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ROLE OF FREE RADICALS AND CALCIUM MOBILIZATION IN THE PENTYLENETETRAZOL-INDUCED KINDLING IN RATS

El-Sayed E. El-Awady[1*], Sawsan A.B. Zaiton[1], Yasser M Moustafa[1], and Soad H. Abou-El-Ela[2]
[1]Department of Pharmacology & Toxicology, [2]Department of Biochemistry, Faculty of Pharmacy,
Suez Canal University

ABSTRACT:

Epilepsy is one of the most common neurologic disorders. The currently available antiepileptic agents do not provide adequate control of the disease in all epileptic patients. The present study was conducted to investigate the role of free radical scavengers e.g., lipoic acid and coenzyme Q10, and the calcium channel blocker, nifedipine, on pentylenetetrazol (PTZ)-induced kindling in rats.

A hundred and sixty male albino rats were used in the current study. Animals were divided into 16 groups, 10 animals each. Kindled group, rats injected with subconvulsive doses of PTZ (30 mg/kg), i.p, three times a week for totals of 13 injections. Control group, rats received 13 injections of normal saline. Groups 3 and 4, kindled rats were pretreated with diazepam (0.5 mg/kg, i.p) or phenytoin (50 mg/kg, i.p). Groups 5-10 received lipoic acid (50 mg/kg, i.p) or coenzyme Q10 (10 mg/kg) alone or in combination with diazepam (0.5 mg/kg, i.p) or phenytoin (50 mg/kg, i.p). Groups 11-16, kindled animals were pretreated with nifedipine (2 mg/kg, i.p) or nifedipine (10 mg/kg, i.p) alone or in combination with diazepam (0.5 mg/kg, i.p) or phenytoin (50 mg/kg, i.p). The convulsive behavior of the animals was evaluated using Racine-scaling method. Biochemical analysis of lipid peroxides levels, glutatione peroxidase, catalase, and superoxide dismutase activities was conducted for blood of all animals.

Both the antioxidants and the calcium channel blocker *per se* demonstrated some protection against PTZ kindling. In addition, lipoic acid potentiated the anticonvulsant effects of diazepam and phenytoin. The biochemical analysis of oxidative stress markers came in line with the anticonvulsant effects of drugs. Lipid peroxides and glutathione peroxidase were most sensitive, whereas catalase and superoxide dismutase were least sensitive to the effects of drugs.

It is concluded that lipoic acid, coenzyme Q10, and nifedipine may have important potentials as adjunct medications with antiepileptic drugs, especially in patients refractory to conventional antiepileptic treatment.

INTRODUCTION

Epilepsy is one of the most common neurologic diseases worldwide with a prevalence of approximately 1% of the total population[1]. Epilepsy continues to be a neurological disorder awaiting safer drugs with improved anticonvulsant and anti-epileptogenic effectiveness. Currently available drugs fail to provide adequate control of epileptic seizures in about one-third of patients and do not prevent progressive epileptogenic changes[2].

Kindling is a recognized model of epilepsy, which was first described by Goddard et al.[3] as the repeated application of initially subconvulsive electrical stimulation of different brain structures resulting in a progressive development of generalized seizures. Kindling can also be obtained by administration of subconvulsive doses of chemicals such as pentylenetetrazol[4] and FG 7142, a benzodiazepine receptor inverse agonist[5].

Repeated administration of a small dose of pentylenetetrazol (PTZ) lead to a progressive decrease in seizure threshold, resulting in generalized seizures[6]. This sort of models, therefore, allows for evaluating neuronal plastcity associated with long-term alterations in neural excitability[7]. This has been associtaed with an increase in susceptibility of the glutamatergic systems[8] and an enhancement of glutamate receptor density.

The enhanced activity of glutamatergic systems induces an increased formation of free oxygen radicals. These may amplify again the basal release of excitatory amino acids and increase the intracelluar calcium resulting in neuronal cell death[9].

Reactive oxygen species have been implicated in the development of seizures under pathological conditions and linked to seizure-induced neurodegeneration. Evidence includes the temporal correlation between free radical generation and the development of seizures in some pathological condition[10] and the protective efficacy of antioxidants treatment against some types of seizures[11].

In Addition, Calcium ions play a central role in the control of neuronal excitability[12]. Accumulating evidence shows that Ca^{2+} channels, modulated by dihydropyridines, play a facilitatory role in experimental seizures[13]. Intensive research has highlighted that calcium is an important factor involved in epileptogenesis and neurotoxicity during status epilepticus[14].

Modulation of calcium influx (by drugs affecting glutamate activation or calcium channel blockers as flunarizine) may suppress chronic seizures-induced glutamate impairment and its consequences. Ca^{2+} channel antagonists have been shown to block various aspects of epileptogenesis and are effective anticonvulsants in a number of in vivo models of epilepsy[15].

