



Journal of Bioscience and Applied Research

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Effects of L-carnitine and *Ginkgo biloba* on Pentylentetrazol-induced liver damage and oxidative stress in rats

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Abstract

Pentylentetrazol (PTZ) kindling is an acknowledged models for epilepsy. The current study aimed to determine the possible protective and ameliorative effects of L-carnitine and *Ginkgo biloba* (GB) against PTZ induced liver injury and oxidative stress in male rats. A total of 80 male albino rats were equally divided into eight groups; 1st group was the control; 2nd and 3rd were GB and L-carnitine groups, respectively; 4th group was pentylenetetrazole rat group and the 5th and 6th groups were pre and post treated PTZ with L-carnitine groups respectively; the 7th and 8th groups were pre and post treated PTZ with GB respectively. Serum ALT, AST, ALP and MDA in liver tissue were significant increase in PTZ group when compare with control, while serum ALB and CAT, SOD, GST and TAC in liver tissue were significant decrease in PTZ group when compare with control. On other hand post and pre-treatment with L-carnitine and GB improved these parameters. Our results revealed that the treatment with L-carnitine improved these parameters more than GB and the results of post treatment were better than pre-treatment

Keywords: Pentylentetrazol; L-carnitine; *Ginkgo biloba*; Liver functions; Oxidative stress

1 Introduction

Epilepsy is the neurological disorder of the human brain characterized by seizures affecting people of all ages, race, social class or geographical boundaries (Hermann et al., 2012). A seizure is an event and epilepsy is the disease involving recurrent un provoked seizures (Fisher et al., 2014). Pentylentetrazol (PTZ) kindling model provides a useful model for post seizure dysfunction, serving as a

screen for potential treatments for the cognitive and emotional deficits that are observed in human epilepsy (Barbalho *et al.*, 2016; Nasir *et al.*, 2012). PTZ is drug used as a γ -aminobutyric acid (GABA) receptor antagonist with high biological membrane penetrance and consequent rapid bioavailability and distribution to all organs including the brain, and very short latency of seizure induction (Spencer *et al.*, 2009).

The *Ginkgo biloba* tree is the oldest tree on earth that appeared in the Jurassic period 170 million years ago and for this reason, is called the "living fossil" (Nuhu, 2014; Tousson *et al.*, 2014). *Ginkgo biloba* have also antioxidant properties, which are mainly connected to their polyphenolic constituents, particularly phenolic acids, flavonoids, proanthocyanidins and catechins (Koczka *et al.*, 2015).

L-carnitine is a vitamin-like dietary compound that is synthesized in the body from and is important in mammalian metabolism, especially for the normal mitochondrial oxidation of fatty acids (Pochini *et al.*, 2013; Ozsoy *et al.*, 2011) Cellular energy metabolism is largely sustained by mitochondrial β -oxidation of fatty acids, especially when carbohydrate stores are depleted after fasting or prolonged exercise. The present study was aimed to investigate the role of *Ginkgo biloba* (GB) and L-carnitine on the biochemical and oxidative stress alterations in Pentylentetrazol (PTZ) induced liver toxicity in male albino rat.

2 Materials and Methods

a. Chemicals:

The reagents Pentylentetrazol (PTZ), thiobarbituric acid, 2,4-dinitrophenylhydrazine, 5,5'-dithiobis(2-

nitrobenzoic acid), (-)-epinephrine, chlorogenic acid and guanidine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA).

L-carnitine: L-carnitine (β -hydroxy- γ -trimethylaminobutyrate) was purchased from Mepacomedifood medicine company from Anshase el ramle, Sharqia, Egypt.

