Biochemical effect of l-Carnitine in Valporate induced hyperammonemia in rabbits

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A B S T R A C T

The present study was undertaken to elucidate the harmful effects of hyperammonemia induced by valporate (VPA) in white male New-Zealand rabbits; Morever, evaluation the potential protective and the therapeutic effect of L-carnitine (L-C). Fifty one white male New-Zealand rabbits were divided into five groups. Group I: (control group): comprised 12 white male New-Zealand rabbits, received no drugs. Group II: (L-C group): comprised 9 white male New-Zealand rabbits, received oral L-C (95 mg/kg body weight/day) daily for 30 days. Group III: (VPA group): comprised 12 white male New-Zealand rabbits, received a single intravenous dose of (130 mg/kg body weight) of VPA for induction of hyperammonemia. Group IV: (protective group): comprised 9 white male New-Zealand rabbits, received L-carnitine as in group II prior VPA administration. Group V: (therapeutic group): comprised 9 white male New-Zealand rabbits, received VPA as in group III prior L-C administration and the treatment was continued for 30 days later. Blood samples as well as liver and brain tissues were collected at 10th, 20th, 30th days from the onset of L-carnitine administration, and after 1 hour and at 10th, 20th, 30th days from the onset of VPA administration for determination of serum (Ammonia (NH_3), Nitric oxide (NO), Gama-aminobutyric acid (GABA), Mono-amine oxidase (MAO)), liver and brain tissue (Catalase (CAT), Super oxide dismutase (SOD), Glutathion reductase (GR), and Malondialdehyde (L-MDA)) activities. The obtained results showed that, VPA- induced hyperammonemia caused a significant increase in serum NH_3, NO, GABA, MAO, and liver and brain tissue MDA concentration. However, liver and brain tissue CAT, SOD, and GR activities were significantly decreased. Administration of L-C was able to mitigate hyperammonemia induced by VPA through decreasing of NH_3, NO, GABA, MAO, as well as liver and brain tissue MDA concentration in addition CAT, SOD, and GR activities in liver and brain tissues were significantly increased. These results suggest that, L-C may be successful in therapeutic of hyperammonemia, hyperammonaemic encephalopathy (HE) and hepatotoxicity (HT).

Key Words: Hyperammonemia; L-carnitine, Valporate, GABA, Antioxidant enzymes.

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1. INTRODUCTION

Hyperammonemia is a condition characterized by elevation in the serum level of ammonia above 40µmols/L (Roth, 2008). High levels of ammonia accumulating as a result of hepatic dysfunction are thought to be the chief causative factor for hepatic encephalopathy. (Kumar et al., 2007). Hyperammonemia also, have deleterious effects on central nervous system function. Depending upon the age and the magnitude and duration of exposure, ammonia toxicity may result in severe neurological symptoms including stupor, seizures, convulsions, mental retardation, neuronal cell damage (Wilkinson and Wilkinson ., 2010). Medications such as valproic acid and its derivates have also been implicated in the development of this condition (Segura et al., 2006). Valproic acid is a broad-spectrum antiepileptic drug that has been used for more than 30 years. Valproic acid is a branched chain carboxylic acid (2-propylpentanoic acid or di-n propylacetic

acid), with a chemical structure very similar to that of short chain fatty acids. Valproic acid has numerous drug interactions and toxicities; include hepatic damage, pancreatitis, teratogenicity, thrombocytopenia, and hyperammonemia (Gerstner et al., 2006). VPA could also exert direct effects on excitable membranes, and alter dopaminergic and serotoninergic neurotransmissions (Loscher, 2002). However, the pharmacokinetic properties are predominantly determined by the mode of VPA administration, and the condition of the patient (particularly age) (Dutta et al., 2004). On the other hand, L-carnitine deficiency is one of the other less common causes of hyperammonemia (McCall and Bourgeois, 2004). L-Carnitine (β- hydroxy-γ-trimethylamino butyrate) is a conditionally essential nutrient for human and animal. L-Carnitine appears essential to ensure proper metabolism of VPA (Evangeliou and Vlassopoulos, 2003), and Carnitine facilitates transport of long-chain fatty acids from the cytosol compartment of the muscle fibre into the mitochondria, where they undergo β-oxidation and produce acetyl-CoA, which enters the Krebs cycle. Indeed, esterification as acylcarnitine is indispensable for transport of long-chain fatty acids through the mitochondrial membrane (Diaz et al., 2000). Also, L-carnitine has a protective effect against the toxic actions of different drugs, which induce oxidative stress and/or carnitine deficiency (Lheureux and Hantson., 2009). Accordingly, the purpose of the present study was to investigate the effect of L-carnitine against Valporate-induced hyperammonemia in rabbits. Also, to determine whether L-carnitine would attenuate the oxidative stress in liver and brain tissues and if it is beneficial for the prevention and treatment of hyperammonemia complications.