The present study was carried out to investigate

* Corresponding author

the anticonvulsant effect of the antioxidants, lipoic acid and coenzyme Q10, and the calcium channel blocker, nifedipine *per se*. In addition, their modulatory effect on the anticonvulsant activity of diazepam or phenytoin in PTZ-kindled rats was assessed.

MATERIALS AND METHODS

Animals:

A hundred and sixty male albino rats, obtained from the National Institute of Drug Control and Research, were used in the current study. Animals had an initial body weight 100-130 g. They were kept under controlled laboratory conditions, normal light-dark cycle, temperature 25-30° C, and relative humidity 55-65 %. The animals were housed as pairs of rats per cage. They had free access to food and water throughout the study period. All the experiments were performed between 10:00 a.m. and 2:00 p.m. to minimize circadian influences on seizure susceptibility.

Drugs and chemicals:

Pentylenetetrazol, PTZ (Sigma), Phenytoin, PHT (Epanutin® ampoule, Abbott laboratory, Chicago, USA), Diazepam, DZP (Valpam® ampoule, Amoun Co. Egypt), Coenzyme Q10, CoQ10 (Sigma), Lipoic acid, LA (Thiotacid® ampoule, Eva laboratory, Egypt), and Nifedipine, NFD (EIPICo. Egypt), Isoflurane (Sigma), EDTA (ADWIC, Egypt), Thiobarbituric acid (Sigma), SOD kit (RANSOD, Randox, UK), and glutathione peroxidase kit (RANSEL, Randox, UK).

Lipoic acid and coenzyme Q10 are sensitive to light, so all glassware used for mixing and injection were opaque and covered with aluminum foil.

Experimental design:

Animals were divided into 16 groups, 10 rats each.

1- Control group: Rats received 13 injections of normal saline in parallel to each PTZ injection.
2- Kindled group: Rats were injected with subconvulsive doses of PTZ (30 mg/kg body weight, i.p.), three times a week for totals of 13 injections[4].
3- Kindled animals pretreated with DZP: Rats were pretreated with DZP at a dose of 0.5 mg/kg, i.p., 60 minutes before each PTZ injection[16].
4- Kindled animals pretreated with PHT: Rats were pretreated with PHT at a dose of 50 mg/kg, i.p., 60 minutes before each PTZ injection[16].
5- Kindled animals pretreated with LA: Rats were pretreated with LA at a dose of 50 mg/kg, i.p., immediately before each PTZ injection.
6- Kindled animals pretreated with CoQ10: Rats were pretreated with CoQ10 at a dose of 10 mg/kg, i.p., immediately before each PTZ injection[17].
7- Kindled animals pretreated with DZP and LA.
8- Kindled animals pretreated with PHT and LA.
9- Kindled animals pretreated with DZP and CoQ10.
10- Kindled animals pretreated with PHT and CoQ10.
11- Kindled animals pretreated with NFD (2 mg/kg): Rats were pretreated with NFD at a dose of 2 mg/kg, i.p., 30 minutes before each PTZ injection[18].
12- Kindled animals pretreated with NFD (10 mg/kg): Rats were pretreated with NFD at a dose of 10 mg/kg, i.p., 30 minutes before each PTZ injection[19].
13- Kindled animals pretreated with DZP and NFD (2 mg/kg).
14- Kindled animals pretreated with PHT and NFD (2 mg/kg).
15- Kindled animals pretreated with DZP and NFD (10 mg/kg).
16- Kindled animals pretreated with PHT and NFD (10 mg/kg).

Doses and treatment regimens of all drugs were also applied in drug combination groups as in individual drug-treatment groups. Selection of doses and injection intervals was made according to our preliminary studies and the pharmacokinetic data reported in the literatures.

Kindling procedures and scoring

Rats were randomly allocated to kindled- and control (saline-injected) groups. Rats were injected with subconvulsive doses of PTZ (30 mg/kg, i.p.), three times a week, for a total of 13 injections. After each injection, the convulsive behavior was observed for 20 minutes and classified using a modified rating scale according to Racine[20], Table 1. Seizure duration is the duration of limbic seizures (stage 1-2) and motor seizures (stage 3-5).

Table 1: Racine rating scale for evaluation of seizures.

