, the corresponding changes in hippocampal 8-hydroxy-2′-deoxyguanosine (8-OHdG) were measured. **Results**: PTZ, (30 mg/kg, i.p.) once every alternate day induced kindling in rats after 31.0±1.4 days. OM3 showed dose-dependent anti-seizure effect. OM3 significantly increased the latency to myoclonic jerks, clonic seizures as well as generalized tonic-clonic seizures, and decreased the number of myoclonic jerks. PTZ kindling induced a significant oxidative stress and cognitive impairment which was reversed by pretreatment with OM3 in a dose-dependent manner. In addition, hippocampal 8-OHdG levels were significantly reduced by OM3 in PTZ kindled rats. **Conclusions**: OM3 ameliorates seizures, cognitive impairment, oxidative stress and hippocampal DNA damage in PTZ kindled rats. These results thus suggest the potential of OM3 as an adjuvant in epilepsy both to prevent seizures as well as to protect against seizure induced memory impairment.
[Basel A. Abdel-Wahab, Samy M. Abd El-Aziz, and Jobran M. Al-Qahtani. **Effects of Omega-3 Fatty Acids on Pentylenetetrazole-induced Kindling, Cognitive Impairment and Oxidative Stress.** *Life Sci J* 2014;11(7):186-196]. (ISSN:1097-8135). http://www.lifesciencesite.com. 23

Key Words: Omega-3, Pentylenetetrazole, Kindling, Memory,Oxidative stress, DNA damage.

1. Introduction

Patients with epilepsy frequently manifest cognitive and affective disorders such as spatial memory deficit and impaired emotional learning (Robinson, 2012). Some studies have demonstrated that prolonged or recurrent seizures cause spatial and emotional deficits (Zhang *et al.,* 2012) but other studies observed that after recurrent seizures, spatial memory remains intact (Detour *et al.,* 2005). Although the brief convulsive seizure typically lasts a minute or less, the postseizure dysfunction may persist beyond the behavioral convulsions, and will affect subsequent learning and memory. Patients with localized epilepsies generally have specific deficits in the cognitive functions controlled by the respective areas, for example, memory impairment associated with temporal lobe epilepsy (Vlooswijk *et al.,* 2011).

The damaging effects of seizures on cognition have been extensively studied in various kindling models (Epps and Weinshenker, 2013). Most obviously, the rats in these experiments have already experienced recurrent seizures with damage to parts of the brain such as the hippocampus and other parts of the limbic system (Potschka, 2012).

Rodent models have also been used to study human declarative memory, predicting the potential effects of seizures on human cognitive performance (Etchamendy *et al.,* 2012). The pentylenetetrazole (PTZ) kindling model provides a useful model for postseizure dysfunction, serving as a screen for potential treatments for the cognitive and emotional deficits that are observed in human epilepsy (Nasir *et al.,* 2012).

Previous reports have established a role for omega-3(OM3) in preventing dementia in humans (Corsinovi *et al.,* 2011). OM3 fatty acids, such as eicosapentaenoic (ECA) acid and docosahexaenoic (DHA) acid, are polyunsaturated fatty acids (PUFAs) that have been associated with many health benefits (Russell and Bürgin-Maunder, 2012). They are manufactured in the body using alpha linolenic acid as a starting point. In the nervous system PUFAs can be released from membrane phospholipids when neurons are stimulated with neurotransmitters and can be metabolized in the brain giving rise to a series

of active products, the eicosanoids, a group of oxygenated C20 compounds, which includes prostaglandins, thromboxanes, leukotrienes and a variety of hydroxy and hydroperoxy fatty acids. These products may act in the intracellular environment as neuronal secondary messengers and may be released in the extra-cellular space and interact with G-protein-coupled receptors on neurons and glial cells, thus influencing neuromodulation and synaptic plasticity (Nicholson *et al.,* 2013).

Fontani *et al.* (2005) have examined the effects of OM3 supplementation on some cognitive and physiological parameters in healthy subjects, and have found that OM3 supplementation is associated with an improvement of attentional and physiological functions particularly those involving complex cortical processing. OM3 fatty acids may play a role in nervous system activity, improve cognitive development and reference memory-related learning, increase neuroplasticity of nerve membranes, contribute to synaptogenesis and are involved in synaptic transmission (Guesnet and Alessandri, 2011).

Animal studies have shown that OM3 may play a role in cognitive development and OM3 fatty acid deficiency impairs the ability to respond to environmental stimulation in rats, which suggests that the provision of OM3to the developing brain may be necessary for normal growth and functional development. An OM3 deficiency in rat brain has been associated with reduced biosynthesis of catecholamines and decreased learning ability, with a lower synaptic vesicle density and stimulates glutaminergic synapses in the hippocampus (Latour *et al.,* 2012) whereas chronic administration of OM3helps to improve reference memory-related learning (Holguin et al., 2008).Different mechanisms have been proposed to explain this effect, e.g. increased hippocampal acetylcholine levels (Minami *et al.,* 1997), anti-inflammatory effects of OM3, and increased neuroplasticity (Connor *et al.,* 2012).

These properties of OM3 suggest its potential as a substance for the prevention of seizures and cognitive impairment in PTZ induced kindling. Therefore, in the present study the effect of chronic OM3 administration on seizures, cognitive impairment,oxidative stress and neuronal DNA damage in PTZ induced kindling in rats was evaluated.

2. Materials and methods

2.1. Animals

Young male Wistar rats, 3-4 weeks old with 150–180 g body weight from the animal house of King Saud University, Riyadh, Saudi Arabia were used in this study. Animals were housed in groups of 10 rats in standard clear polycarbonate cages with food and water available *ad libitum*. Animals were kept on a 12-h light–dark schedule (6:00 am–6:00 pm), and all behavioral testing was conducted during the light phase from 9:00 am to 12:00 pm. All experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The experimental protocol was approved by the Institutional Animal Use and Care Committee.

2.2. Chemicals

Omega-3 and PTZ were purchased from Sigma (Sigma Aldrich, USA).PTZ was dissolved in physiological saline prior to the experiments. Thiobarbituric acid, glutathione, NADPH,Ellman's reagent [5,5-dithiobis-2-nitrobenzoic acid (DTNB solution)] andBovine serum albumin (BSA),were purchased from Fluca Co., (Germany). Total Antioxidant Assay kit, and 8-hydroxy-2′-deoxyguanosine (8-OHdG) assay kit was purchased from Cayman's Chemical Co., (USA). All other chemicals were of analytical grade.