2. MATERIALS AND METHOD

2.1. Experimental animals:

Fifty one white male New-Zealand rabbits, weighting 2.0 – 3.0 kg were used in this study. Rabbits were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was ad- libitum. The animals were left 14 days for acclimatization before the beginning of the experiment.

2.2. L-Carnitine:

L-Carnitine is colorless powder (purity ~99%) was manufactured by Arab Co. for Pharmaceuticals and Medical Plants-MEPACO-Egypt. L-carnitine was dissolved in distilled water and administrated orally to rabbits daily at a dose of 95 mg/kg body weight for 30 days *(Paget and Barnes, 1964).*

2.3. Valporate:

Valporate is white to off-white crystalline powder (purity ~99%) was manufactured by Global Napi Pharmaceuticals-Egypt under license of Sanofi Avents-France. VPA was dissolved in water and injected via ear vein of rabbits in single intravenous dose of 130 mg/kg body weight *(Paget and Barnes, 1964).*

2.4. Experimental design:

Fifty one rabbits were randomly divided into five main groups, placed in individual cages and classified as follow: Group I: (control group): comprised 12 white male New-Zealand rabbits, received no drugs. Group II: (L-C group): comprised 9 rabbits, received L-carnitine orally (95 mg/kg body weight/day) daily for 30 days. Group III: (VPA group): comprised 12 rabbits, received single intravenous dose (130 mg/kg body weight) of VPA for induction of hyperammonemia. Group IV: (protective group): comprised 9 rabbits, received oral L-carnitine (95 mg/kg body weight/day orally) daily for 30 days prior VPA administration (130 mg/kg body weight). Group V: (treated group): comprised 9 rabbits, received single intravenous dose of VPA (130 mg/kg body

weight) prior administration of L-C (95 mg/kg body weight/day orally), and the treatment was continued for 30 days later.

2.5. Sampling:

2.5.1. Blood samples:

Blood samples were collected one hour after administration of VPA in VPA group with control group and at 10th, 20th, 30th days from the onset of VPA administration in VPA group and protective group and at 10th, 20th, 30th days from the onset of L-C administration in L-C group, treated group . Serum was separated by centrifugation at 2500 r.p.m for 15 minutes. The clean, clear serum was collected by automatic pipette and received in dry sterile tubes and kept in a deep freeze at -20ºC until used for subsequent biochemical analysis. All sera were analyzed for NH_3, NO, GABA, MAO.

2.5.2. Tissue samples (liver and brain tissue):

At the end of each experimental period the rabbits were sacrificed by cervical decapitation. The liver and brain was quickly removed, cleaned by rinsing with cold saline and stored at -20°C for subsequent biochemical analyses.

2.5.3. Liver and brain tissue preparation:

Briefly, Liver and brain tissues were cut, weighted and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant were used for the determination of the following parameters: superoxide dismutase (SOD), catalase (CAT) glutathione reductase (GR), and L- Malondialdehyde (L-MDA).

2.6.Biochemical analysis:

Serum NH3, NO, GABA, MAO, and liver and brain tissue L-MDA, SOD, CAT, GR, were determined according to the methods described by (Neely and Phillipson, 1988); *(Vodovotz, 1996)*; *(Arru et al., 2010)*; *(Mc Eween, 1969)*; *(Gross et al., 1967)*; *(Luck, 1974)*; *(David and Richard, 1983)*; *(Moron et al., 1979)*; respectively.