Symptoms	Score
No seizure response.	0
Immobility, eye closure, ear twitching, twitching of vibrissae, sniffing, facial clonus.	1
Head nodding associated with more severe facial clonus.	2
Clonus of one forelimb.	3
Bilateral forelimb clonus without rearing.	3.5
Bilateral forelimb clonus with rearing.	4
Falling on a side (without rearing), loss of righting reflex accompanied by generalized clonic seizures.	4.5
Rearing and falling on back accompanied by generalized clonic seizures.	5

Biochemical analysis:

After the last PTZ injection (fully kindled), all rats were anaesthetized with isoflurane and blood samples were obtained from the orbital sinus. Blood was collected on EDTA (29 µg/ml blood) and centrifuged to obtain plasma and erythrocytes. Plasma was kept in a deep freezer at -80°C until the analysis of lipid peroxides using the method described by Yagi[21],

based on the reaction with thiobarbituric acid. Erythrocytes were washed three times with normal saline and centrifuged at 3000 rpm for 10 minutes. The obtained erythrocyte pellets were then separated. The erythrocyte lysate was obtained by resuspending the erythrocytes in ice-cold distilled water. The lysate was kept in a deep freezer at -80° C until used for enzymatic assay. Total glutathione peroxidase activity was determined using reduced glutathione and cumene hydroperoxide as described by Paglia et al.[22]. Total superoxide dismutase activity was determined using xanthine and xanthine oxidase method[23]. Catalase activity was measured using hydrogen peroxide as a substrate according to the method of Aebi[24]. Protein concentration was measured as described by Lowry et al.[25].

Statistical analysis:

Values are expressed as mean ± SEM. One-way analysis of variance, ANOVA, followed by Bonferroni's multiple comparison tests were applied for statistical analysis. For all comparisons, differences were considered significant at $p < 0.05$.

RESULTS

In the present study, repeated administration of subconvulsive doses of PTZ (30 mg/kg) for totals of 13 injections produced kindling with a mean seizure score 3.6±0.04 (Fig. 1). That was significantly different from the control group, which received saline.

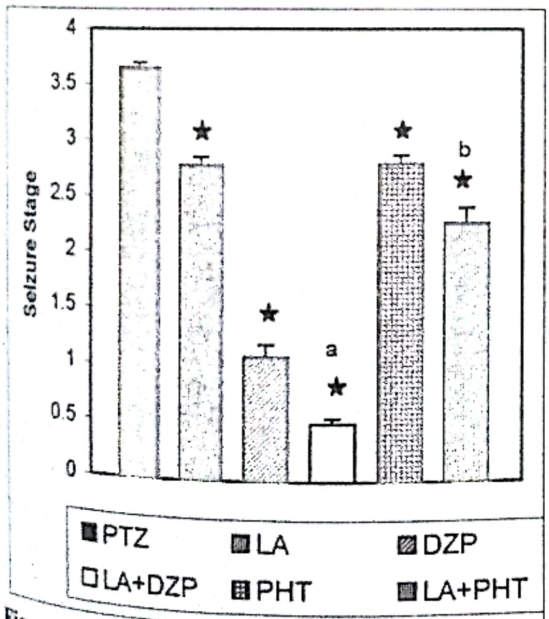

Figure 1: The anticonvulsant effects of LA and its combinations with DZP and PHT in PTZ-kindled rats.
*: Significantly different from PTZ-kindled group at p<0.05.
a: Significantly different from DZP group at $p < 0.05$
b: Significantly different from PHT group at $p < 0.05$

Biochemical analysis of blood demonstrated significant differences between PTZ-group and the control group. The kindled group exhibited elevated levels of lipid peroxides (PTZ, 6.65±0.89 vs. saline, 2.25±0.05, Table 2), glutathione peroxidase (PTZ, 10.62±2.36 vs. saline 1.03±0.13), and catalase (PTZ, 10.04±1.29 vs. saline, 4.54±0.67). There was no change in SOD activity from control in any of the treatment groups (SOD, 5.55 unit/ml).

Table 2: The effect of LA and its combination with DZP and PHT on lipid peroxides level (LP), glutathione peroxidase (GPx), and catalase activities in PTZ-kindled rats.

	LP	GPx	CAT
Saline	2.25±0.05*	1.03±0.13*	4.54±0.67*
PTZ	6.65±0.89	10.62±2.36	10.04±1.29
LA	2.94±0.36*	5.31±0.81*	6.88±0.78*
DZP	3.44±0.38*	3.84±0.73*	7.7±0.84
LA+DZP	2.77±0.31*,a	2.76±0.55*	5.19±0.66*,a
PHT	5.22±0.80	8.36±0.68	8.36±1.31
LA+PHT	2.74±0.29*,b	3.27±0.62*,b	5.35±0.21*,b

*: Significantly different from PTZ at $p<0.05$.
a: Significantly different from DZP at $p<0.05$.
b: Significantly different from PHT at $p<0.05$.

Pretreatment with LA could partially protect the animals against the convulsive properties of PTZ (LA, 2.78±0.07 vs. PTZ, 3.6±0.04, $p<0.05$, Fig. 1). However, DZP showed a better protection against PTZ-induced kindling (DZP, 1.1±0.1). The combination of LA and DZP produced a further depression in seizure score , which was significantly different from DZP alone (LA+DZP, 0.50±0.05, Fig. 1).

