Effects of L-Carnitine and Ginkgo Biloba on Spleen in Experimentally-Induced Epileptic Seizures Disease in Rats

Mohamed F. F Bayomy¹, Elab Tousson Ehab¹ and Basiony Fouad El-sendiony²*

¹Department of Zoology, Menoufiya University, Egypt
²Department of Zoology, Tanta University, Egypt

Abstract

Pentylenetetrazole (PTZ) model is playing important role to understand the pathophysiology of epileptic seizures and leading to a theory in which a repeated injection of PTZ causes seizure gradually. It is used widely in antiepileptic drug studies. The current study aimed to determine the possible protective and ameliorative effects of L-carnitine and Ginkgo biloba Leaf Extracts (GLE) on spleen against PTZ induced epileptic seizures disease in rats. A total of 80 male albino rats were equally divided into eight groups: 1st group was the control; 2nd and 3rd were GLE and L-carnitine groups, respectively; while the 4th group was pentylenetetrazole rat group and the 5th and 6th groups were pre GLE and L-carnitine groups respectively; the 7th and 8th groups were post GLE and L-carnitine, respectively. PTZ leads to an increase in Malondialdehyde (MDA) and to a decrease in Glutathione Reductase (GSH), Super Oxide Dismutase (SOD), Glutathione Peroxidase activity (GPX) and catalase. Marked disruption of spleen structure and loss in distinction between the white and red pulps, a marked decrease in the lymphocyte population in the white pulp. In the spleen sections in PTZ group, Faint or mild positive reactions of PCNA-ir were detected in spleen of the white and red pulps, a marked decrease in the lymphocyte population in the white pulp. In the spleen sections in PTZ group, Faint or mild positive reactions of PCNA-ir were detected in spleen of PTZ rat group. Pre- and post treatment with GLE and L-carnitine were improved the biochemical, histological and immunohistochemical alterations in spleen that treated with PTZ.

Keywords: Pentyleneetetrazole; Seizures; Ginkgo biloba; L-carnitine; PCNA-ir

Introduction

Epilepsy is a brain defect including sudden and repetitive seizures that cut off normal functions for brain [1]. Epilepsy is the clinical pathological neurological defects that affect people of all ages, social degree, geographical outlines, or strain [2]. The activity of Seizure can be controlled with current drugs however they don’t prevent or treatment epilepsy. Anti-seizure drugs have lowering seizure activity or their side effects which are the result of long therapy overcome their therapeutic advantages [3]. The impairments of functional CNS, which occur during seizures have been suggested to be related to the oxidative damages of brain tissues [4,5]. The beginning of seizures appears to occur when a small group of abnormal neurons undergo prolonged depolarizations associated with the rapid firing of repeated action potentials. A clinical seizure occurs when the electrical discharges of a huge number of cells become abnormally linked together, causing a storm of electrical activity in the brain. Seizures then spread to adjacent areas of the brain [6].

Many experimental models for seizure have been developed in order to understand the important mechanisms of epileptic defects and to discover new therapies for seizure management. Pentylenetetrazole (PTZ) is a pharmaceutical agent that displays activity as the respiratory stimulant and central nervous system. It is considered a non-competitive gamma-aminobutyric acid antagonist [7]. PTZ has been used experimentally to study seizure phenomenon and to identify pharmaceuticals that may control seizure susceptibility. It is the most choose drug to develop chemical kindled seizure and it is reported that PTZ-kindled seizures imitative primary generalized epilepsy in aspect of ILEA classification [8].

