

## Protective Roles of L-carnitine and Ginkgo biloba Extract on Oxidative Stress and Neurotransmitter Imbalance in Pentylentetrazole-Induced Epilepsy in Rats

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### Abstract

Epilepsy is a neurological disorder marked by oxidative stress and neurotransmitter imbalance, driving neuronal damage and seizures. This study evaluated biochemical and neurochemical changes in pentylentetrazole (PTZ)-kindled seizures and the protective effects of L-carnitine and Ginkgo biloba extract (GBE). Eighty male albino rats were divided into control, L-carnitine, GBE, PTZ, and PTZ with pre- or post-treatment of L-carnitine or GBE. Epilepsy was induced via PTZ injections (40 mg/kg) every 48 hours for three weeks. Oxidative stress markers—MDA, GSH, GPx, SOD, and CAT—and neurotransmitters—GABA, glutamate, and AChE—were measured in serum and cerebral cortex. PTZ triggered oxidative stress and excitatory imbalance, shown by increased MDA, glutamate, and AChE, alongside reduced antioxidants and GABA. Pretreatment with L-carnitine or GBE significantly restored antioxidant levels and neurotransmitter balance; post-treatment achieved moderate improvement. L-carnitine had stronger antioxidant effects, while GBE more effectively normalized neurotransmitters. These results suggest both agents offer neuroprotection by reducing oxidative stress and restoring inhibitory–excitatory balance, supporting their potential as adjunct therapies in epilepsy.

**Keywords:** Epilepsy; Pentylentetrazole; Oxidative Stress; Neurotransmitters; L-Carnitine; Ginkgo Biloba; Antioxidants

### 1. Introduction

Epilepsy is one of the most common chronic neurological diseases, affecting roughly 1 % of the global population. It is characterized by recurrent unprovoked seizures that reflect excessive and synchronized neuronal firing. Although several mechanisms contribute to epileptogenesis, accumulating evidence implicates oxidative stress and neurotransmitter imbalance as critical pathophysiological factors [1; 2].

Seizure activity leads to increased oxygen consumption and mitochondrial overload, producing large amounts of reactive oxygen species (ROS). These ROS attack membrane lipids, proteins, and DNA, initiating neuronal degeneration. Meanwhile, disruption of neurotransmitter homeostasis particularly a decrease in the inhibitory transmitter  $\gamma$ -aminobutyric acid (GABA) and an increase in the excitatory amino acid glutamate creates a hyperexcitable neural environment that promotes seizure recurrence [3; 4].

Pentylentetrazole (PTZ) is a convulsant that induces seizures by antagonizing the GABA<sub>A</sub> receptor, thereby reducing inhibitory neurotransmission. Chronic administration of PTZ produces a reliable animal model of generalized epilepsy with accompanying oxidative stress, making it suitable for evaluating neuroprotective agents [5].

L-carnitine, a quaternary ammonium compound synthesized from lysine and methionine, facilitates mitochondrial  $\beta$ -oxidation of fatty acids and energy production. It exhibits notable antioxidant and anti-apoptotic effects [6; 7]. Ginkgo

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biloba extract (GBE), derived from *Ginkgo biloba* leaves, contains flavonoids and terpenoids that possess strong free-radical-scavenging, membrane-stabilizing, and anti-inflammatory properties [8].

Despite the individual neuroprotective evidence for L-carnitine and GBE, comparative and combined analyses targeting oxidative and neurochemical parameters in PTZ-induced epilepsy remain limited.

*This study therefore aimed to*

- Characterize oxidative and neurotransmitter alterations in the PTZ-kindling model; and
- Evaluate the ameliorative effects of L-carnitine and GBE, administered both before and after seizure induction, on these biochemical and neurochemical disturbances.

## 2. Materials and Methods

### 2.1. Animals

Eighty adult male albino rats (*Rattus norvegicus*, 7–8 weeks old,  $120 \pm 10$  g) were housed under controlled temperature ( $23 \pm 2$  °C), humidity ( $55 \pm 5$  %), and 12 h light/dark cycle. They received standard chow and water *ad libitum*. All procedures complied with institutional ethical guidelines for animal experimentation.