Ginkgo biloba: The plant *Ginkgo biloba* is dietary supplement for improving circulation and memory. The extract was purchased from (EMA Pharma pharmaceutical, Nozha, Nasrcity, Cairo, Egypt).

b. Experimental animals

The experimental protocol was approved by Local Ethics Committee and Animals Research. The experiments were performed using 80 male albino rats (*Rattus norvegicus*) weighing 120 ± 10 g and of 7-8 weeks age. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard diet and water was available *ad libitum*. The rats were equally divided into eight groups. 1st group was control group in which rats never received any treatment. 2nd group was given L-carnitine (300 mg/kg body weight) once per day every other day for 9 days according to Tousson et al. (2014). 3rd group was given GB (100 mg/kg body weight) once per day every other day for 9 days according to Rodriguez de Turco et al. (1993). 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. (2007). 5th group that 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. 6th group that 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. 7th group that treated with PTZ (40 mg/kg) once every 48 h for 9 days after GB (100 mg/kg) treated once every 48 h for 9 days. 8th group that injected with PTZ (40 mg/kg) once every 48 h for 9 days then treated with GB (100 mg/kg) once every 48h for 9 days.

c. Biochemical analysis

Tissue Homogenate

Prior to dissection, perfuse tissue with a PBS (phosphate buffered saline) solution, pH 7.4. Containing 0.16 mg / ml heparin to remove any red blood cells and clots. The tissue was then homogenized in 5 – 10 ml cold buffer (50 mM potassium phosphate, pH 7.4. 1 mM EDTA) per gram tissue, centrifuge at 4,000 rpm for 15 minutes at 4 °C, remove the supernatant for assay and store on ice. The samples were then freeze at - 80°C.

Enzymatic and non-enzymatic antioxidant assays

Malondialdehyde (MDA) assay was detected by TBARS analysis and measured as reported by Ohkawa et

al. (1979). Catalase (CAT) activity was detected according to the methods of Aebi (1984). Superoxide dismutase (SOD) was detected according to the methods of Nishikimi (1972). Glutathione-S-transferase (GST) was determined according to the method of Beutler et al. (1963). Total antioxidant capacity (TAC) activity was determined according to the method of Koracevic et al. (2001). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the method of Reitman and Frankel (1957). Serum Albumin concentration was determined according to the method of Bowers and Wong (1980). Serum alkaline phosphatase activity was determined according to the method of Belfield and Goldberge (1971).

Statistical Analysis:

Data were expressed as mean values \pm SEM and statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc., USA). The criterion for statistical significance was set at $p < 0.05$.

3 Results

Table (1) shows that SOD, CAT, GST and TAC levels in PTZ group showed significant decrease when compared with control, L-carnitine and GB groups. On the other hand, MDA levels in PTZ group showed significant increase when compared with control, L-carnitine and GB groups. In contrast, MDA levels in pre-treated PTZ group with L-carnitine or with GB groups (as in G5&G7) were significantly decreased when compared with PTZ group while catalase, SOD, GST and TAC levels in pre-treated PTZ group with L-carnitine or with GB groups (as in G5&G7) were significantly increased when compared with PTZ group. MDA levels in post treated PTZ group with L-carnitine or with GB groups (as in G6&G8) were significantly decreased when compared with PTZ group while catalase, SOD, GSH and TAC levels in post treated PTZ with L-carnitine or with GB groups (as in G6&G8) were significantly increased when compared with PTZ group (Table 1). Table (2) shows that; a significant decrease in ALB levels in PTZ group while, serum ALT, AST and ALP levels in PTZ group were significantly increased when compared with control, L-carnitine and GB groups. In contrast, serum ALB levels were significantly increased in pre-treated PTZ group with L-carnitine or with GB groups (as in G5&G7) when compared with PTZ group while serum ALT, AST and ALP levels in pre-treated PTZ group with L-carnitine or with GB groups (as in G5&G7) were significantly decreased when compared with PTZ group (Table 2). Serum ALB levels was significant increase in post treated PTZ group with L-carnitine or with GB groups (as in G6&G8) when compared with PTZ group while ALT, AST and ALP levels in post treated PTZ group with L-carnitine or with GB groups (as in G6&G8) were significantly decreased when compared with PTZ group (Table 2).

Table 1: Changes in antioxidant and oxidative stress in different groups.