Although PHT exhibited anticonvulsant effect, its effect was not significantly different from that of LA (PHT, 2.80±0.07, Fig. 1). The combination of the two drugs produced a reduction in seizure score that was significantly different from either treatment alone (LA+PHT, 2.27±0.13, Fig. 1).

Table 2 shows that lipid peroxides level in PTZ kindled group was significantly higher than all other treatment groups except PHT group (PTZ, 6.65±0.89 vs. PHT, 5.22±0.8). Similar phenomenon was observed with glutathione peroxidase activity; PHT failed to induce significant change (PHT, 8.36±0.68, Table 2). Moreover, only LA and its combination with DZP or PHT exhibited significant differences from PTZ group in catalase activity (Table 2). This catalase activity observed with the combination of LA and DZP or PHT was also significantly different from DZP or PHT, respectively (Table 2). Furtermore, the lipid peroxides level observed with the combination of LA with DZP or PHT was also significantly different from DZP or PHT, respectively (Table 2).

Figure 2 illustrates the effects of CoQ10, DZP, PHT, and the combination of CoQ10 with DZP or PHT on PTZ-kindled rats. CoQ10 successfully decreased the seizure stages (CoQ10, 2.61±0.11). DZP offered a better protection against PTZ kindling (DZP, 1.1±0.1, Fig. 2). The combination of CoQ10 and DZP

was not significantly different from DZP (CoQ10+DZP, 0.89±0.14, Fig. 2). Although PHT induced a significant depression in seizure stages (PHT, 2.8±0.07), PHT and its combination with CoQ10 did not differ from CoQ10 alone.

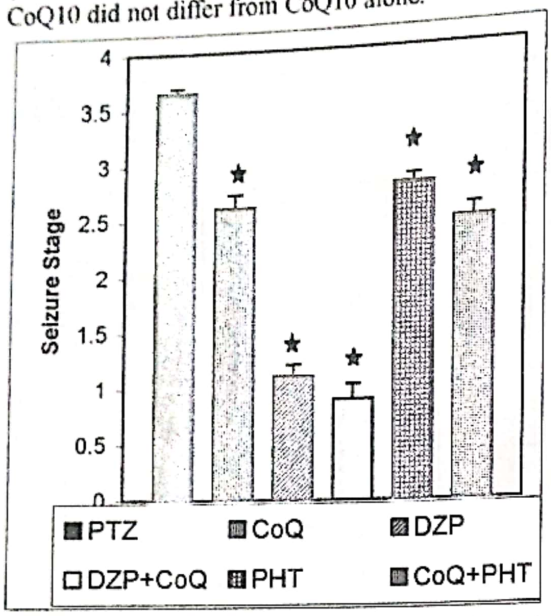

Figure 2: The anticonvulsant effects of CoQ10 and its combinations with DZP and PHT in PTZ-kindled rats.
*: Significantly different from PTZ-kindled group at $p<0.05$.

The biochemical data was in line with the behavioral effects of various drug treatments. PHT was least effective on any of the measured parameters. Table 3 shows that all treatments, with the exception of PHT, could significantly reduce the elevated levels of lipid peroxides. In addition, the combination of PHT and CoQ10 was signficantly different from PHT alone (Table 3).

Table 3: The effect of CoQ10 and its combination with DZP and PHT on lipid peroxides level (LP), glutathione peroxidase (GPx), and catalase activities in PTZ-kindled rats.

	LP	GPx	CAT
Saline	2.25±0.05*	1.03±0.13*	4.54±0.67*
PTZ	6.65±0.89	10.62±2.36	10.04±1.29
CoQ10	4.20±0.61*	6.27±1.01	6.92±0.89
DZP	3.44±0.38*	3.84±0.73*	7.7±0.84
CoQ10+DZP	3.02±0.46*	2.72±0.74*	6.07±0.80*
PHT	5.22±0.80	8.36±0.68	8.36±1.31
CoQ10+PHT	3.45±0.45*,b	3.35±0.55*,b	5.86±0.79*

*: Significantly different from PTZ at $p<0.05$.
b: Significantly different from PHT at $p<0.05$.

Glutathione peroxidase activity was less sensitive to both CoQ10 and PHT; only the combination treatments with CoQ10 and PHT or DZP were significantly different from PTZ group (Table 3). Similar results were obtained with Catalase, the combination treatment of CoQ10 and DZP or PHT were different from PTZ (Table 3).

The present study revealed a protective effect of NFD (2 mg/kg) against PTZ-induced seizures. The small dose of NFD (2 mg/kg) could depress the seizure score (NFD, 2.96±0.33 vs. PTZ, 3.6±0.04, $p<0.05$, Fig. 3). This effect was not significantly different from the effect of PHT. However, their combination was beneficial (PHT+NFD, 2.02±0.03, Fig. 3), the combination was significantly different from PHT alone. In contrast, this dose of NFD failed to enhance the anticonvulsant effect of DZP (DZP, 1.11±0.1 vs. DZP+NFD, 0.84±0.05, Fig. 3).