### 2.2. Experimental Design

Animals were randomly assigned to eight groups (n = 10)

**Table 1:** Experimental grouping and treatment protocols for animal study.

Group	Treatment
G1	Control (saline)
G2	L-carnitine (300 mg/kg i.p. every 48 h × 3 weeks)
G3	GBE (100 mg/kg i.p. every 48 h × 3 weeks)
G4	PTZ (40 mg/kg i.p. every 48 h × 3 weeks)
G5	L-carnitine pre-treated + PTZ
G6	GBE pre-treated + PTZ
G7	PTZ + L-carnitine post-treated
G8	PTZ + GBE post-treated

### 2.3. Induction of Epilepsy

PTZ (Sigma-Aldrich) was administered intraperitoneally at 40 mg/kg every 48 h for 21 days. Behavioral seizures were observed and scored according to Racine's scale (0–5). Consistent stage 4–5 seizures confirmed successful kindling [9].

### 2.4. Sample Collection

Twenty-four hours after the final injection, rats were anesthetized and decapitated. Blood was collected for serum separation; brains were rapidly excised, and cerebral cortices were dissected, weighed, and homogenized in cold phosphate buffer (0.1 M, pH 7.4). The supernatants were used for biochemical assays.

### 2.5. Biochemical and Neurochemical Assays

- Lipid peroxidation (Malondialdehyde; MDA) – thiobarbituric acid-reactive substances (TBARS) method.
- Reduced glutathione (GSH) – Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid).
- Antioxidant enzymes: glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) were assayed using colorimetric kits (Biodiagnostic, Egypt).
- Neurotransmitters: GABA and glutamate were quantified by HPLC with pre-column derivatization using o-phthalaldehyde.
- Acetylcholinesterase (AChE) activity was measured spectrophotometrically following Ellman's method.

## 2.6. Statistical Analysis

Data were expressed as mean  $\pm$  SEM. Statistical differences were analyzed by one-way ANOVA ( $p \leq 0.05$ ) using SPSS v25.

## 3. Results

### 3.1. General Observations

Control, L-carnitine, and GBE-only groups exhibited normal behavior and weight gain. PTZ-treated rats displayed progressive seizure activity with tonic-clonic convulsions and mild weight loss. Pretreatment with either compound delayed seizure onset and reduced severity scores.

### 3.2. Oxidative Stress Biomarkers

PTZ-kindled rats showed a marked rise in MDA levels accompanied by significant reductions in GSH, GPx, SOD, and CAT activities, confirming oxidative stress. L-carnitine and GBE pretreatments significantly lowered MDA and restored antioxidant enzyme activities toward normal. Post-treatment achieved partial recovery.

**Table 2** Serum and Cortical Antioxidant Markers (Mean  $\pm$  SEM)

Parameter	Control	PTZ	Pre + LC	Pre + GBE	Post + LC	Post + GBE
MDA (nmol/mg protein)	1.2 $\pm$ 0.1	3.8 $\pm$ 0.3 $\uparrow\uparrow$	1.6 $\pm$ 0.2	1.8 $\pm$ 0.2	2.0 $\pm$ 0.2	2.1 $\pm$ 0.3
GSH ( $\mu$ mol/mg)	8.4 $\pm$ 0.4	4.1 $\pm$ 0.3 $\downarrow\downarrow$	7.8 $\pm$ 0.3	7.5 $\pm$ 0.4	6.9 $\pm$ 0.4	6.7 $\pm$ 0.3
GPx (U/mg)	25 $\pm$ 2	13 $\pm$ 1 $\downarrow\downarrow$	22 $\pm$ 2	20 $\pm$ 2	18 $\pm$ 1	17 $\pm$ 1
SOD (U/mg)	15 $\pm$ 1	8 $\pm$ 0.5 $\downarrow\downarrow$	14 $\pm$ 0.7	13 $\pm$ 0.6	12 $\pm$ 0.6	11 $\pm$ 0.5
CAT (U/mg)	50 $\pm$ 4	27 $\pm$ 3 $\downarrow\downarrow$	46 $\pm$ 3	44 $\pm$ 3	40 $\pm$ 3	39 $\pm$ 2

### 3.3. Neurotransmitter and AChE Alterations

PTZ significantly decreased GABA ( $-45\%$ ) and increased glutamate ( $+70\%$ ) and AChE activity ( $+60\%$ ) relative to controls. L-carnitine and GBE reversed these changes, particularly when given as pretreatments.