2.3. Experimental protocol

The animals were randomly divided into five groups with ten animals in each group. Group I (control group) received 0.9% saline i.p. every other day (3.5 ml/kg, 13 injections total). Group II (PTZ group) received saline pretreatment along with PTZ (30 mg/kg, i.p.) every other day. Groups III, IV and V (PTZ + OM3 groups) received OM3 pretreatment in doses of 30 mg/kg, i.p. PTZ and 200, 300 and 500 mg/kg, p.o. OM3 respectively, in alternate day treatment. In these groups, OM3 was given 30 min before PTZ. Cognition was assessed after 24 h of the last PTZ injection. At the end of cognition assessment animals were rapidly sacrificed by decapitation and brains were collected for biochemical measurements.

2.4. PTZ- induced kindling in rats

For PTZ kindling, a subconvulsant dose of PTZ 30 mg/kg body weight was injected intraperitoneally on every second day. The animals were observed for 30 min after each PTZ administration. The latency to myoclonic jerks and the generalized tonic clonic seizures (GTCS), as well as the duration of GTCS were recorded. Seizure stage was evaluated using the following scale: Stage 0: no response; Stage 1: hyperactivity and vibrissae twitching; Stage 2: head nodding, head clonus and myoclonic jerk; Stage 3: unilateral forelimb clonus; Stage 4: rearing with bilateral forelimb clonus; Stage 5: generalized tonic-clonic seizure (GTCS) with loss of postural control. The PTZ injections were stopped when the animals showed adequate kindling, i.e. seizure score of 5 on three consecutive injections. The first incidence of

seizure with score five was observed between Day 27 and Day 31. Thus, in no case did the PTZ schedule exceed Day 35.

2.5. Passive avoidance test

Memory retention deficit was evaluated using a step-through passive avoidance apparatus as described earlier (Reeta *et al.,* 2009).The learning box consisted of two compartments, one light (white compartment, 20×20×30 cm) and the other dark (black compartment, 20×20×30 cm). A guillotinedoor opening (6×6 cm) was made on the floor in the center of the partition between the two compartments. The floor of the dark compartment consists of stainless-steel grids to produce the foot shock. All animals were allowed to habituate in the experimental room for at least 30 minutes prior to the experiments. After habituation, each animal was gently placed in the light compartment of the apparatus; after 5 seconds, the guillotine door was opened and the animal was allowed to enter the dark compartment. The latency to the animal's entry into the dark compartment was recorded. Animals that waited more than 100 seconds to cross into the dark compartment were eliminated from the experiments. Once the animal crossed with all four paws into the next compartment, the guillotine door was closed and the rat was returned to its home cage.The acquisition trial was carried out 30 minutes after the habituation trial. The animal was placed in the light compartment, the guillotine door was opened 5 seconds later, and as soon as the animal crossed into the dark compartment, the door was closed and a foot shock (50 Hz, 5 seconds, 0.2-mA intensity) was immediately delivered to the grid floor of the dark room with an insulated stimulator. After 20 seconds, the rat was removed from the apparatus and placed in its home cage. Training was terminated when the rat remained in the light compartment for 120 consecutive seconds. One day following training, the retention tests were performed to evaluate memory. Each animal was placed in the light compartment for 20 seconds, the door was opened, and the step through latency to entry into the dark compartment was measured. The session ended either when the animal entered the dark compartment or when it remained in the light compartment for 300 seconds. During these sessions, no electric shock was applied.

2.5. Measurement of oxidative stress parameters

2.5.1. Tissue preparation

The brain was rinsed in ice-cold saline; the whole hippocampus was separated, washed with ice-cold saline, blotted carefully, weighed and then homogenized in phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000× g at 4 °C for 15 min and the supernatant was collected for biochemical assays.

2.5.2. Determination of lipid peroxidation

Lipid peroxidation was estimated by the measurement of malondialdehyde (MDA) levels. It is an end product of lipid peroxidation and its level was determined spectrophotometrically by use of thiobarbituric acid reactive substances method previously described by Ohkawa *et al.* (1979).

2.5.3. Determination of total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) was measured by using Total Antioxidant Assay kit purchased from Cayman's Chemical Company, (USA). Aqueous and lipid soluble antioxidants are not separated, thus the combined antioxidant activities of all constituents including vitamins, proteins, lipids, glutathione, uric acid are assessed. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS® (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) to $ABTS^{®\bullet-}$ by metmyoglobin. All procedures were carried out as described in the manufacturer manual.

2.5.4. Determination of intracellular GSH

The GSH content of the neutralized supernatant of hippocampal homogenate was assayed using Ellman's reagent [5,5-dithiobis-2-nitrobenzoic acid (DTNB solution)] according to the method of Griffith (1980).

2.5.5. Determination of GSH-Px activity

Glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia and Valentine (1967). The enzymatic reaction which contained β-nicotinamide adenine dinucleotide phosphate (NADPH), GSH, glutathione reductase and a sample or standard was initiated by addition of hydrogen peroxide. The change in the absorbance was measured spectrophotometrically. A standard curve was plotted for each assay.

2.5.6. Determination of 8-hydroxy-2′-deoxyguanosine (8-OHdG) level

Cayman's 8-hydroxy-2′-deoxyguanosine (8-OHdG) assay kit purchased from Cayman's Chemical Co., (USA) was used. It is a competitive assay that can be used for the quantification of 8-OHdG in serum and tissue homogenate. It recognizes both free 8-OHdG and DNA-incorporated 8-OH-dG. This assay depends on the competition between 8-OHdG and 8-OHdG-acetylcholinesterase (AChE) conjugate (8-OHdGTracer) for a limited amount of 8-OHdG monoclonal antibody. All procedures were carried out in accordance with the provider manual.

2.5.5. Determination of total protein

The protein content in the supernatant of hippocampal homogenate was measured by method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

2.7. Statistical analysis

Results are expressed as mean ± S.E.M. Statistical analysis was performed using one way analysis of variance (ANOVA) with Tukey's post hoc test. All statistical analyses were performed using SPSS statistical software package version 13.0. A $p< 0.05$ was considered as significant.