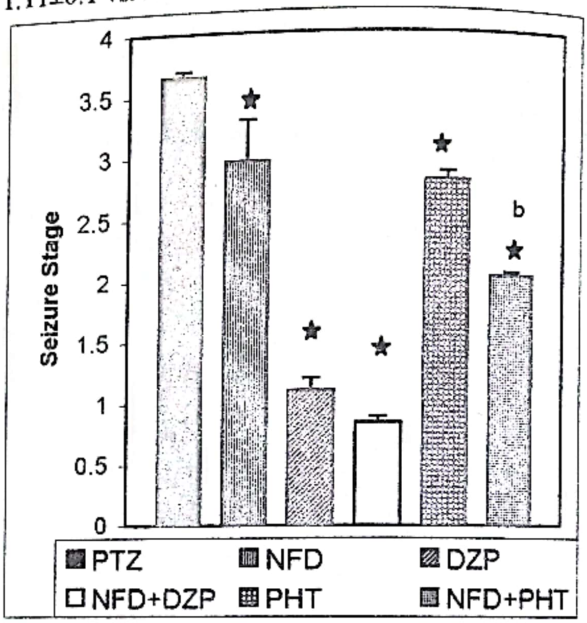

Figure 3: The anticonvulsant effects of NFD (2 mg/kg) and its combinations with DZP and PHT in PTZ-kindled rats
*: Significantly different from PTZ-kindled group at $p<0.05$.
b: Significantly different from PHT group at $p< 0.05$

Table 4: The effect of NFD (2 mg/kg) and its combination with DZP and PHT on lipid peroxides level (LP), glutathione peroxidase (GPx), and catalase activities in PTZ-kindled rats.

	LP	GPx	CAT
Saline	2.25±0.05*	1.03±0.13*	4.54±0.67*
PTZ	6.65±0.89	10.62±2.36	10.04±1.29
NFD	4.80±0.78*	8.36±1.41	9.31±1.36
DZP	3.44±0.38*	3.84±0.73*	7.7±0.84
NFD + DZP	3.5±0.50*	3.74±0.62*	7.58±1.01
PHT	5.22±0.80	8.36±0.68	8.36±1.31
NFD + PHT	4.32±0.66*,b	3.98±0.63*,b	8.12±1.09

*: Significantly different from PTZ at $p<0.05$.
b: Significantly different from PHT at $p<0.05$.

Biochemical analysis of oxidative stress markers indicated that NFD (2 mg/kg) and its combinations with DZP or PHT produced a significant reduction in lipid peroxides levels as compared to PTZ group

(NFD, 4.8±0.78 vs. PTZ, 6.65±0.89, p<0.05, Table 4). In addition, the nonsignificant effect of PHT on glutathione peroxidase activity became significant when combined with NFD (Table 4). None of the treatment groups had significant effect on neither catalase nor SOD activities.

The higher dose of NFD (10 mg/kg) was more effective in suppressing seizure stage (NFD, 2.28±0.34, Fig. 4). In contrast to the low dose, NFD (10 mg/kg) enhanced the anticonvulsant activity of DZP (DZP, 1.11±0.1 vs. DZP+NFD, 0.44±0.05, p<0.05, Fig. 4). Similar observation was noted with PHT; NFD potentiated the anticonvulsant effect of PHT (PHT, 2.8±0.07 vs. PHT+NFD, 1.86±0.05, p<0.05, Fig. 4).

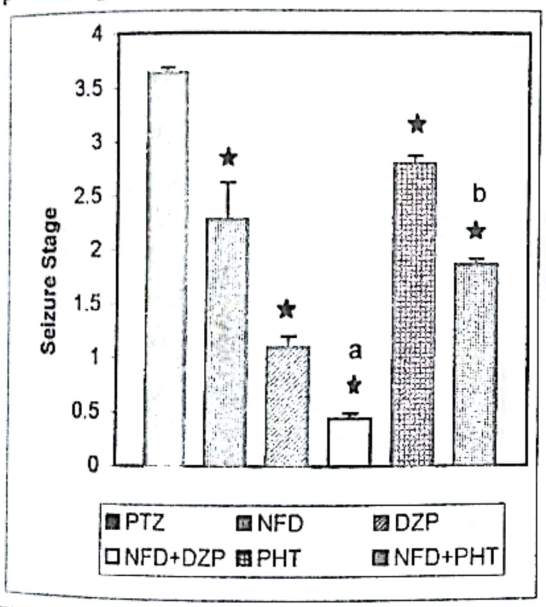

Figure 4: The anticonvulsant effects of NFD (10 mg/kg) and its combinations with DZP and PHT in PTZ-kindled rats.
*: Significantly different from PTZ-kindled group at p<0.05.
a: Significantly different from DZP group at p< 0.05
b: Significantly different from PHT group at p< 0.05

Table 5: The effect of NFD (10 mg/kg) and its combination with DZP and PHT on lipid peroxides level (LP), glutathione peroxidase (GPx), and catalase activities in PTZ-kindled rats.