2.7.Statistical analysis:

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS, 13.0 software, 2009). Values of $P<0.05$ were considered to be significant.

3. RESULTS

The obtained data in table (1) revealed that administration of a single intravenous dose of VPA to normal rabbits exhibited a significant increase in serum (NH3, NO, GABA and MAO) levels after 1 hour and at 10th, 20th, 30th days when compared with control group. However, oral administration of L-C daily for 30 days cause significant reduction in serum (NH3, NO, GABA and MAO) at 10th, 20th, 30th days compared with that of VPA group (positive control). The obtained results presented in table (2) revealed that administration of a single intravenous dose of VPA to normal rabbits exhibited a significant decrease in both of brain and liver (CAT, SOD and GR) activities.

However a significant increase was recorded in brain and liver (MDA) concentration after 1 hour and at 10th, 20th, 30th days when compared with control group. Administration of L-C daily for 30 days cause significant increase in both of brain and liver (CAT, SOD and GR) activities and ameliorate brain and liver (MDA) levels.

4. DISCUSSION

The obtained results demonstrated that intravenous VPA injection significantly

Table (1): Effect of L-Carnitine on serum (NH_3, NO, GABA and MAO) levels in experimentally induced hyperammonaemia in rabbits.

Duration periods	Groups	Parameters			
		NH_3 (mg\dl)	NO (mmol\ L)	GABA (ng\ ml)	MAO (ng\ ml)
After 1 hour	Control	53.17 ± 0.55^{A}	12.83 ± 0.49^{A}	9.90 ± 0.40^{A}	5.40 ± 0.06^{A}
	VPA	101.00 ± 0.58^{B}	33.97 ± 1.56^{B}	31.47 ± 0.79^{B}	21.20 ± 0.42^{B}
After 10 days	Control	53.77 ± 0.79^{C}	12.73 ± 0.62^{B}	9.87 ± 0.34^{B}	5.37 ± 0.09^{B}
	VPA	93.33 ± 0.88^{E}	31.33 ± 0.88^{D}	28.77 ± 0.54^{E}	19.31 ± 0.58^{E}
	L-C	48.33 ± 0.88^{A}	11.17 ± 0.44^{A}	8.43 ± 0.28^{A}	3.10 ± 0.06^{A}
	Protective	63.33 ± 1.20^{D}	22.33 ± 0.88^{C}	22.34 ± 1.45^{D}	16.47 ± 1.02^{D}
	Therapeutic	51.00 ± 0.58^{B}	23.00 ± 1.53^{C}	21.03 ± 0.55^{C}	13.46 ± 0.57^{C}
After 20 days	Control	53.67 ± 0.76^{C}	12.53 ± 0.33^{B}	9.97 ± 0.28^{B}	5.60 ± 0.06^{C}
	VPA	83.33 ± 1.67^{D}	28.83 ± 0.60^{D}	26.85 ± 0.44^{D}	15.25 ± 0.8^{E}
	L-C	46.67 ± 2.40^{B}	10.37 ± 0.09^{A}	6.97 ± 0.52^{A}	2.53 ± 0.32^{B}
	Protective	42.67 ± 0.88^{A}	18.21 ± 0.77^{C}	9.70 ± 0.89^{B}	0.60 ± 0.25^{A}
	Therapeutic	44.28 ± 2.80^{A}	19.00 ± 0.58^{C}	16.07 ± 0.52^{C}	13.22 ± 0.78^{D}
After 30 days	Control	53.70 ± 0.79^{C}	12.73 ± 0.41^{B}	9.90 ± 0.32^{B}	5.43 ± 0.12^{B}
	VPA	76.00 ± 0.58^{D}	26.00 ± 0.58^{E}	25.49 ± 0.29^{E}	13.12 ± 0.69^{C}
	L-C	33.33 ± 0.88^{A}	7.67 ± 0.88^{A}	4.17 ± 0.44^{A}	0.87 ± 0.12^{A}
	Protective	54.00 ± 0.58^{C}	18.01 ± 0.59^{D}	16.58 ± 1.11^{D}	13.00 ± 0.58^{C}
	Therapeutic	37.67 ± 1.45^{B}	14.07 ± 0.58^{C}	11.58 ± 0.96^{C}	0.67 ± 0.09^{A}

Data are presented as (Mean ± S.E). SE: Standard error. A, B, C: Mean values with different superscript letters in the same column are significantly different at ($P\leq0.05$).