3. Results

3.1. Effect of OM3 on the development of PTZ kindling

Repeated administration of a subconvulsant dose of PTZ (30 mg/kg, i.p.) on alternate days resulted in increasing convulsive activity leading to GTCS stage 5 on two consecutive trials on 31.0±1.4 days in the control rats. One way ANOVA showed a significant difference in the development of kindling amongst the treated groups [F (3, 22) =3.587, p=0.018]. OM3 caused a modest dose-dependent delay in the development of kindling. GTCS stage 5 was observed after 33.4±0.8, 35.0±1.6 and 37±1.7 days of PTZ injection in the OM3 200, 300 and 500 mg/kg administered groups, respectively. On post hoc analysis, it was found that the 300 and 500 mg/kg doses of OM3 significantly delayed the course of development of kindling by PTZ (*p<0.05) whereas the delay in the development of kindling by OM3 at dose of 200 mg/kg was not statistically significant (Fig. 1A). In the PTZ group and the OM3 200 mg/kg/day group, 34.4% and 15.8% mortality, respectively was observed. There was no mortality in the control animals and the groups administered higher OM3 doses (300 and 500 mg/kg).

3.2. Effect of OM3 on seizures in PTZ kindled rats

Latency to myoclonic jerks, latency to clonic seizures, latency to GTCS, number of myoclonic jerks, duration of GTCS and seizure score were used to assess the severity of seizures in rats. OM3 caused dose-dependent increase in the latency of myoclonic jerks [F(3,22) =275.4, p =0.0001] as well as the latency to GTCS [F(3,22)=276.6, p =0.0001] and a decrease in number of myoclonic jerks [F(3,22)=104.33, p =0.0001] as compared to the PTZgroup. Pretreatment with OM3 caused a significant increase in the latency to myoclonic jerks from56.7±3.8 s in the PTZ group to 74.3 ± 2.9 s, 94.6 ±2.7 s and 128.8±2.9 s in the groups administered OM3 200, 300 and 500 mg/kg, respectively (Fig. 1D). The number of myoclonic jerks decreased from 54.4 ±3.3 in the PTZ group to 23.9 ±1.7, 22.8±1.7 and 16.3±1.4 in the groups administered OM3 200, 300 and 500 mg/kg, respectively (Fig. 1C). A significant difference in the onset of clonic seizures was observed [F(3,22)=248.5, p =0.0001]. Post hoc analysis revealed that OM3 at doses of 200, 300 and 500 mg/kg significantly increased the latency to clonic seizures from 81.2±2.7 s in the PTZ group to 100.6±2.6 s (**$p < 0.01$), 109.5±2.8 s (***$p < 0.001$) and 315.3±4.6 s (***$p < 0.001$) in OM3 200, 300 and 500 mg/kg treated groups, respectively (Fig. 1B). OM3 significantly increased the latency of GTCS from 238.4±12.6 s in the PTZ group to 317.3±8.6 s, 383.4±9.7 s, and 465.4±15.6 s in the groups administered OM3 200, 300 and 500 mg/kg, respectively (Fig. 1E). There was a significant difference in the duration of GTCS amongst the different groups [F(3,22)= 14.22, p =0.0001]. OM3 decreased the duration of GTCS from 17.4±1.8 s in the PTZ group to 6.3±1.4 s (**p <0.01) and 2.4±0.3 s (***p <0.001) in the groups administered 300 and 500 mg/kg ofOM3, respectively. However, the reduction in the GTCS duration at the 200 mg/kg dose of OM3 was not statistically significant (Fig. 1F).

3.2. Effects of OM3 on passive avoidance task (step-through paradigm) in PTZ kindled rats

Passive avoidance task assesses the ability of the animals to retain and recall information. The mean initial latency did not differ significantly amongst the different groups [F(4,49)=1.540, p =0.232] whereas the retention latency was significantly different between the groups [F(4,49)= 4.683, p =0.003]. Tukey's post hoc test revealed that PTZ induced kindling led to a significant decrease in retention latency in the passive avoidance paradigm as compared to the control group. The retention latency decreased from 135.8±5.4 s in the control rats to 92.7±6.8 s in the PTZ group (*$p < 0.05$). When OM3 was administered with PTZ, it produced significant dose-dependent increase in retention latency as compared to the PTZ group. The retention latency increased from 92.7±6.8 s in PTZ administered rats to 135.5±7.8 s, 141.53±8.6 s (*$p <$ 0.05) and 153.6±9.7 s (**$p < 0.01$) in the groups administered OM3200, 300 and 500 mg/kg, respectively (Fig. 2).

3.3. Effects of OM3 on hippocampal oxidative stress in PTZ kindled rats

Results of changes in MDA and antioxidant defense system, TAC, GSH levels and GSH-Px activity in hippocampus of PTZ kindled ratstreated with OM3 are shown in Table 1.

Results revealed a significant effect of PTZ kindling and OM3 treatment on hippocampal MDA [F(4, 49) = 5.688, p =0.0009] levels. Post hoc analysis indicated that PTZ kindling significantly increased hippocampal MDA (**$p < 0.01$) levels compared with control animals. In groups treated with OM3, there was a significant decrease in hippocampal MDA (*p <0.05) with doses200and 300mg/kg and (**$p < 0.01$) with the dose 500 mg/kg of OM3 relative to PTZ group.

The results revealed a significant effect of PTZ kindling and OM3 treatment on hippocampal TAC level [F(4, 49) = 5.026, $p < 0.002$]. Post hoc analysis indicated that PTZ kindling significantly (**$p < 0.01$) decrease hippocampal TAC level relative to control animals. Treatment with OM3 increased hippocampal TAC level (*p <0.05) with the dose 200 mg/kg and (**$p < 0.01$) with the doses 300 and 500 mg/kg of OM3 relative to PTZ group.

GSH plays an important role in protecting the cells againstoxidative damage by scavenging the free radicals. It is used as the marker for oxidative stress. In the present study, there was a significant difference in hippocampal GSH levels between groups [F(4,49)= 5.866, p =0.0007]. The hippocampalGSHlevel was significantly lower in the PTZ group as compared to the control group (**p <0.01). OM3resisted thedecreased in hippocampal GSH level induced by PTZ kindling. There was insignificant change in GSH level with 200 mg/kg dose of OM3, while significant increase was observed with higher doses, (*p <0.05) with300 mg/kg and (**p <0.01) with 500 mg/kg of OM3 compared to the PTZ group.