Many plants, known for their anticonvulsant activity are subjected to phytochemical and pharmacological studies [9]. Among these herbal products are L-carnitine and Ginkgo biloba extract [10,11]. L-carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine [12]. In eukaryotic cells, it is required for the transport of fatty acids from the intramembranous space in the mitochondria, into the mitochondrial matrix during the breakdown of lipids for the generation of metabolic energy [13]. Recent studies suggest that
L-carnitine may play an important role in oxidative/antioxidative balance and has an antiperoxidative effect on several tissues [11]. Two main pharmacologically active groups’ compounds were present in Ginkgo biloba are the flavonoids and the terpenoids. Flavonoids exert antiepileptic activity by modulating the GABA-A-Cl-channel complex. Thus, flavonoids may have a modulating role in the treatment of neurodegenerative diseases due to their phenolic nature, since they can disrupt cellular oxidative processes in the central nervous system [14]. GLE decreases tissue levels of reactive oxygen species, inhibits membrane lipid peroxidation [15]. Therefore, the present paper aimed to study the protection and ameliorating role of Ginkgo biloba Leaf Extract (GLE) and L-carnitine on spleen in the physiological, histopathological and immunohistochemical alterations in pentylenetetrazole induced epilepsy in rats [16].

Materials and Methods

Animals

The experiments were performed using 80 male albino rats (Rattus norvegicus) weighing 120 ± 10 g and of 7±8 week’s age. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard diet (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch; Egyptian Company of Oils and Soap Kafir–El Zayat Egypt) and water was available ad libitum. The temperature in the animal room was maintained at 23 ± 2ºC with a relative humidity of 55 ± 5%. Light was available ad libitum. The animals were divided into eight groups (10 rats each). 1st group was control group in which rats never received any treatment. 2nd group was positive control rats (-ve control + L-carnitine (300 mg/kg body weight) once per day) for 9 days according to Tousson et al. [3] while 3rd group was positive control rats (-ve control + GLE (100 mg/kg body weight)) once per day every other day for 9 days according to Rodriguez de Turco et al. [10]. 4th group was the experimental group that treated with pentylenetetrazol (PTZ; 40 mg/kg body weight) once per day every other day for 9 days according to Akula et al. [17]; Dhir et al. [18] and Waggas and Al-Hasani [19]. 5th group the rats were treated with PTZ (40 mg/kg) once every 48 h for 9 days after L-carnitine (300 mg/kg) treated once every 48 h for 9 days. 6th group the rats were treated with PTZ (40 mg/kg) once every 48 h for 9 days after Ginkgo biloba (100 mg/kg) leaf extract treated once every 48 h for 9 days. 7th group the rats were injected with PTZ (40 mg/kg) once every 48 h for 9 days then treated with L-carnitine (300 mg/kg) once every 48 h for 9 days. and 8th group the rats were injected with PTZ (40 mg/kg) once every 48 h for 9 days then treated with Ginkgo biloba (100 mg/kg) leaf extract once every 48 h for 9 days.

Sample preparation

Blood samples from each rat were collected from inferior vena cava of each rat in non heparinized glass tubes. Glass tubes were incubated at room temperature for 10 minutes and left to clot then centrifuged at 3000 rpm for 10 min and the serum were collected, and kept in clean stopper plastic vial at −80°C until estimation of serum Malondialdehyde (MDA), Reduced Glutathione (GSH), Glutathione Peroxidase activity (GPX), Superoxide Dismutase (SOD) and Catalase.

Histopathological investigation

Immediately after decapitation, animals were dissected, spleen from animals of different groups were quickly removed, washed in 0.9% saline solutions and samples of bone marrow were fixed in 10% neutral buffered formalin. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin (mp. 50 ºC to 58ºC). Sections of 7 microns thickness were cut using rotary microtome and mounted on clean slides. Sections were stained with Ehrlich’s haematoxylin and counterstained with eosin as a routine method by Bancroft and Stevens [20].

Immunohistochemical detection of (PCNA-ir)