	LP	GPx	CAT
Saline	2.25±0.05*	1.03±0.13*	4.54±0.67*
PTZ	6.65±0.89	10.62±2.36	10.04±1.29
NFD	3.99±0.57*	7.31±1.25	8.5±1.25
DZP	3.44±0.38*	3.84±0.73*	7.7±0.84
NFD + DZP	3.18±0.40*	3.58±0.68*	7.16±0.99
PHT	5.22±0.80	8.36±0.68	8.36±1.31
NFD + PHT	3.54±0.47*,b	3.84±0.68*,b	7.31±0.93

*: Significantly different from PTZ at p<0.05.
b: Significantly different from PHT at p<0.05.

The biochemical profile of the high dose of NFD (10 mg/kg) was not different from that of the low dose. NFD (10 mg/kg) produced a significant reduction in lipid peroxides (NFD, 3.99±0.57 vs. PTZ, 6.65±0.89, Table 5). Moreover, the combination of NFD and PHT produced significant reduction in lipid peroxides level as compared to PHT alone (Table 5). This effect was absent with the enzymes glutathione peroxidase, catalase, and SOD.

Table 6 summerizes the anticonvulsant effects of various drug treatments used in the current study. The inhibitory effect of these drugs on PTZ-induced kindling was expressed as a percentage of the maximal seizure score. the antioxidant drugs, LA and CoQ10, and NFD could demonstrate some anticonvulsant effects in the present model of seizures. The combinations of these drugs with either DZP or PHT were more effective than the individual treatment with DZP or PHT. The biochemical results came in line with the anticonvulsant effects of drugs. Lipid peroxides was the most sensitive parameter, whereas SOD was least sensitive to the effects of the applied drugs.

Table 6: The aniconvulsant activities of various drug treatments are expressed as anticonvulsant %, where (the maximum convulsive score – observed convulsive score) is calculated as a percentage of the maximum convulsive score (5).

Group	Score ± SEM	Anticonvulsant % (5 – Score)/5 × 100
Diazepam (0.5 mg/kg)	1.09 ± 0.10	78.2
Phenytoin (50 mg/kg)	2.8 ± 0.07	44
Lipoic acid (50 mg/kg)	2.78 ± 0.07	44.4
Coenzyme Q10 (10 mg/kg)	2.61 ± 0.11	47.8
Nifedepine (2 mg/kg)	2.96 ± 0.33	40.8
Nifedepine (10 mg/kg)	2.28 ± 0.33	54.4
Diazepam + Lipoic acid	0.5 ± 0.05	90
Diazepam + Coenzyme Q10	0.88 ± 0.14	82.4
Diazepam + NFD (2 mg/kg)	0.84 ± 0.05	83.2
Diazepam+ NFD (10 mg/kg)	0.43 ± 0.05	91.4
Phenytoin + Lipoic acid	2.27 ± 0.13	54.6
Phenytoin + Coenzyme Q10	2.47 ± 0.11	50.6
Phenytoin + NFD (2 mg/kg)	2.02 ± 0.03	59.6
Phenytoin + NFD (10 mg/kg)	1.86 ± 0.05	62.8

DISCUSSION

Pharmacotherapy of epilepsy utilizes chronic administration of anticonvulsants with the intent to prevent the occurrence of convulsive seizures. Nevertheless, no effective prophylaxis or pharmacotherapeutic cure of epilepsy is currently available[26]. Efforts have been done to intervene in the development of epilepsy-epileptogenesis through administration of antiepileptic agents[27]. However, Anticonvulsant therapy, is neither universally effective nor invariably safe.

Specific knowledge about epileptogenesis may share new light regarding methods for prophylaxis or treatment. Investigations with various animal models have provided experimental evidences for the heterogeneity of events inherent to epileptogenesis[28]. In vivo chronic models of epilepsy, in which repeated administration of a subconvulsive electrical or chemical stimulus gradually develops an epileptic state, provides an appropriate approach for quantifying epileptogenesis[29].

Kindling is the term used to describe the phenomenon whereby repeated administration of an initially subconvulsant chemical stimulus ultimately results in the generation of seizure activity[30]. Examples of chemicals producing kindling in rats and mice include PTZ, FG 7142, and picrotoxin[31].

Various mechanisms for the genesis of seizures have been proposed, however, overexcitation of excitatory amino acid, glutamate, and inhibition of GABAergic system have gained much acceptance[32]. Glutamatergic transmission, particularly N-methyl-D-aspartate (NMDA) receptors, seems to be critical to the plastic changes associated with kindling, ultimately leading to decreased seizure threshold[33].