Table (2): Effect of L-Carnitine on both of brain and liver tissues of (CAT, SOD, GR and MDA) activities in experimentally induced hyperammonaemia in rabbits.

Duration periods	Groups	CAT (U\ gm tissue)		SOD (U\ gm tissue)		GR (U\gm tissue)		MDA (mmol\ gm tissue)	
		Brain	Liver	Brain	Liver	Brain	Liver	Brain	Liver
After 1 hour	Control	47.07±0.64^{B}	41.93±0.67^{B}	42.00±0.58^{B}	34.73±0.43^{B}	4.10±0.06^{B}	5.77±0.09^{B}	98.17±0.73^{A}	97.63±0.66^{A}
	VPA	24.86±1.98^{A}	23.7±0.96^{A}	35.43±1.55^{A}	14.00±1.53^{A}	0.70±0.06^{A}	1.70±0.15^{A}	121.17±0.73^{B}	130.00±0.58^{B}
After 10 days	Control	46.78±0.21^{B}	42.90±1.59^{C}	42.27±0.37^{B}	35.06±1.39^{D}	4.13±0.03^{D}	5.47±0.13^{D}	98.00±0.46^{B}	97.57±0.62^{B}
	VPA	24.00±0.58^{A}	21.97±0.48^{A}	27.06±0.58^{A}	13.25±1.38^{A}	0.61±0.00^{A}	1.30±0.60^{A}	123.83±0.44^{D}	126.67±0.88^{D}
	L-C	59.37±1.23^{D}	50.14±0.75^{D}	48.67±1.86^{D}	46.24±3.01^{E}	5.60±0.06^{E}	6.50±0.15^{D}	89.10±0.49^{A}	84.13±1.95^{A}
	Protective	34.83±1.09^{B}	33.27±0.45^{B}	46.33±1.20^{CD}	27.67±1.20^{C}	1.68±0.06^{B}	2.53±0.19^{B}	111.03±0.61^{C}	119.00±0.58^{C}
	Therapeutic	34.00±1.32^{B}	32.30±0.62^{B}	44.40±0.31^{BC}	24.13±1.66^{B}	2.10±0.06^{C}	3.17±0.12^{C}	111.67±0.88^{C}	119.27±0.48^{C}
After 20 days	Control	47.33±0.44^{C}	42.27±0.54^{C}	43.30±0.36^{B}	34.67±1.45^{D}	4.23±0.09^{D}	5.63±0.15^{D}	98.00±0.58^{B}	98.27±0.55^{B}
	VPA	21.00±0.58^{A}	19.17±0.44^{A}	24.00±0.58^{A}	13.12±1.26^{AE}	0.52±0.02^{A}	1.47±0.09^{A}	121.00±0.58^{E}	119.00±0.58^{D}
	L-C	66.00±0.58^{D}	54.34±1.42^{D}	56.04±0.54^{D}	55.45±0.48^{B}	6.60±0.06^{E}	7.10±0.06^{E}	85.00±1.15^{A}	81.67±1.20^{A}
	Protective	34.17±0.60^{B}	33.00±0.58^{B}	63.00±1.53^{E}	32.92±0.64^{C}	1.40±0.06^{B}	2.73±0.08^{B}	113.13±0.59^{D}	109.00±0.58^{C}
	Therapeutic	34.00±0.50^{B}	32.10±0.55^{B}	46.57±1.19^{C}	26.33±3.19^{B}	2.37±0.09^{C}	3.60±0.06^{C}	109.17±0.44^{C}	108.17±0.60^{C}
After 30 days	Control	45.17±0.44^{C}	42.13±0.70^{C}	44.07±0.52^{B}	35.33±0.44^{C}	4.13±0.02^{D}	5.41±0.05^{D}	97.50±1.04^{B}	97.50±0.29^{B}
	VPA	14.00±0.58^{A}	14.60±0.21^{A}	19.67±1.20^{A}	10.31±0.86^{A}	0.44±0.03^{A}	0.90±0.06^{A}	119.17±0.44^{E}	116.00±0.58^{D}
	L-C	71.00±0.58^{D}	61.17±0.73^{D}	68.67±4.10^{D}	62.67±1.45^{D}	7.03±0.15^{E}	7.73±0.12^{E}	80.60±0.31^{A}	78.07±0.64^{A}
	Protective	31.00±0.58^{B}	28.50±0.87^{B}	51.33±1.20^{C}	33.32±1.44^{C}	1.70±0.06^{B}	2.80±0.06^{B}	111.00±0.58^{D}	104.00±0.58^{C}
	Therapeutic	32.00±0.84^{B}	28.50±1.04^{B}	51.33±0.88^{C}	30.94±2.46^{B}	2.37±0.03^{C}	3.47±0.18^{C}	102.67±1.45^{C}	103.67±1.45^{C}