Expression of Proliferating Cell Nuclear Antigen Immunoreactivities (PCNA-ir) was detected using Avidin Biotin Complex (ABC) method [21]. Paraffin sections (5 μm thick) of fixed rat spleen that mounted on gelatin chromium–coated glass slides were dewaxed and rehydrated sections were washed in distilled water for 5 min, rinsed in PBST for 10 min and incubated with 10% normal goat serum for 15 min to reduce non-specific background staining. Then, the sections were incubated with anti-rabbit PCNA for 1-2 hours at room temperature. The sections after 5 times baths in PBST were incubated with biotinylated goat anti rabbit immunoglobulin (Nichirei, Tokyo, Japan). The sections after 5 times baths in PBST were further incubated with Avidin Biotin Complex (ABC: Nichirei, Tokyo, Japan) for 1 hour at RT. The criterion for a positive reaction confirming the presence of PCNA is cells with brown nuclear staining. For the negative control, the primary antibody was omitted to guard against any false positive results which might develop from a non-specific reaction. Brightness, contrast were adjusted using Adobe Photoshop software. Image analysis was adjusted using PAX-it image analysis software.
L-carnitine (G2) and GLE (G3) groups. On the other hand, Glutathione Reductase (GSH), Superoxide Dismutase (SOD), Glutathione Peroxidase activity (GPX) and catalase in pentylenetetrazole group (G4) showed significant decreases when compared with control (G1), GLE (G2) and L-carnitine (G3) groups. In contrast, MDA levels in pre-treated PTZ with L-carnitine and GLE groups (G5 & G6) were significantly decreased when compared with PTZ group (G4) while GSH, SOD, GPX and catalase levels in pre-treated PTZ with L-carnitine and GLE groups (G5 & G6) were significantly increased when compared with PTZ group (G4). MDA levels in post-treated PTZ with L-carnitine and GLE groups (G7 & G8) were significantly decreased when compared with PTZ group (G4) while GSH, SOD, GPX and catalase levels in post-treated PTZ with L-carnitine and GLE groups (G7 & G8) were significantly increased when compared with PTZ group (G4).

Histopathological findings in the spleen

Representative coronal sections of H & E stain obtained from the male rat spleen in different groups under study are shown at the light microscope levels in Figures 6A-H. Coronal sections in spleen of control (A); L-carnitine (B) and GLE (C) groups revealed a normal histopathological structure. D: Rat spleen sections of PTZ group revealed marked disruption of spleen structure and loss in distinction between the white and red pulps: E: spleen sections in pro-treated PTZ with L-carnitine revealed moderate decrease in the lymphocyte population in the white pulp and congestion of splenic sinuses in the red pulp. F: Spleen sections in pro-treated PTZ with GLE revealed moderate decrease in the lymphocyte population. G: spleen sections in post-treated PTZ with L-carnitine revealed white pulp appeared as a normal accumulation of cellularity and the red pulp showed an increase in the number of the reticular cells and macrophages. H: Spleen sections in post-treated PTZ with GLE revealed increased in the number of inflammatory cell components and little congestion of splenic sinuses were seen in the red pulp.

Statistical analysis

Data were expressed as mean values ± SEM and statistical analyses were performed using SPSS statistical version 16 software package (SPSS Inc., USA). The criterion for statistical significance was set at P <0.01.

Results

Figures 1-5 show that serum MDA levels in PTZ group (G4) showed significant increase when compared with control (G1), L-carnitine (G2) and GLE (G3) groups. On the other hand, Glutathione Reductase (GSH), Superoxide Dismutase (SOD), Glutathione Peroxidase activity (GPX) and catalase in pentylenetetrazole group (G4) showed significant decreases when compared with control (G1), GLE (G2) and L-carnitine (G3) groups. In contrast, MDA levels in pre-treated PTZ with L-carnitine and GLE groups (G5 & G6) were significantly decreased when compared with PTZ group (G4) while GSH, SOD, GPX and catalase levels in pre-treated PTZ with L-carnitine and GLE groups (G5 & G6) were significantly increased when compared with PTZ group (G4). MDA levels in post-treated PTZ with L-carnitine and GLE groups (G7 & G8) were significantly decreased when compared with PTZ group (G4) while GSH, SOD, GPX and catalase levels in post-treated PTZ with L-carnitine and GLE groups (G7 & G8) were significantly increased when compared with PTZ group (G4).