GSH-Px is an important enzyme for inactivation of peroxy radicals utilizing GSH. Hippocampal GSH-Px activity was significantly reduced in the PTZ group, reflecting the exhaustion of antioxidant enzymes by PTZ kindling [F(4, 49) = 4.210; $p < 0.0056$]. OM3 in the tested doses showed a dose-related increase in GSH-Px activity that was depressed by PTZ kindling. Hippocampal GSH-Px activity increased significantly (*$p < 0.05$) with doses200 and 300 mg/kg and (**$p < 0.01$) with the highest dose 500 mg/kg compared with PTZ group.

3.4. Effects of OM3on hippocampal 8-OHdG level in PTZ kindled rats

8-hydroxy-2'-deoxyguanosine (8-OHdG) is a product of oxidatively damaged DNA formed by hydroxy radicaland singlet oxygen. 8-OHdG can be detected in tissue, serum, urine and other biomaterials.The 8-OHdG level in the hippocampus of animals exposed to PTZ kindlingwas significantly increased (**$p < 0.01$) relative to control indicating increased oxidative DNA damage in hippocampal neurons by PTZ kindling. OM3 in the tested doses showed a neuroprotective effect against neuronal DNA damage, where the level of 8-OHdG was significantly reduced by treatment with OM3 in animals exposed to PTZ kindling(*$p < 0.05$) with the doses 200 and 300 mg/kg and (**$p < 0.01$) with the highest dose 500 mg/kg compared with PTZ group [F(4, 49) = 4.602; $p < 0.0034$] (Fig. 3).

4. Discussion

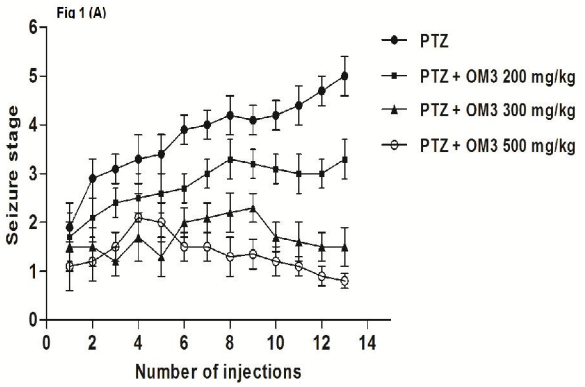
In the present study, we investigated the effect of OM3on the development of PTZ-induced kindling in young rats. Pretreatment with OM3in doses 200, 300 and 500 mg/kg attenuated seizure severity from the beginning of the kindling procedure by lowering the mean seizure stage, seizure latency and duration. These effects of OM3 at higher doses are more significant.

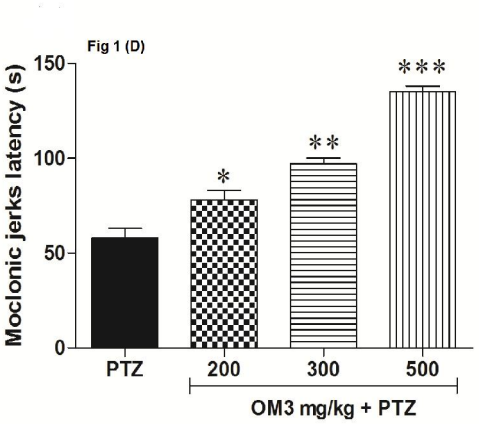
In addition, pretreatment with OM3 before administration of PTZ every other day, and prior to training, is associated with enhanced memory retrieval in rats. OM3(300 and 500 mg/kg) significantly increased retrieval of memory in the retention tests of a passive avoidance task, compared with PTZ-treated group. The cognitive enhancing effect of OM3was also observed at 200 mg/kg.

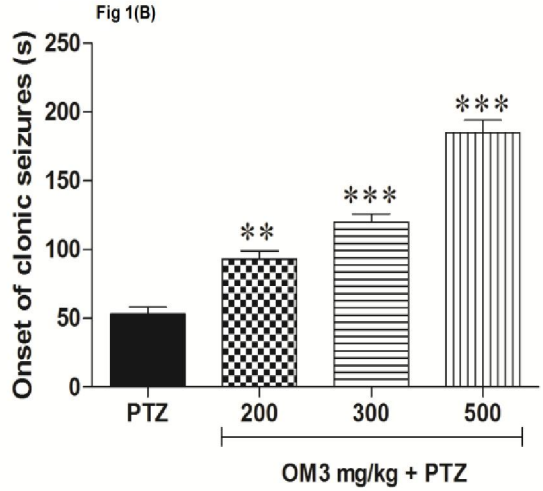
Epilepsy as a major neurological disorder, is still awaiting safer drugs with improved antiepileptic effect and lesser side effects. Apart from epilepsy itself causing cognitive impairment, antiepileptic drugs (AEDs) have been shown to induce cognitive impairment in patients with epilepsy (Shannon and Love, 2007). Therefore, there is a strong need for effective AEDs with multiple actions and no/minimal side effects on cognitive function or antagonizing the side effects of other drugs.

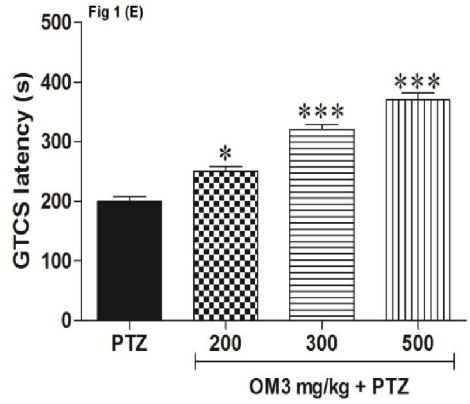
The effects of epilepsy on cognition have been reviewed in several articles (Vlooswijk *et al.,* 2011; Zhang *et al.,* 2012), suggesting the presence of at least a mild decline in intellectual performance in children and adults with epilepsy (Robinson 2012).Animal models for kindling provide an acceptable approach to quantify epileptogenesis (Epps *et al.,* 2013). PTZ kindling represents a model of primary generalized epilepsy (Ammon-Treiber *et al.,* 2007).