Data are presented as (Mean ± S.E). SE: Standard error. A, B, C: Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

increased serum (NH_3, NO, GABA and MAO) levels when compared with control group. These results were nearly similar to those recorded by (Vossler et al., 2002) who reported that Valporate increases ($P \leq 0.05$) ammonia levels through both hepatic (due to decreased hepatic urea production through valporate induced inhibition of liver carbamoyl phosphate synthetase I) and renal (due to stimulation by valporate of glutaminase activity in the renal cortex) mechanisms. The recorded increase in ammonia level in VPA- administrated rabbits may be related to Sodium valporate and one of its metabolites, propionate, which are potent inhibitors of mitochondrial enzymes and inhibits carbamyl phosphate synthatase-1, the enzyme involved in the first step of removal of ammonia from the body (Duarte et al., 1993). Also, (Peker et al., 2009), found a significant increase ($P \leq 0.05$) of NO in the serum of epileptic children by VPA. The recorded increase in ammonia level in VPA- administrated rabbits may be related to subsequent studies using in vivo cerebral micro dialysis showed that acute ammonia toxicity led to activation of the MDA receptor-NO-cGMP (NO, nitric oxide; cGMP, cyclic GMP) signal transduction pathway in brain as suggested by (Hermenegildo et al., 2000). It was reported that VPA-mediated neuroprotection may to some extent involve increases in GABA in the basal ganglia (Chen et al., 2007). The recorded increase in GABA level in VPA- administrated rabbits may be related to the accepted action of VPA that increases brain levels of the inhibitory neurotransmitter GABA by inhibiting GABA transaminase and thus preventing GABA metabolism (Rosenberg, 2007). The recorded increase in MAO activities in VPA-administration rabbits may be related to VPA induces MAO A via the activation of Akt/FoxO1 signaling pathway. Otherwise, VPA has shown the capability to induce selective cellular signaling pathways including PI3K/Akt and extracellular signal-regulated kinase pathways, which eventually activate downstream transcription factors such as c-Jun, c-Fos, and β-catenin via differential regulations of mediators such as glycogen synthase kinase-3β (GSK-3β), to control the transcription of target genes (Kostrouchova et al., 2007). Also, VPA may induce *MAO A* by modulating the acetylation status of *MAO A* promoter. It has been shown previously the capability of it to increase Sp1 acetylation (Marinova et al., 2009) and activates *MAO A* gene expression and demonstrate the molecular mechanisms via the activation of Akt/FoxO1 signaling pathway (Shih et al., 2011). Finally, VPA has shown previously the capability to increase Sp1 acetylation, a key transcriptional activator of MAO A by directly binding to the Sp1 sites in the MAO A core promoter. This further suggests the possibility that Sp1 and other potential VPA-responsive MAO A regulators may also contribute to the induction of MAO A by VPA via diverse mechanisms such as histone acetylation (Shih et al., 2011). The action mechanism of VPA involves the regional changes in the concentration of the neurotransmitter γ-aminobutyric acid (GABA), (Perucca, 2002). Once VPA inside the mitochondria, VPA must be converted to an active intermediate, valproyl-CoA, in the presence of ATP and CoA, in order to gain access to the β-oxidation system. The activation of VPA is the only step in VPA degradation requiring ATP (Aires et al., 2007). Furthermore, VPA reduces the release of the epileptogenic γ-hydroxybutyric acid and attenuates the neuronal excitation induced by N-methyl-D-aspartate (NMDA)-type glutamate receptors. Regarding the significant decrease in serum (NH_3, NO, GABA and MAO) concentrations after administration of L-carnitine as compared to that of VPA group (positive control). The obtained results came in accordance with those recorded by (Malaguarnera et al., 2003) who declared a protective effect of L-carnitine against ammonia-precipitated encephalopathy in cirrhotic patients. The possible beneficial effect of carnitine may