Figures 6A-H: Photomicrographs of rat spleen sections in the different experimental groups stained with Haematoxylin & Eosin. A-C: Rat spleen sections in control (A); L-carnitine (B) and GLE (C) groups revealed a normal histopathological structure. D: Rat spleen sections of PTZ group revealed marked disruption of spleen structure and loss in distinction between the white and red pulps: E: spleen sections in pro-treated PTZ with L-carnitine revealed moderate decrease in the lymphocyte population in the white pulp and congestion of splenic sinuses in the red pulp. F: Spleen sections in pro-treated PTZ with GLE revealed moderate loss in the distinction between the white and red pulps with a moderate decrease in the lymphocyte population. G: spleen sections in post-treated PTZ with L-carnitine revealed white pulp appeared as a normal accumulation of cellularity and the red pulp showed an increase in the number of the reticular cells and macrophages. H: Spleen sections in post-treated PTZ with GLE revealed increased in the number of inflammatory cell components and little congestion of splenic sinuses were seen in the red pulp.
L-carnitine is occurring normally as endogenous compound in all mammalian and the most important function is that act as a transporter of long chain fatty acids into mitochondria for β-oxidation [24]. L-carnitine, among a large group of target neuroprotective agents, is of particular interest in neurons due to its role in amino acid synthesis, ATP metabolism, mitochondrial fatty acid transport, as well as its antioxidant effects [25–27].

Discussion

Epilepsy is a disorder characterized by repeated seizures due to defects in the brain cells. The onset of seizures may be at any time during life and occur sporadically or frequently. Some suffer from it their whole lives and others only for a few years. Some of the epilepsies are confined to particular age groups [22].

Strong linkage between PTZ-induced seizures and oxidative stress. PTZ kindling causes alterations in antioxidant defense systems of the brain [23].

GbE protects against development of seizures (i.e. against Pentylenetetrazole (PTZ)-induced kindling in mice) [28]. EGb761 inhibits membrane lipid peroxidation and decreases tissue levels of reactive oxygen species [29].

This study conducts a biochemical, histological and immunohistochemical investigations into whether L-carnitine and Ginkgo biloba have a protective and ameliorative effect on spleen against pentylenetetrazole-induced epilepsy in male rats. The results of the studies indicate that pentylenetetrazole causes oxidative tissue damage by increasing lipid peroxidation in the serum and decreasing the level of serum antioxidant enzymes.

A significant rise in serum MDA levels was observed after PTZ administration when compared to control animals. Similarly, Kutluhan et al. [30] have reported increased levels of MDA in brain tissues in a mouse-seizure model after PTZ administration.

A significant reduction in serum MDA levels was observed when treated with L-carnitine. This reduction is possibly due to the modulatory activity of L-carnitine in the antioxidant enzymes in the brain of adult rats. Similarly, Ahmed and Mahmoud et al. [31] have reported that L-carnitine administration has decreased levels of MDA in brain tissues in a mouse-seizure model.

Treatment with pre and post GB significantly reduced MDA level. Abdel-Wahab and Metwally have reported decreased levels of...
MDA as effects of GB of inhibition of lipid peroxidation. The present findings indicate that the effects of GB against oxidative damage may be due to its antioxidant and free radical-scavenging activity [32].

The present study demonstrated that a significant decrease in serum GSH reductase levels was observed after PTZ administration. Previous study was reported, PTZ induced seizure shows marked reduction of antioxidant enzymes like glutathione reductase [33,34].

In this study, administration of pre and post L-carnitine induced significant increase in the GSH. This result is in agreement with the study made by Heba and Sameh who found that administration of L-carnitine induced significant increase in GSH content and this might be the active role of L-carnitine in protection against oxidative stress [35].

Pre and Post GLE treatments led to increasing levels of GSH when compared to PTZ group. These results are similar to others observed in a study made by Pinakinik et al. [36], Ginkgo biloba, a known plant with its antioxidant property, probably strengthened the antioxidant status as evidenced by the observed increase in the GSH levels.

In the present study, the result demonstrating significant decrease in GPX levels was observed after PTZ administration. Sekar Babu et al. [37] documented that changes in glutathione peroxidase activity were inversely correlated with intensity of lipid peroxidation.