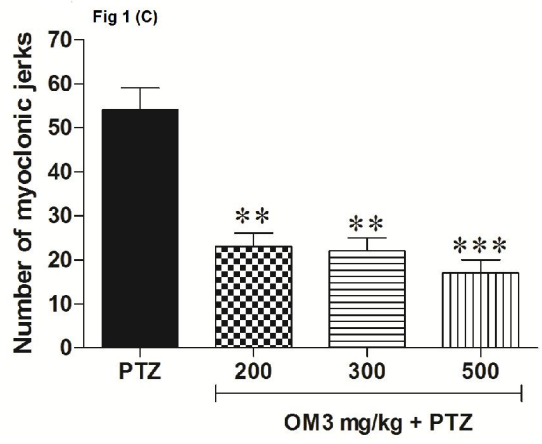
In the present study, sub-convulsive dose of PTZ when given intraperitoneally on alternate days induced kindling in the control rats. The groups which were administered OM3 showed dose-dependent protection against seizures. OM3 significantly increased the latencies to myoclonic jerks, clonic seizures and GTCS as well as duration of GTCS as compared to the PTZ kindled rats (Figs. 1B and 1D-1F). The number of myoclonic jerks was also decreased by OM3 in a dose-dependent manner (Fig. 1C). OM3 produced maximum seizure protective effect in the dose of 500 mg/kg. The results of the present study are consistent withprevious reported outcomes indicated that acute administration of Docosahexaenoic acid (DHA), an OM3fatty acid, has been reported to raise seizure thresholds and increases resistance to PTZ-induced seizures in rats (Taha *et al.,* 2010).











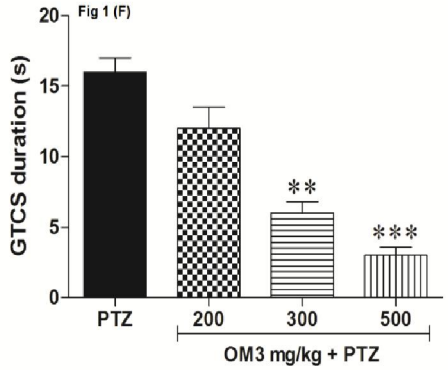


Fig 1. Effect of repeated administration of omega-3(OM3) in doses 200, 300 and 500 mg/kg, p.o. on the development of pentylenetetrazole (PTZ) kindling (25 mg/kg, i.p, 13 injections total) in rats. (A) Mean seizure score. (B) Onset of clonic seizures (C) Number of myoclonic jerks. (D) Latency to myoclonic jerks. (E) Latency to GTCS. (F) Duration of GTCS.Values are expressed as mean ±S.E.M.(n=10).

* $p < 0.05$ vs. PTZ-treated group. ** $p < 0.01$ vs. PTZ-treated group. *** $p < 0.001$ vs. PTZ-treated group.

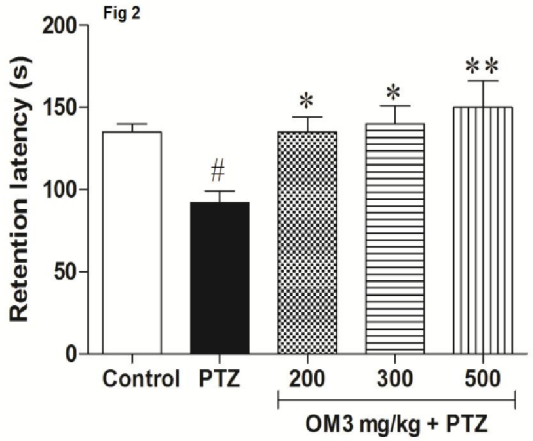


Fig 2. Effect ofrepeated administration of omega-3(OM3) in doses 200, 300 and 500 mg/kg, p.o.,on step-through latency of passive avoidance reflex test of PTZ-kindled rats.
Results in each group represent mean ±SEM (n = 10).
# $p < 0.05$ vs. control group. * $p < 0.05$ vs. PTZ-treated group. ** $p < 0.01$ vs. PTZ-treated group.

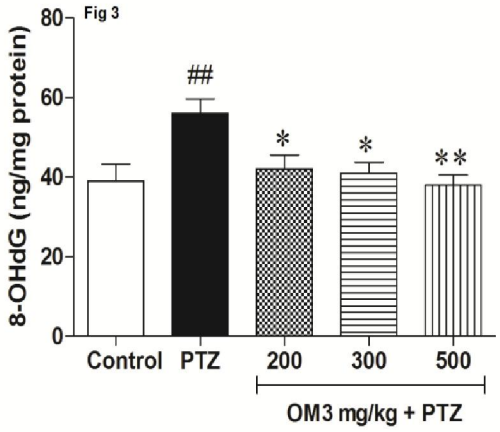


Fig 3. Effect of repeated administration of omega-3(OM3) in doses (200, 300 and 500 mg/kg, p.o.) on hippocampal 8-hydroxy-2-deoxyguanosine (8-OHdG) level of PTZ kindled rats.
Results in each group represent mean ±SEM (n = 10).
# $p < 0.05$ vs. control group. * $p < 0.05$ vs. PTZ-treated group. ** $p < 0.01$ vs. PTZ-treated group.

In the present study, the protective effect of OM3 was evaluated against cognitive impairment induced by seizures in the PTZ kindled rats. OM3 exhibited dose-dependent protective effect against seizures, oxidative stress and cognitive impairment inrats. The biological processes in the brain that contribute to impairment in cognitive function have been reported to be influenced by ongoing seizure activity and AEDs treatment (Marsh *et al.,* 2006).

There are several possible explanations for deterioration of cognitive processes in kindled rats. One of several explanations refers to the degenerative processes in the brain structures, secondary to seizure related ischemia and hypoxia (Björkman *et al.,* 2010). It has been shown that kindled seizures are associated with a selective degeneration of cortical and limbic structures including hippocampal areas, involving loss of neurons, neuronal and glial growth, and hypertrophy of astrocytes and sprouting of new connections (Fang and Lei, 2010). Moreover, seizure activity and AEDs have caused increased level of free radicals and reduced activity of antioxidant defense mechanisms (Júnior *et al.,* 2009). This imbalance between oxidant and antioxidant defense mechanism in the body may result into seizures and cognitive deficit.