be related to an improved pyruvate oxidation, Krebs cycle and flux through glutamate dehydrogenase. The latter could then explain the lowering of blood ammonia levels that follows L-carnitine administration. The best-known function on L-carnitine is the facilitation of β-oxidation by transforming activated long chain fatty acids into mitochondria. Thus when mitochondrial β-oxidation is inhibited, not only is acetyl-CoA, but also the activating effect of acetyl-CoA is further inhibited by none sterified acyl-CoA esters, an effect that further decreases gluconeogenesis. High levels of acyl-CoA derivates also may inhibit ureagenesis (resulting in hyperammonemia and the tricarboxylic acid cycle) (Corkey et al., 1988). Also, (Ergun et al., 2001) found that L-carnitine treatment produced a significant reduction in NO levels. Similarly, (Aydogdu et al., 2004) reported that recent experimental and clinical studies have shown that mitochondrial dysfunction secondary to a disruption of carnitine homeostasis may play a role in decreased NO. Moreover, (Fariello and Shug, 2000) found that GABA competitively inhibits carnitine transport in brain slices. Also, (Smeland, 2012) have found that acetyl-L-carnitine treatment increases noradrenalin and serotonin levels in brain which accompanied by decrease in the levels of GABA in the brain. It was indicated that MAO induces the oxidative stress which reduced by L-carnitne administration (Jenkins, 2000). Otherwise, (McCall and Frei, 1999) revealed that the massive release of 5-HT from pre-synaptic storage vesicles induced by 3,4 Methyl enedioximetham-phetamine followed by monoamine oxidase B (MAO-B) metabolism, significantly increases oxidative stress at the mitochondrial level. L-Carnitine and its ester, acetyl-L-carnitine (ALC), facilitate the transport of long chain free fatty acids across the mitochondrial membrane enhancing neuronal anti-oxidative defense. Concerning, the results demonstrated in table (2) which revealed that intravenous VPA injection significantly decreased both brain and liver (CAT, SOD and GR) activities and significantly increased both of brain and liver (MDA) concentration when compared with control group. These results were nearly similar to these recorded by (Cengiz et al., 2000) who reported that VPA decrease the levels of liver and brain SOD. Also, (Solowiej and Sobaniec, 2003) found that 25 children treated with VPA, exhibited a significant decrease in SOD activity. Furthermore, (Zhang et al., 2011) reported a significant decrease in liver and brain antioxidant activity of SOD and CAT with increasing VPA. Moreover, (Chaudhary and Parvez, 2012) found a significant reduction of GR, SOD, and CAT activity with VPA administration that approved by (Pipalia et al., 2011) who showed that intravenous administration of VPA resulted in significant decrease in tissue catalase and tissue glutathione reductase. The increase in superoxide dismutase in the brain appears to be due to the intensified generation of reactive oxygen species (Vidyasagar et al., 2004). The intra cellular antioxidant system comprises of different free radical scavenging antioxidant enzymes along with some non enzyme antioxidant. CAT and GR constitute the first line of cellular antioxidant defense enzymes. Thus, to eliminate free radicals, these cellular antioxidants play an important role and equilibrium exists between these enzymes under normal conditions. When excess free radicals are produced, this equilibrium is lost and consequently oxidative insult is established (Manna et al., 2007). The significant decrease in GR, CAT, and SOD activities observed in the present study might lead to elevated levels of H_2O_2 and oxidative stress that has been shown to cause mitochondrial dysfunction which is implicated in many neurological disorders (paradies et al., 2001). Otherwise, (Kodai et al., 2007) recorded an increased level of MDA with VPA administration. Also, (Hamed and Abdellah, 2004) reported that epileptic patients treated with VPA have increased MDA levels that approved by