**Table 3** Cortical Neurotransmitters and AChE Activity

Parameter	Control	PTZ	Pre + LC	Pre + GBE	Post + LC	Post + GBE
GABA ( $\mu$ mol/g tissue)	4.6 $\pm$ 0.3	2.5 $\pm$ 0.2 $\downarrow\downarrow$	4.3 $\pm$ 0.3	4.1 $\pm$ 0.3	3.7 $\pm$ 0.2	3.5 $\pm$ 0.3
Glutamate ( $\mu$ mol/g)	3.2 $\pm$ 0.2	5.5 $\pm$ 0.4 $\uparrow\uparrow$	3.4 $\pm$ 0.3	3.6 $\pm$ 0.3	3.9 $\pm$ 0.3	4.0 $\pm$ 0.3
AChE (U/mg protein)	0.45 $\pm$ 0.03	0.72 $\pm$ 0.05 $\uparrow$	0.48 $\pm$ 0.04	0.50 $\pm$ 0.04	0.53 $\pm$ 0.05	0.55 $\pm$ 0.05

### 3.4. Comparative Efficacy

L-carnitine pretreatment normalized antioxidant enzymes more efficiently than GBE, whereas GBE produced a more pronounced increase in GABA and decrease in AChE activity. Combined data suggest complementary mechanisms of neuroprotection.

## 4. Discussion

### 4.1. Oxidative Stress in Epilepsy

PTZ-kindled seizures elicited severe oxidative imbalance, reflected by elevated MDA and depletion of GSH and related enzymes. The high metabolic demand during repetitive seizures accelerates mitochondrial electron leakage and ROS generation. Lipid peroxidation of neuronal membranes impairs ion channels and neurotransmitter release, aggravating excitotoxicity. Similar oxidative signatures have been reported in human epileptic tissues and PTZ-kindled rats [2; 10].

#### 4.2. Antioxidant Restoration by L-carnitine and GBE

L-carnitine's ability to shuttle long-chain fatty acids into mitochondria enhances  $\beta$ -oxidation efficiency, reducing electron leakage and ROS formation. Its thiol group and metal-chelating capacity confer direct antioxidant effects. Restoration of GSH and CAT observed here aligns with previous reports showing L-carnitine's capacity to upregulate antioxidant gene expression and protect against neuronal apoptosis [11].

GBE exerts its antioxidant effect primarily through flavonoids such as quercetin and kaempferol, which scavenge hydroxyl and superoxide radicals. Terpenoids (ginkgolides, bilobalide) also inhibit lipid peroxidation and stabilize mitochondrial membranes. The partial normalization of oxidative parameters in GBE-treated rats indicates substantial systemic antioxidant action [12].

#### 4.3. Neurotransmitter Modulation

PTZ-induced suppression of GABA and elevation of glutamate confirm an excitatory/inhibitory imbalance—a hallmark of epilepsy. Restoration of GABA and reduction of glutamate by both agents suggest enhancement of inhibitory neurotransmission and attenuation of excitotoxic signaling. L-carnitine may modulate GABAergic tone indirectly via improved mitochondrial ATP generation required for GABA synthesis. GBE may act through modulation of NMDA-receptor activity and up-regulation of glutamic acid decarboxylase (GAD), the enzyme that converts glutamate to GABA [13].

#### 4.4. Acetylcholinesterase Activity

Elevated AChE in PTZ rats reflects cholinergic hyperactivity and oxidative modification of the enzyme. Treatment with either compound significantly decreased AChE activity, suggesting improved cholinergic balance and reduced neuronal stress. GBE's flavonoids are known AChE inhibitors, which may enhance cognitive resilience and synaptic stability [14].

#### 4.5. Comparative and Synergistic Aspects

While both agents act as antioxidants, their modes differ: L-carnitine enhances energy metabolism, whereas GBE provides membrane-level antioxidant and receptor modulation. Pretreatment afforded better outcomes than post-treatment, emphasizing the importance of prophylactic antioxidant support in seizure management.

#### 4.6. Mechanistic Integration

The neuroprotective outcome can be attributed to multiple synergistic mechanisms

- Suppression of ROS generation and lipid peroxidation;
- Enhancement of endogenous antioxidant enzyme synthesis;
- Restoration of GABA/glutamate equilibrium;
- Regulation of cholinergic activity via AChE inhibition; and
- Stabilization of neuronal membranes and mitochondria.

By attenuating oxidative injury and neurotransmitter dysregulation, L-carnitine and GBE prevent the cascade leading from oxidative stress to neuronal death.

#### 4.7. Clinical Relevance

Chronic antiepileptic drug use often exacerbates oxidative stress. Natural antioxidants with neurochemical stabilizing properties could therefore serve as adjuvant therapies to improve seizure control and mitigate systemic side effects. The current results support future translational studies and possible combination therapies.