Although the increased neuronal excitability, which is presumably the basis of kindling, may be associated with increased excitatory, and more specifically NMDA-mediated glutamate, neurotransmission, it may also be linked to decreased inhibitory neurotransmission[34]. Pharmacological evidence shows that compounds that modulate GABAergic function via the $GABA_A$ receptor alter the susceptibility to PTZ-induced seizure activity in rats[35] and mice[36].

Neuronal cell death that may result from the increased activity of glutamatergic systems, plays a crucial role in epilepsy. It has been shown that the glutamatergic transmitter system is modified following the PTZ kindling[37]. The activation of glutamate receptors and the increase in intracellular calcium, the hallmarks of excitotoxicity, have been documented during epileptiform events in various models of epilepsy[38]. Free radicals have been reported to be generated due to the glutamate-mediated increase ofcalcium ions[39], and to be a prerequisite for excitotoxic neuronal death[40]. Accordingly, the antioxidative therapies have been shown to decrease cell death associated with kainate-induced seizures[41] and iron-induced epilepsy[42]. Moreover, Schmidt[43] found that vitamin E did retard the course of PTZ kindling. N-omega-nitro-L-arginine methyl ester was significantly able to suppress the PTZ kindling development[44]. One possible reason for this may be the reduction of the kindling-induced hydroxyl radical formation. It may be assumed that further reasons exist for the increased hydroxyl radical formation in kindled animals during PTZ seizure, such as a reduced activity of antioxidant enzymes.

In the present study, the antioxidants, LA and CoQ10, exhibited some protection against PTZ-induced seizures. This effect was accompanied by a reduction in oxidative stress; however, there was no change in SOD activity. These data suggest that SOD be not utilized as the major free radical scavenging system. More likely, although as yet unproven, alternative scavenging systems, such as glutathione peroxidase or catalase, come into play. Glutathione is an essential tripeptide, and an endogenous antioxidant found in all animal cells. It reacts with free radicals and can protect from singlet oxygen[45]. The present results indicated that antioxidants and NFD and their combinations with DZP or PHT maintained glutathione concentrations close to normal values. In contrast, PTZ group showed an elevated antioxidant enzyme activity, which took place as a response to the presumed status of oxidative stress. In agreement with this view, Koch et al.[46] showed that rat liver after chronic ethanol, a free radical generator, feeding exhibited an increased manganese superoxide dismutase (MnSOD) activity with an upregulation of the enzyme at the mRNA level. The same study showed that chronic intake of ethanol also led to a significant decrease in the content of vitamin E in both the liver mitochondrial and microsomal fractions.

Kainic acid has been shown to induce free radical formation by different pathways. Since kainic acid is a glutamate agonist, it can act to activate glutamate receptor to incite the entry of calcium intracellularly[47]. The influx of intracellular calcium can stimulate free radical formation through various mechanisms including mitochondrial dysfunction and activation of nitric oxide synthase[48]. In fact, epileptiform bursts are often associated with influx of Ca^{2+}into nerve cells[49] and a decrease in the extracellular concentration of Ca^{2+}precedes the onset of seizures in many experimental models of epilepsy[50].

It has been proposed that Ca^{2+} currents may contribute to epileptogenesis by a) undergoing bursting in pacemaker cells, b) enhancing postsynaptic excitatory responses in dendrites and somatic nerve cells, c) providing post-burst re-excitation[51]. Ca^{2+} is not only important in genesis and spread of seizures but is also involved in neuronal injury that is caused as a result of repeated seizures.

Since the entry of Ca^{2+} into neurons seems to play an important role in epileptogenesis, the use of Ca^{2+}channel blockers in the treatment of these seizures may be successful. In fact, blockers of voltage dependent Ca^{2+}channels display anticonvulsant activity in various models of experimental convulsions[52] and in human[53]. Accordingly, various antiepileptic drugs have been shown to reduce the transmembrane flux of Ca^{2+} at their therapeutic levels or at high concentrations[54].

The calcium channel blocker, nimodipine, has been found to be effective against seizures induced by high doses of pilocarpine[55] and kainic acid[56]. Meyer et al.[57] showed that nimodipine when administered in a dose of 5 mg/kg orally for 5 days, increased the seizure-threshold by 50-60% in rabbits. When nimodipine was combined with DZP, it produced synergistic anticonvulsant activity against PTZ-induced seizures.

The present results indicated that combination of the Ca^{2+} channel blocker, NFD, with DZP produced a synergistic and potent action as compared to DZP alone. The broader implication of this report suggests a role for Ca^{2+}channel blockers as an adjunctive therapy in epilepsy. Their degree of anticonvulsant activity may not render them useful as anticonvulsants *per se*, NFD may be used as an adjunct medication with DZP to provide a greater clinical effectiveness against epileptic seizures.