In this study, PTZ caused a significant decrease in retention latency in the passive avoidance task which indicates impairment of memory of rats. In the groups that were administered OM3, the rats exhibited significantly increased retention latencies in the passive avoidance paradigm as compared to the animals administered PTZ alone (Fig 2).These improvements in cognition of kindled rats with OM3 were dose-dependent with the maximum benefit at 500 mg/kg dose. These results are in agreement with a recent study (Liu et al., 2012)in which the authors related the cognitive improvement in PTZ –induced seizure model after supplementation with DHA to the antioxidant properties of DHA.

The protective effect of OM3 on cognitive impairment observed in the present study may be due to its anticonvulsant effect. In the present study, OM3 showed anti-seizure activity which may be at least partially responsible for the improvement in cognitive function in the groups treated withOM3. This antiepileptic potential of OM3 has been reported in earlier studies in acute PTZ model (Taha *et al.,* 2010). Animal studies and a preliminary clinical observation suggest that nutritional supplementation with OM3 fatty acids may be useful in the non-pharmacological treatment of patients with epilepsy. OM3 fatty acids increase seizure thresholds and lower inflammatory mediators, which are increased in these patients (Yuen *et al.,* 2012)

Brain and other neural tissues are particularly rich in long-chain PUFAs, which serve as specific precursors for eicosanoids that play important roles in normal central nervous system (CNS) cells development and function (Bradbury *et al.,* 2011). OM3 PUFAs are able to directly and indirectly modulate neurological activity on many different levels, through a number of overlapping mechanisms. OM3 PUFAs are central components of glial and neuronal membrane phospholipids, and take part in

brain membrane remodeling and synthesis, and in signal transduction (Duda, 2012). In particular DHA can modulate membrane fluidity (synaptic plasticity), participate in signal transduction, and the biodynamic activity of neuronal membranes (Bazan, 2003). Proteins in the bilayer have crucial cellular functions as they operate as receptors and transporters. OM3 fatty acids modify membrane fluidity by shifting cholesterol from the membrane, and determine an optimal membrane fluidity as it is required for neurotransmitter binding and signaling within the cell (Bazan 2003). In addition, DHA stimulates the expression of peroxisomal enzymes. These are essential for plasmalogen synthesis, which in turn is essential for myelin formation. Thus, DHA stimulates remyelination (Bradbury, 2011). Decreased DHA serum content has been found to correlate with cognitive impairment and Alzheimer disease (AD) (Lukiw *et al.,* 2005).

OM3 PUFAs can also modulate dopaminergic, serotoninergic and cholinergic neurotransmission. For example, chronic OM3 deficiency significantly decreases dopamine storage vesicle formation, and dopamine levels in the rat frontal cortex. Conversely, OM3 supplementation significantly increases dopamine levels in this area (Ohara, 2006.). Moreover, OM3 deficiency alters cholinergic transmission that plays an important role in cognitive functions in the rat hippocampus. The deprivation also causes a 10% reduction in muscarinic acetylcholine receptor binding. (Aïd *et al.,* 2003).

The role of oxidative stress in epilepsy and cognitive impairment is well established (Corvino *et al.,* 2013), Therefore, the present study focused mainly on the oxidative stress and role of antioxidant properties of OM3.MDA is an end product of free radical-mediated oxidative cell damage and lipid peroxidation(Garcia *et al.,* 2005). In the present study, PTZ kindling increased the level of MDA and decreased the GSH,total antioxidant (TAC) levels and antioxidant enzyme GSH-Px in rat hippocampus. PTZ thus caused an imbalance between oxidant stress and antioxidant defense system which may be at least partially responsible for seizures and cognitive impairment. This is supported by Liu *et al.* (2012) who observed that increased oxidative stress by PTZ kindling may be one of the factors responsible for the cognitive impairment seen with chronic seizures.

Co-administration of OM3 prevented the rise in brain MDA levels in a dose-dependent manner. The significant decrease in brain MDA levels with concomitant OM3 administration as compared to PTZ alone treated rats indicates an attenuation of lipid peroxidation. These results are in agreement with previous results indicating the ability of OM3 to inhibit lipid peroxidation (Bazan, 2005).

A significant decrease in GSH level was observed in PTZ kindled rats as compared to the control group. Additionally, a significant reversal in the brain GSH levels was observed in the groups co-administered OM3. OM3 (500 mg/kg, p.o) perse caused a significant increase in the hippocampal GSH level when compared to the control group. This antioxidant effect of OM3 in PTZ kindled rats is supported by the recent findings of Liu *et al.*(2012) wherein oral supplementation of DHA decreased the MDA, and increased the catalase and glutathione -S-transferase levels in rat cerebrum and cerebellum. Moreover, theresults of the present study are in agreement with reports of recent study wherein OM3increases the cellular GSH, restores its levels and prevent oxidative damage by enhancing transcription of genes for glutamate cysteine ligase, and apoptotic indices in oxidative stress conditions (Patten *et al.,* 2012).

The observed decrease in the total antioxidant (TAC) defense in hippocampi of animals exposed to PTZ kindling is in agreement with previous studies. Seizure activity is characterized by a decreased antioxidant status, as evidenced by lowered tryptophan, tyrosine, albumin, zinc and vitamin E (Rauca *et al.,* 2004). Furthermore, TAC is lower in patients with epilepsy than in healthy volunteers (Mahle and Dasgupta, 1997).

OM3 in this study is not only able to increase the concentration of antioxidant substances but also increased the activity of antioxidant enzyme GSH-Px depressed by PTZ kindling (Dhir *et al.,* 2007). These results are in agreement with the results obtained by (Kusunoki *et al.,* 2013) indicting the ability of OM3 to increase the activity of antioxidant enzymes.

Increased oxidative stress induced by PTZ kindling may increase the incidence of neuronal DNA damage. This finding is clear in this study that showed a significant increase in hippocampal levels of 8-OHdG; the biomarker for oxidative DNA damage. In normal conditions, free radicals attack nuclear and mitochondrial DNA causing oxidation of nucleosides and consequently, mutagenic DNA lesions. One of these lesions is 8-OHdG; the end product of the hydroxylation of guanine. The DNA lesions are consequently removed by the base excision repair (BER) pathway, which prevents replication of DNA lesions. Moreover, free radicals inhibit the BER system through direct interactions with cellular repair proteins (Feng *et al.,* 2006). Since the BER pathway removes the mutagenic 8-OHdG lesions, the inhibitory effects of oxygen free radicals pathways on BER activity may potentiate mutagenesis and DNA damage.