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### 5. Conclusion

Pentylenetetrazole-induced epilepsy provokes significant oxidative stress and neurotransmitter imbalance in rat cerebral cortex. L-carnitine and Ginkgo biloba extract effectively counter these pathological changes through potent antioxidant and neuromodulatory mechanisms. Pretreatment with either compound provides substantial protection, whereas post-treatment aids recovery. Their complementary actions—metabolic support by L-carnitine and receptor-level antioxidant defense by GBE—highlight their promise as adjunctive, non-toxic strategies for preventing oxidative neuronal damage in epileptic conditions.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that they have no conflict of interest to disclose.

### *Statement of ethical approval*

Animals used for research only within an ethical framework.

## References

- [1] Tousson E., Bayomy F. M. and El-Sendiony F. B. (2015). Effects of L-carnitine and Ginkgo biloba on cerebral cortex in experimentally induced epileptic seizures disease in rat. *Journal of Bioscience and Applied Research*.1: 277-289.
- [2] Sarkisian M.R. (2001). Pentylentetrazole kindling model of epilepsy. *Brain Res Rev* 35: 231–243.
- [3] Johnston M. V. (2007). Seizures in childhood. In: Kleigman RM, Behrman RE, Jenson HB, Stanton BP. *Nelson Text Book of Pediatrics 18th Edition* Philadelphia: Saunders Elsevier; . p.2457-8.
- [4] Rasgado L. A. V., Reyes G. C. and Díaz F. V. (2013). Effect of convulsant drugs in GDH activity and oxygen consumption in mouse brain. *Journal of Medicine and Medical Sciences*. 4(1) pp. 34-42.
- [5] Bayomy F. M., B., and Tousson E. (2018). Effects of L-carnitine and Ginkgo biloba on spleen in experimentally induced epileptic seizures disease in rats. *Annals of Epilepsy and Seizures*. 1(1):1003.
- [6] Smith J. V. and Luo Y. (2004). Studies on molecular mechanisms of Ginkgo biloba extract. *Appl Microbiol Biotechnol* 64:465–72.
- [7] Tousson E., Bayomy F. M. and El-Sendiony F. B. (2020). Ameliorating role of L-carnitine and Ginkgo biloba extract on Pentylentetrazol induced bone marrow injury in epileptic seizures disease in rats. *GSC Advanced Research and Reviews*, 05(02), 001-011.
- [8] Moreno S., Carvalho J. J., Nascimento A. L. R., Freitas R. S., Diré G. F., Lima E. A., Lima-Filho G. L., Rocha E. K. and Bernardo-Filho M. (2004). Biodistribution of sodium pertechnetate and light microscopy of organs isolated from the rats: Study of the effects of a Ginkgo biloba extract. *Pak. J. Nutr.*, 3:64-7.
- [9] Waggas A. and Al-Hasani R. (2010). Neurophysiological study on possible protective and therapeutic effects of Sidr (*Zizyphus spina-christi*) leaf extract in male albino rats treated with pentylentetrazol. *Saudi Journal of Biological Sciences*, 17(4): 269- 274.
- [10] Ilhan A. et al. (2006). Protective effects of Ginkgo biloba extract against PTZ-induced seizures in rats.
- [11] Silva-Adaya D., Perez-De La Cruz V. and Herrera-Mundo M. N. (2008). Excitotoxic damage, disrupted energy metabolism, and oxidative stress in the rat brain: antioxidant and neuroprotective effects of L-carnitine. *J Neurochem*; 105:677–89.
- [12] Abdel-Wahab B. A. and Metwally M. E. (2011). Ginkgo biloba Enhances the Anticonvulsant and Neuroprotective Effects of Sodium Valproate Against Kainic Acid-induced Seizures in Mice. *Journal of Pharmacology and Toxicology*, 6: 679-690.
- [13] Halliwell B. and Gutteridge M. C. (2009). "Oxygen is poisonous, an introduction to oxygen toxicity and free radicals," in *Free Radicals in Biology and Medicine*, B. Halliwell and M. C. Gutteridge, Eds., pp. 1–20, Clarendon Press, Oxford, UK, 2nd edition.
- [14] Kehr J., Yoshitake S., Ijiri S., Koch E., Noldner M. and Yoshitake T. (2012). Ginkgo biloba leaf extract (EG b 761®) and its specific acylated flavonol constituents increase dopamine and acetylcholine levels in the rat medial prefrontal cortex: possible implications for the cognitive enhancing properties of EGb 761®. *Int Psychogeriatr*; 24(S1): S25- S34.