The potentiation of anticonvulsant activity could be related to central blockade of calcium ion entry. Calcium channel subtype, L-channel, is modulated by dihydropyridine agonists and antagonists[49]. Therefore, the anticonvulsant effects may be due to antagonism of Ca^{2+} influx through dihydropyridine L-channel. It is currently accepted that GABA neurons are inhibitory through the modulation of the L-channel and enhancing the inward Cl^- conductance. Moreover, they also provide a site at which the endogenous Ca^{2+}antagonist could exert its anticonvulsant effects[58].

The current results indicated that NFD potentiated the anticonvulsant activity of PHT. Though the exact mechanism of Ca^{2+}channel blockers is not clear, it may be speculated that they potentiate the antiepileptic activity of phenytoin possibly by blockade of voltage-dependent Ca^{2+}channels[59] and thus exert a synergistic effect with phenytoin. Phenytoin exerts an antiepileptic effect by stabilization of neuronal membrane thereby prolonging the recovery of activated sodium channels and by inhibiting post-tetanic potential probably due to Ca^{2+}influx inhibition. Hence, antiepileptic effect was potentiated as seen in different models of experimental epilepsy[60].

Electrophysiological evidence has indicated that Ca^{2+} currents in hippocampal cells are sensitive to voltage-dependent Ca^{2+} channel agonists and antagonists[61]. Moreover, the central actions of voltage-dependent Ca^{2+}channel blockers show anticonvulsant activity, depending on the kind of experimental convulsions investigated. Thus, the protection offered by NFD in kindled rats is in accordance with the literature[62].

Taken together, kindling is a multifactorial complex that involves changes at different levels, e.g., seizures, free radical generation, physiological and neurochemical alterations, and changes in behavior. Once these pathway are better understood, it will provide better rationale design for interventional strategies to reduce brain injury associated with seizure disorders and epilepsy.

The antioxidants, lipoic acid and coenzyme Q10, and the Ca^{2+}channel blocker, nifedipine, may have an important potential as an adjunct medication with antiepileptic drugs. They will be helpful in those patients in whom conventional therapies have been inadequate, or patients who are refractory to anticonvulsant treatment, or in cases of intreatable epilepsy.

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دور المشتقات النشطة وحركة الكالسيوم في التشنجات المزمنة المحثة بالبنتيلين تترازول في الجرذان

السيد العوضى[1*] ، سوسن زيتون[1] ، ياسر مصطفى[1] وسعاد أبو العلا[2]

[1] قسم الأدوية والسموم –[2] قسم الكيمياء الحيوية – كلية الصيدلة – جامعة قناة السويس

تهدف الدراسة الحالية لبحث تأثير مضادات الأكسدة – حامض الليبويك وكوانزيم كيو ١٠ – وكذلك تأثير غالقة قناة الكالسيوم – نيفديبين – علي التشنجات المزمنة المحثة بمادة البنتيلين تترازول في الجرذان.

استخدم في هذه الدراسة مائة وستون جرذا . قسمت هذه الحيوانات إلى ست عشرة مجموعة بواقع ١٠ حيوانات في كل مجموعة – استخدمت مادة البنتيلين تترازول – بالحقن في تجويف البطن – بجرعة قدرها ٣٠ مجم / كجم ثلاث مرات أسبوعيا وبإجمالي ١٣ جرعة لعمل تشنجات مزمنة في الحيوانات . عولجت مجموعات الحيوانات بمادة الديازيبام (٠,٥ مجم / كجم) أو مادة الفينيتوين (٥٠ مج/كجم) منفردة أو مجتمعة مع مضادات الأكسدة ، حامض الليبويك (٥٠ مجم / كجم) أو كوانزيم كيو ١٠ (١٠ مجم / كجم) ، أو مع غالقة قناة الكالسيوم نيفديبين (٢جم / كجم) ، أو نيفديبين (١٠مجم / كجم) . استخدمت طريقة راسين في تقييم التشنجات في الحيوانات . تم عمل تحليل لنسبة الدهون المؤكسدة والانزيمات المضادة للاكسدة في الدم.

وجد من هذه الدراسة أن مضادات الأكسدة وغالقة قناة الكالسيوم استطاعت أن توفر بعض الحماية ضد التشنجات المزمنة والمحثة بالبنتيلين تترازول . وقد اضافت هذه المواد إلى التأثير الواقي لمضادات الصرع المستخدمة. وقد جاءت نتائج التحليل لمؤشرات الإجهاد التأكسدي لتؤكد دور الإجهاد التأكسدي في الصرع ، وقد كان مؤشر الدهون المؤكسدة والجلوتاثيون بيرأوكسيداز اكثر حساسية لتأثير المواد المستخدمة من الكتالاز والسوبر أوكسيد ديسميوتاز.