The decrease in free radicals production via the OM3 antioxidant properties and its ability to

reactivate the antioxidant defenses may contribute in the observed decrease in hippocampal 8-OHdG level increased by PTZ kindling after pretreatment with OM3. OM3 through its antioxidant properties exerts a protective effect on hippocampal neurons, decrease the number of apoptotic cells and protect neurons from injury of hypoxemia and ischemia (Ozsoy *et al.,* 2011). In addition, *in vitro*, OM3 was found to exert a direct neurotrophic effect on cholinergic and other neurons (Bousquet *et al.,* 2009). These findings are in agreement with our results that showed a reduction in hippocampal level of 8-OHdG, increased by PTZ after treatment with OM3.

Conclusions

In conclusion, the present study demonstrates that OM3 iseffective in preventing PTZ induced kindling seizures, cognitive impairment, oxidative stress and neuronal DNA damage in young rats, thereby showing the promise of OM3 as a possible chemopreventive agent. This study thus suggests the potential of OM3 as an adjuvant to AEDs in epileptic patients particularly in children with dual advantages of better seizure control as well as less cognitive impairment.

Acknowledgement

This work was financially supported by Najran University Program for Health and Medical Research Grants, Grant No. (NU 6/10). The work of this study was carried out in College of Medicine, Najran University, Najran, Saudi Arabia.

Corresponding author:
Samy M. Abd El-Aziz
Department of Physiology, College of Medicine, Najran University, Najran, Saudi Arabia.
samoalsafty@gmail.com

References

1. Aïd S, Vancassel S, Poumès-Ballihaut C, Chalon S, Guesnet P, Lavialle M. Effect of a diet-induced n-3 PUFA depletion on cholinergic parameters in the rat hippocampus. J Lipid Res. 2003;44(8):1545-51.
2. Ammon-Treiber S, Grecksch G, Angelidis C, Vezyraki P, Höllt V, Becker A. Pentylenetetrazol-kindling in mice overexpressing heat shock protein 70. Naunyn Schmiedebergs Arch Pharmacol. 2007 Apr;375(2):115-21.
3. Björkman ST, Miller SM, Rose SE, Burke C, Colditz PB. Seizures are associated with brain injury severity in a neonatal model of hypoxia-ischemia. Neuroscience., 2010; 166(1): 157-67.
4. Bradbury J. Docosahexaenoic acid (DHA): an ancient nutrient for the modern human brain. Nutrients., 2011;3(5):529-54.
5. Bazan NG Synaptic lipid signaling: significance of polyunsaturated fatty acids and platelet-activating factor. J Lipid Res., 2003;44(12):2221-33.
6. Bazan NG Neuroprotectin D1 (NPD1): a DHA-derived mediator that protects brain and retina against cell injury-induced oxidative stress. Brain Pathol., 2005;15(2):159-66.
7. Bousquet M, Gibrat C, Saint-Pierre M, Julien C, Calon F, Cicchetti F. Modulation of brain-derived neurotrophic factor as a potential neuroprotective mechanism of action of omega-3 fatty acids in a parkinsonian animal model. Prog Neuropsychopharmacol Biol Psychiatry., 2009;33(8):1401-8.
8. Connor S, Tenorio G, Clandinin MT, Sauvé Y. DHA supplementation enhances high-frequency, stimulation-induced synaptic transmission in mouse hippocampus. Appl Physiol Nutr Metab. 2012 Oct;37(5):880-7.
9. Corsinovi L., Biasi F., Poli G., Leonarduzzi G., Isaia G. Dietary lipids and their oxidized products in Alzheimer's disease. Mol. Nutr. Food Res., 2011;55 Suppl 2:S161-72.
10. Corvino V, Marchese E, Michetti F, Geloso MC. Neuroprotective strategies in hippocampal neurodegeneration induced by the neurotoxicant trimethyltin. Neurochem Res. 2013;38(2):240-53.
11. Detour J, Schroeder H, Desor D, Nehlig A. A 5-month period of epilepsy impairs spatialmemory, decreases anxiety, but spares object recognition in the lithium-pilocarpine model in adult rats. Epilepsia, 2005 ;46(4):499-508.
12. Dhir A, Naidu PS, Kulkarni SK. Neuroprotective effect of nimesulide, a preferential COX-2 inhibitor, against pentylenetetrazol (PTZ)-induced chemical kindling and associated biochemical parameters in mice. Seizure., 2007;16(8):691-7.
13. Duda MK. Polyunsaturated fatty acids omega-3 as modulators of intracellular signaling pathways. Postepy Biochem., 2012;58(2):149-54.
14. Ellman GL, Courtney DK, Andres V, Feathstone RM. A new and rapid colorimetric determination of acetylcholinestrase activity. *Biochem Pharmacol.* 1961;7:88-95.
15. Epps SA, Weinshenker D. Rhythm and blues: Animal models of epilepsy and depression comorbidity. Biochem Pharmacol. 2013 Jan 15;85(2):135-46.