(Pipalia et al., 2011) who reported that intravenous administration VPA resulted in significant increase in tissue MDA. The increased level of hepatic MDA observed in this study could be explained by the fact to the cytotoxic activity of VPA that resulted in generation of hydrogen peroxide and production of highly reactive hydroxyl radical (Na L et al., 2003). The obtained results demonstrated in table (2) indicated that oral L-carnitine administration significantly increased both brain and liver (CAT, SOD and GR) activities and significantly decreased their (MDA) concentration as compared to that of VPA group (positive control). These results were came in accordance with those recorded by (Kumaran et al., 2003), (Aydogdu et al. 2006) and (Boonsanit et al. 2006) who reported that L -carnitine could increase levels of different antioxidant enzymes (e.g., SOD, catalase, glutathione reductase). Also, (Kalaiselvi and Panneerselvam, 1998) showed that carnitine supplementation enhances the activities of brain and liver anti-oxidant enzymes, such as SOD, CAT and GR levels. Lower CAT activity may have resulted from increased superoxide radical production arising from decreased SOD activity as reported in (Zama et al., 2007) who showed that superoxide radicals can directly inhibit the CAT activity. Pretreatment with L-C increased hepatic SOD and CAT activities apparently due to decreased lipoperoxidation. Increase in hepatic SOD and CAT activities accelerated the removal of ROS therefore reducing hepatic injury. Otherwise, (Augustyniak and Skrzydlewska, 2009) reported that MDA was ($P \leq 0.05$) reduced by 10.4-33.69% with supplementation of L-carnitine. In addition, (Sener et al., 2004) have shown that L-carnitine administration prevents MDA formation that approved by (Ergun et al., 2001) who reported that L-carnitine administration inhibits tissue MDA formation. Decreasing malonaldehyde concentration that observed in the present study in response to L-carnitine supplementation might be attributed at least partly to an increased rate of the transport of long-chain fatty acids into the mitochondria. Dietary L-carnitine supplementation promotes the β-oxidation of these fatty acids to generate ATP energy and improves energy utilization. Consequently, L-carnitine supplementation to diets reduces the amount of long-chain fatty acids availability for esterification to triacylglycerol and storage in the adipose tissue (Xu et al., 2003). Co-administration of L- carnitine with VPA produced excellent protection from VPA- induced damage which was evident biochemically by reduction of liver enzymes activities, MDA and antioxidant enzyme activities to approximately near normal values (Bykov et al., 2004). Thus, it could be concluded that, L-C pretreatment provided an effective protection against HE, HT and oxidative damage in liver and brain induced by VPA in rabbits, since this compound was able to ameliorate serum biochemical parameters, enzymatic and non-enzymatic antioxidant defense system. Therefore, we recommended that, administration of diet rich in the natural L-carnitine is very important for preventing adverse effects of hyperammonemia.