Groups	MDA (n mole/gm tissue)	CAT (U/g)	SOD (U/gm)	GSH (U/gm)	TAC (m Mole/L)
G1	10.77±0.47 ^b	1.78±0.030 ^b	1090±24.22 ^b	2.50±0.09 ^b	0.71±0.06 ^b
G2	12.00±0.57 ^b	2.48±0.030 ^b	1028±34.7 ^b	3.56±0.09 ^b	0.58±0.06 ^b
G3	12.17±0.70 ^b	2.20±0.036 ^b	943.3±25.7 ^b	4.18±0.16 ^b	0.61±0.04 ^b
G4	25.83±0.87 ^a	0.96±0.004 ^a	593.2±20.51 ^a	1.63±0.18 ^a	0.08±0.006 ^a
G5	17.0±0.73 ^{ab}	1.35±0.042 ^{ab}	783.3±22.31 ^{ab}	2.90±0.09 ^{ab}	0.33±0.04 ^{ab}
G6	11.50±0.34 ^{ab}	2.32±0.054 ^{ab}	949.8±30.4 ^{ab}	3.93±0.19 ^{ab}	0.55±0.04 ^{ab}
G7	13.00±0.96 ^{ab}	1.31±0.060 ^{ab}	758.2±19.61 ^{ab}	2.88±0.06 ^{ab}	0.50±0.07 ^{ab}
G8	13.00±0.51 ^{ab}	1.35±0.004 ^{ab}	923.2±28.6 ^{ab}	3.23±0.13 ^{ab}	0.165±0.007 ^{ab}

Data are expressed as mean ± S.E.M of 10 observations. (^a) significant difference compared to control group. (^b) highly significant difference compared to PTZ group, where G1, control group; G2, L-carnitine group; G3, GBgroup; G4, PTZ group; G5&G6, pre- and post- treated PTZ with L-carnitine groups respectively; G7&G8, pre- and post- treated PTZ with Ginkgo biloba respectively.

Table 2: Changes in liver functions in different groups

Groups	ALT (U/L)	AST(U/L)	ALB (g/dl)	ALP(U/L)
G1	35.70±0.66 ^b	190±8.60 ^b	3.41±0.08 ^b	373.3±19.54 ^b
G2	41.00±0.7 ^b	224±9.2 ^b	2.98±0.12 ^b	431.7±14.9 ^b
G3	40.80 ±0.7 ^b	224±9.2 ^b	3.25±0.07 ^b	433.3±13.33 ^b
G4	77.2±3.09 ^a	329±20.76 ^a	2.48±0.09 ^a	651.7±18.8 ^a
G5	44.28±1.54 ^b	274±15.88 ^{ab}	2.83±0.10 ^{ab}	496.5±9.50 ^{ab}
G6	41.20±0.86 ^b	278±11.58 ^{ab}	3.21±0.07 ^b	445.0±9.27 ^{ab}
G7	43.8±1.32 ^b	243±16.74 ^{ab}	3.00±0.07 ^b	493.3±10.22 ^{ab}
G8	43.8±1.24 ^b	280±15.81 ^a	2.71±0.07 ^b	475±17.63 ^{ab}

Data are expressed as mean ± S.E.M of 10 observations. (^a) Significant difference compared to control group. (^b) highly significant difference compared to PTZ group, where G1, control group; G2, L-carnitine group; G3, GB group; G4, PTZ group; G5&G6, pre- and post- treated PTZ with L-carnitine groups respectively; G7&G8, pre- and post- treated PTZ with *Ginkgo biloba* respectively.