16. Etchamendy N., Konishi K., Pike G.B., Marighetto A., Bohbot VD. Evidence for a virtual human analog of a rodent relational memory task: a study of aging and fMRI in young adults. Hippocampus, 2012;22(4):869-80.
17. Fang F., Lei H. Increased hippocampal T2 in a rat model of pentylenetetrazol-induced kindling correlates with seizure scores. J Neurol Sci., 2010; 292(1-2):16-23.
18. Feng, Z., Hu, W., Marnett, L.J., Tang, M.S.,. Malondialdehyde, a major endogenous lipid peroxidation product, sensitizes human cells to UV-and BPDE-induced killing and mutagenesis through inhibition of nucleotide excision repair. Mutat. Res., 2006; 601(1–2): 125–36.
19. Fontani G., Corradeschi F., Felici A., Alfatti F., Migliorini S., Lodi L. Cognitive and physiological effects of Omega-3 polyunsaturated fatty acid supplementation in healthy subjects. Eur. J. Clin. Invest., 2005;35(11):691-9.
20. Garcia YJ, Rodríguez-Malaver AJ, Peñaloza N. Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebellar slices. J Neurosci Methods, 2005;144(1):127-35.
21. Griffith, O.W. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem., 1980;106:207-12.
22. Guesnet P., Alessandri JM. Docosahexaenoic acid (DHA) and the developing central nervous system (CNS) - Implications for dietary recommendations. Biochimie. 2011;93(1):7-12.
23. Holguin S., Huang Y., Liu J., Wurtman R. Chronic administration of DHA and UMP improves the impaired memory of environmentally impoverished rats. Behav. Brain. Res. 2008;191(1):11-6.
24. Júnior HV, de França Fonteles MM, Mendes de Freitas R. Acute seizure activity promotes lipid peroxidation, increased nitrite levels and adaptive pathways against oxidative stress in the frontal cortex and striatum. Oxid Med Cell Longev, 2009;2(3):130-7.
25. Kusunoki C, Yang L, Yoshizaki T, Nakagawa F, Ishikado A, Kondo M, et al. Omega-3 polyunsaturated fatty acid has an anti-oxidant effect via the Nrf-2/HO-1 pathway in 3T3-L1 adipocytes. Biochem Biophys Res Commun. 2013 Jan 4;430(1):225-30.
26. Latour A, Grintal B, Champeil-Potokar G, Hennebelle M, Lavialle M, Dutar P, et al., Omega-3 fatty acids deficiency aggravates glutamatergic synapse and astroglial aging in the rat hippocampal CA1. Aging Cell. 2012 Oct 31. [Epub ahead of print].
27. Liu S.H., Chang C.D., Chen P.H., Su J.R., Chen C.C., Chaung H.C. Docosahexaenoic acid and phosphatidylserine supplementations improve antioxidant activities and cognitive functions of the developing brain on pentylenetetrazol-induced seizure model. Brain Res., 2012;1451:19-26.
28. Lowry O., Rosenbrough N., Farr A., Randall R.. Protein measurement with the Folinphenol reagent. J. Biol. Chem., 1951;193: 265-275.
29. Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, et al. A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. J Clin Invest. 2005 ;115(10):2774-83.
30. Mahle C, Dasgupta A. Decreased total antioxidant capacity and elevated lipid hydroperoxide concentrations in sera of epileptic patients receiving phenytoin.Life Sci. 1997;61(4):437-43.
31. Marsh E.D., Brooks-Kayal AR, Porter BE. Seizures and antiepileptic drugs: does exposure alter normal brain development? Epilepsia. 2006 Dec;47(12):1999-2010.
32. Minami M, Kimura S, Endo T, Hamaue N, Hirafuji M, Togashi H, Dietary docosahexaenoic acid increases cerebral acetylcholine levels and improves passive avoidance performance in stroke-prone spontaneously hypertensive rats. Pharmacol. Biochem. Behav. 1997;58(4):1123-9.
33. Nasir S.A., Sharma A., Khanam R., Vohora D. Effect of medroxyprogesterone on development of pentylenetetrazole-induced kindling in mice. Neuroscience. 2012, 5;207:283-7.
34. Nicholson T, Khademi H, Moghadasian MH. The role of marine n-3 fatty acids in improving cardiovascular health: a review. Food Funct., 2013 Jan 16. [Epub ahead of print]
35. Ohara K. The n-3 polyunsaturated fatty acid/dopamine hypothesis of schizophrenia. Prog Neuropsychopharmacol Biol. Psychiatry., 2007;31(2):469-74.
36. Ohkawa H., Ohishi N., Yagi K.,. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 1979; 95(2): 351-58.
37. Ozsoy O, Seval-Celik Y, Hacioglu G, Yargicoglu P, Demir R, Agar A, *et al.,* The influence and the mechanism of docosahexaenoic acid on a mouse model of Parkinson's disease. Neurochem Int., 2011;59(5):664-70.
38. Paglia D.E., Valentine W.N. Studies on the quantitative and qualitative characterization of

erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 1967; 70: 158-69.
39. Patten AR, Brocardo PS, Christie BR. Omega-3 supplementation can restore glutathione levels and prevent oxidative damage caused by prenatal ethanol exposure.J Nutr Biochem. 2012 Jul 25. [Epub ahead of print]
40. Potschka H. Animal models of drug-resistant epilepsy. Epileptic Disord., 2012;14(3):226-34.
41. Reeta KH, Mehla J, Gupta YK. Curcumin is protective against phenytoin-induced cognitive impairment and oxidative stress in rats. Brain Research, 2009;1301: 52–60.
42. Robinson S.J. Childhood epilepsy and autism spectrum disorders: psychiatric problems, phenotypic expression, and anticonvulsants. Neuropsychol Rev., 2012 ;22(3):271-9.
43. Rauca C, Wiswedel I, Zerbe R, Keilhoff G, Krug M. The role of superoxide dismutase and alpha-tocopherol in the development of seizures and kindling induced by pentylenetetrazol - influence of the radical scavenger alpha-phenyl-N-tert-butyl nitrone. Brain Res. 2004;1009(1-2):203-12.
44. Russell F.D., Bürgin-Maunder C.S. Distinguishing health benefits of eicosapentaenoic and docosahexaenoic acids. Mar. Drugs, 2012;10(11):2535-59.
45. Shannon HE, Love PL. Effects of antiepileptic drugs on learning as assessed by a repeated acquisition of response sequences task in rats. Epilepsy Behav., 2007;10(1):16-25.
46. Taha AY, Jeffrey MA, Taha NM, Bala S, Burnham WM. (2010). Acute administration of docosahexaenoic acid increases resistance to pentylenetetrazol-induced seizures in rats. Epilepsy Behav;17(3):336-43.
47. Vlooswijk MC, Vaessen MJ, Jansen JF, de Krom MC, Majoie HJ, Hofman PA, *et al.,* Loss of network efficiency associated with cognitive decline in chronic epilepsy. Neurology. 2011;77(10):938-44.
48. Yuen AW, Flugel D, Poepel A, Bell GS, Peacock JL, Sander JW. Non-randomized open trial of eicosapentaenoic acid (EPA), an omega-3 fatty acid, in ten people with chronic epilepsy.Epilepsy Behav. 2012 Mar;23(3):370-2.
49. Zhang Q, Ding D, Zhou D, Lin W, Wu Q, Sun J, *et al.,* Cognitive dysfunction in people with convulsive seizures in rural China. Epilepsy Behav., 2012;24(4):435-8.

4/15/2014