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التأثير الكيميائي الحيوي للإل- كارنتين فى زيادة نسبة الأمونيا المحدث بالفالبورات فى الأرانب

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الملخص العربي

أجريت هذه الدراسة بهدف معرفة الأثار الضارة الناجمة عن زيادة نسبة الأمونيا المحدثة بالفالبورات وتقييم التأثير الوقائى والعلاجى للإل- كارنتين على التغيرات فى مستوى الأمونيا، أكسيد النيتريك، الجاما أمينو حمض بيوتيريك، والمونو أمين أكسيديز، والإنزيمات المضادة للأكسدة (سوبر أكسيد ديسميوتيز، كاتاليز، جلوتاثيون ريداكتاز)، تركيز إل-مالون داى ألدهيد فى دم وأنسجة (كبد ومخ) الأرانب المحدث فيها زيادة نسبة الأمونيا بالفالبورات. ولهذا الغرض استخدم عدد 51 من ذكور الأرانب النيوزيلاندية البيضاء تتراوح أوزانها من 2-3 كيلو جرام قسمت إلى خمس مجموعات كالآتى: المجموعة الأولى (المجموعة الضابطة): اشتملت على 12 أرنب لم تعط أى أدوية. المجموعة الثانية (مجموعة إل- كارنتين): تكونت من 9 أرانب تم تجريعها إل- كارنتين يومياً عن طريق الفم بجرعة مقدارها 95 مليجرام لكل كيلو جرام من وزن الجسم لمدة 30 يوم. المجموعة الثالثة (مجموعة فالبورات): أشتملت على 12 أرنب تم حقنهم فى الوريد جرعة واحدة من الفالبورات مقدارها 130 مليجرام لكل كيلو جرام من وزن الجسم. المجموعة الرابعة (المجموعة الوقائية): اشتملت على 9 أرانب تم تجريعها إل-كارنتين كما فى المجموعة الثانية لمدة 30 يوم وفى اليوم 31 تم حقن جرعة واحدة من الفالبورات فى الوريد مقدارها 130 مليجرام لكل كيلو جرام من وزن الجسم. المجموعة الخامسة (المجموعة العلاجية): اشتملت على 9 أرانب تم حقنها فى الوريد جرعة واحدة من الفالبورات كما فى المجموعة الثالثة واستمر العلاج بالإل- كارنتين بنفس الجرعة السابقة لمدة 30 يوم. تم تجميع عينات الدم والأنسجة فى اليوم العاشر والعشرين والثلاثين من تعاطى إل- كارنتين وبعد ساعة واحدة وفى اليوم العاشر والعشرين والثلاثين من تعاطى الفالبورات. وقد أسفرت نتائج التحليل الكيميائى الحيوى عن وجود زيادة معنوية فى كل من الأمونيا وأكسيد النيتريك والجاما أمينو حمض بيوتيريك والمونو أمين أكسيديز بمصل الدم بالإضافة إلى الزيادة المعنوية فى تركيز إل- مالون داى ألدهيد فى نسيج الكبد والمخ مع حدوث إنخفاض معنوى فى نشاط الكاتاليز والسوبر أكسيد ديسميوتيز والجلوتاثيون ريداكتاز فى نسيج الكبد والمخ فى مجموعة الفالبورات. كما أوضحت النتائج أن المجموعتين الرابعة والخامسة إنخفض فيهما مستوى الأمونيا وأكسيد النيتريك والجاما أمينو حمض بيوتيريك والمونو أمين أكسيديز بالإضافة إلى الإنخفاض المعنوى فى تركيز إل- مالون داى ألدهيد فى نسيج الكبد والمخ فى حين زاد نشاط الكاتاليز والسوبر أكسيد ديسميوتيز والجلوتاثيون ريداكتاز فى نسيج الكبد والمخ. وأوضحت الدراسة أن استخدام الإل- كارنتين كمادة واقية مضادة للأكسدة كان لها دوراً هاماً فى التقليل من الإجهاد التأكسدى فى نسيج الكبد والمخ، كما أن له دوراً فى المعالجة والوقاية من مشكلات زيادة نسبة الأمونيا عن طريق منع الإبطال المحدث بالفالبورات لمفعول عملية البيتا- أكسدة للدهون. لذلك توصى الدراسة بضرورة إستغلال الإل- كارنتين كمادة وقائية مضادة للأكسدة وإدخاله كمادة فعالة فى صناعة العقاقير الطبية المستخدمة فى وقاية وعلاج زيادة نسبة الأمونيا وما يترتب عليها من التسمم الكبدى و اعتلال الكبد والمخ.

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