4 Discussion

Recurrent and prolonged seizures and anticonvulsant drugs may increase the concentration of ROS in the body, which may result in substantial deleterious effects on an individual's health. The liver is an organ that is sensitive to oxidative damage (Akbas et al., 2005). Seizures may lead to lipid peroxidation and decreased antioxidant defense mechanisms in hepatocytes (Dillioglugi et al., 2010). These types of damage may lead to epilepsy complications, such as fulminant liver failure (Akbas et al., 2005). Furthermore, anticonvulsant drugs may elevate liver enzyme levels, to deplete hepatic enzymatic antioxidant defenses, which may result in hepatotoxic effects or liver injury (Ochoa and Riche, 2012). Epileptic patients also present alterations in the redox status of their serum samples (Costello and Delanty 2004). Pentylentetrazole-induced seizure activity mimics the increased oxidative stress in the brain by altering membrane phospholipid metabolism and ultimately resulting in the release of free radicals (Corvino et al., 2013). This work aimed to study the role of *Ginkgo biloba*(GB) and L-Carnitine on the biochemical and oxidative stress alterations in Pentylentetrazol (PTZ) induced liver toxicity in male albino rat.

The current results indicated that pentylentetrazole causes oxidative tissue damage by increasing lipid peroxidation and decreasing the level of antioxidant

enzymes in the liver tissues. Our results agree with Akbas et al. (2005); Abdullah et al. (2008) and Dillioglugil et al. (2010) who reported that an increased in the levels of MDA in liver tissues in a mouse-seizure model after PTZ administration.

A significant reduction in MDA levels was observed in rat liver when treated with L-carnitine. This reduction is possibly due to the modulatory activity of L-carnitine in the antioxidant enzymes in the liver of adult rats. This indicates that L-carnitine inhibits liver damage as free radical scavenger. Similarly, Also; Tousson et al. (2015) find that; L-carnitine administration has decreased levels of MDA in brain tissues in rat seizure model. SOD and catalase are the main enzymes of the enzymatic antioxidant defense system, responsible for protection against the increase in ROS production (Naziroglu et al., 2007). Catalase serves as an antioxidant defense mechanism and is primarily responsible for the removal of H₂O₂ from cells form of oxygen and a normal cellular metabolite. GST is one of the endogenous detoxifying antioxidant enzymes that lead to the formation of oxidized glutathione conjugates. Reduced GSH participates in the detoxification of xenobiotics as a substrate for the enzyme GST. Glutathione and other thiol containing proteins play a crucial key role in cellular defense against toxicity (Halliwell and Gutteridge 1999).

In the present study; a significant decrease in SOD, catalase, GST and TAC levels were observed after PTZ administration. Previous study was reported, PTZ induced seizure shows marked reduction of antioxidant enzymes like glutathione reductase (Nieoczym et al., 2008). Also our results agree with Noaishi et al. (2013) who reported that decrease in catalase and GST due to oxidative stress in white rat. Evaluation of tissue/organ total antioxidant capacity is valuable since it provides a gross estimation of how the body can react against oxidative and nitrosative injuries Constantini and Verhulst (2009).

In the assessment of liver injury the analysis of enzyme levels such as ALT and AST are largely used and located in the cytosol of hepatocytes. Necrosis or membrane damage releases the enzyme into the circulation and hence it can be measured in the serum. Clinical diagnosis of disease and damage to the structural integrity of liver is commonly assessed by monitoring the status of serum ALT and AST activities. The present study demonstrated significant decrease in ALB and significant increase in ALT, AST, and ALP levels were observed after PTZ administration. These results are accepted with those of Thapa and Walia (2007) who showed marked reduction in enzyme (ALT, AST and ALP) in the PTZ-induced kindled group which leads to the production of free radicals and existence of oxidative stress in the brain of the kindled mice. The present study demonstrating a significant increase in ALB while significant decrease in ALT, AST and ALP levels were observed in pre and post treatment with GB. These results are in accepted by Sato *et al.* (2009) who showed that GB can also enhance activities of enzymes, (ALT, AST and ALP), but not accepted with ALB which show no significant changes. This indicates the antioxidant action of GB. The present study also demonstrated that a significant increase in ALB while significant decrease in ALT, AST and ALP levels were observed in pre and post treatment with L-carnitine. These results are in accordance with the results of Canbaz *et al.* (2007) who showed that L-carnitine enhances activities of ALT, AST and ALP.

5 References

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