In this study, administration of pre and post treatment with L-carnitine induced significant increase in the GPX content. This is similar to another study which showed that I-carnitine augmented the antioxidant defense capacity. This effect was mediated by an up regulation of antioxidant enzymes, an increase in the glutathione peroxidase levels [38].

Pre and post GBE induced significant increase in the GPX content. GBE can also enhance activities of antioxidant enzymes, such as Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX) and catalase, thereby indirectly contributing as an antioxidant [39].

The present study demonstrated that a significant decrease in SOD levels was observed after PTZ administration. These results are in accordance with those of who showed marked reduction in antioxidant enzyme (SOD) in the PTZ-induced kindled group which leads to the production of free radicals [40].

The present study demonstrating a significant increase in SOD levels was observed in pre and post treatment with GLE. These results are in accordance with those documented by Atmaca et al. [41] which showed that GLE can also enhance activities of antioxidant enzymes, such as Superoxide Dismutase (SOD). This indicates the antioxidant action of GLE.

The present study also demonstrated that a significant increase in SOD levels was observed in pre and post treatment with L-carnitine. These results are in accordance with the results of Li JL who showed that L-carnitine enhances activities of Superoxide Dismutase (SOD) [41]. This indicates the influence of L-carnitine as an antioxidant for this enzyme.

The present study demonstrated that a significant decrease in catalase levels which were observed after PTZ administration. Senthil and Rajkapoor reported that PTZ administration significantly decreased catalase activity in rate [42].

It was found that L-carnitine, pre and post treatments, was able to reverse action of PTZ on catalase activity and caused significant increase in the activity of catalase. Previous study by Cao et al. [43] showed that L-carnitine increase the activity of catalase, this indicate the ability of L-carnitine as a powerful antioxidant. Therefore, it may be useful as a supplementary therapy for chronic illnesses involving excessive oxidative stress.

Pre and post treatments with Ginkgo biloba were able to reverse action of PTZ against catalase activity and cause significant increase in the activity of catalase. Shaoping et al. [4], showed that GLE cause significant increase in the activity of catalase. This GLE might scavenge the free radicals and enhance antioxidant enzyme activities.

Spleen section in PTZ group revealed marked disruption of spleen structure and loss in distinction between the white and red pulps a marked decrease in the lymphocyte population in the white pulp. In contrast, a moderate degree of improvement with moderate decrease in the lymphocyte population in the white pulp and mild vasodilatation and congestion of splenic sinuoids in the red pulp were seen in pro-treated PTZ with L-carnitine and GLE. Spleen sections in post treated PTZ with GLE and L-carnitine revealed a moderate to a good degree of improvement respectively with more or less normal structure, the white pulp appeared as a normal accumulation of cellularity and the red pulp showed an increase in the number of the reticular cells and macrophages.

Faint or mild positive reaction of PCNA-ir was detected in the spleen of PTZ rat group. Spleen sections in pro-treated PTZ with L-carnitine and GLE revealed mild to moderate positive reaction for PCNA-ir. Spleen sections in both post-treated PTZ with L-carnitine and with GLE revealed mild and moderate reaction respectively for PCNA-ir.

This similar with other study was showed that Histologic analysis of the spleens exposed to pilocarpine revealed altered white and red pulp anatomy and an increase in CD3+ T cells [44].

Manal who reported that Ginkgo biloba extract has a relative improvement in the structure of the spleen as histology and immunohistochemistry after noise stress had an immunosuppressive effect [45].

L-carnitine showed an immunostimulatory effect as L-carnitine group showed more or less normal tissues architectures on histopathological examination [46]. This results are in agreement with previous study reported antioxidant effect of L-carnitine as removing of the free radicals reduce the inflammatory stress [47,48].

It can be concluded that pre and post-treatment pentylenetetrazol with Ginkgo biloba and L-carnitine prevented oxidative damage by inhibiting ROS production and improving antioxidant enzymes [49]. This study suggests that neuroprotective properties of Ginkgo biloba and L-carnitine through their ability to reduce glutamate efflux, thus leading to a decrease in the excitotoxic effects of this neurotransmitter [50].

References


