

Full Length Research Paper

The amelorative effect of L-arginin and omega-3 fatty acid against sodium valproate induced hepatotoxicity

Dalia M. Amin¹, Marwa T. Abaza¹, Walaa M. Sarhaan², Amany I. Ahmed³ and Amr A. Moustafa^{3*}

¹Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Zagazig University, Egypt.

²Department of Medical Biochemistry, Faculty of Medicine, Zagazig University, Egypt.

³ Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Received 15 May, 2018; Accepted 20 July, 2018

Sodium valproate (VPA) used in epilepsy and has hepatotoxic effect in mammals. This work is to assess the role of L-arginin and omega-3 fatty acid against VPA oxidative stress on liver. This study was carried out on 70 rats divided into 7 groups each of 10 rats treated orally once daily 6days/ week; Group I (control), Group II (L-arginin): each rat was received 300 mg/kg L-arginin, Group III (omega-3): each rat received 300 mg/kg omega-3, Group IV (VIA): Each rat received 400 mg/kg VPA. Group V (VPA and L-arginin): Each rat received the previous doses. Group VI (VPA and omega-3): as before. Group VII (VPA, L-arginin and omega-3 group): as before. There was a significant increase in the mean values of AST, ALT, ALP and TBL in VPA treated group. Upon supplementation with L-arginin or omega-3 to VPA treated rats there was a significant decrease in the mean values of AST, ALT, ALP and TBL. Microscopic examination of liver section of VPA treated rat revealed vacuolated hepatocytes, focal necrosis, and enlarged portal tract. VPA toxicity induced severe liver damage in rats, and administration of either L-arginin or omega-3 protecting liver tissues.

Key words: L-arginin, omega-3 and sodium valproate.

INTRODUCTION

Sodium valproate (VPA), an eight carbon branched chain fatty acid which commonly prescribed as antiepileptic drug for epilepsy, bipolar disorders and migraines (Tolou-Ghamari and Palizban (2015). It is effective and widely used, but hepatotoxic in both animals and humans (Defoort et al., 2006). Chronic use of sodium valproate causing damaging complications (Khan et al., 2013). Severe side effects caused by VPA treatment such as

thrombocytopenia, platelet aggregation and pancreatitis (Saleh et al., 2012). The mechanism of liver toxicity is mainly oxidative stress with increase reactive oxygen species (ROS) attenuating antioxidant capacity (Ochs-Balcom et al., 2006). Many natural substances have been tried as antioxidants to overcome the free radical hazardous effects.

Omega-3 fatty acid cannot be synthesized by humans

*Corresponding author. E-mail: daliaamin013@gmail.com.

and should be taken by diet named according to the location of double bond from the carbon of the terminal methyl called omega carbon (Plourde and Cunnane, 2007). The unsaturated fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) belong to the omega-3 fatty acid category. These sources of omega-3 are fish oil and oceanic fish (Wergedahl et al., 2009). The omega-3 fatty acid has protective effects are by induction of cytochrome CYP7A1 expression and the activity of cholesterol catabolism to bile acids (Kim et al., 2012). In a study by Haast and Kiliaan (2015) a direct correlation was found between Omega-3 fatty acid intake and reduced liver deterioration and damage (Haast and Kiliaan, 2015).

L-arginine was first isolated from the extract of a lupine (*Lupinus* spp.) seedling. *Lupinus* is a genus in the legume (Fabaceae) plant family (Utagawa, 2004). Arginine serves as a precursor for the synthesis of nitric oxide (NO), a free radical molecule that is synthesized in all mammalian cells from L-arginine by NO synthase (NOS) (Ivanova et al. (2014). Nitric oxide (NO) appears to be a major form of the endothelium-derived relaxing factor (EDRF). Nitric oxide (NO) and (EDRF) share similar chemical and pharmacological properties and is derived from the oxidation of a terminal guanidine group of L-arginine (Tapiero et al., 2002). Nitric oxide (NO) produced from L-arginine by endothelial NO synthase (eNOS) plays an important role in regulating endothelium-dependent vasodilatation, preventing the adhesion of blood cells and platelets along the endothelial cell layer of blood vessels, and inhibiting vascular smooth muscle cell proliferation. Nitric oxide (NO) also shows scavenging effects against oxygen radical species, including the prevention of oxidation of LDL-cholesterol (Morita et al., 2014). The aim of this work is to assess the protective role of L-arginine and omega-3 fatty acids against the effect of sodium valproate (VPA) induced toxicity and oxidative stress in the liver.

MATERIALS AND METHODS

Omega-3 fatty acid

It was obtained from Wako Chemical Co, (Japan). It was provided in a liquid form.

L- arginine

L-arginine is solid white crystalline; it is available in a glass bottle powder containing 25 gm of L arginine purchased from Euromedex, Egypt. The solution is prepared by dissolving 10gm of powder in 100 ml water.

Sodium valproate (Depakine®)

Sanofi-France was purchased from Global Napi Pharmaceuticals,

Egypt.

Distilled water

The dissolvent for all previous chemicals.

Animals

The study was done for 12 weeks and the rats were divided into 7 groups each of 10 rats: This study was carried on 40 adult albino rats, each weighing 150 g. The animals were obtained from Animal House of the Faculty of Veterinary Medicine, Zagazig University. The study had been designed in the Faculty of Medicine, Zagazig University. All animals received care in compliance with the Animal Care Guidelines and Ethical Regulations in accordance with "The Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources (1996). In order to exclude fallacies the following environmental conditions were standardized according to Cuschieri and Backer (1977):

- 1) The climate in the animal house and in the cage with proper ventilation.
- 2) Low noise level was maintained as noise may affect the behavior of the animals.
- 3) The bedding, wood shaving had to be sufficient to absorb urine and also changed frequently to keep the animals clean.

Accordingly the animals were kept in galvanized iron mesh cage with solid bottoms, which contained wood shaving as bedding, 10 rats per cage to avoid overcrowding, as well as isolation were avoided. The rats were kept in this environment for two weeks before starting experimentation to be adapted to any possible stress secondary to transportation procedure from the animal supplier or due to sudden environmental modification and exclude any diseased animals (Semler, 1992). The rats received balanced food, rich in all stuffs necessary to maintain their health before and during drug administration. It consists of *ad libitum*. Distilled water was offered in separate clean containers. This study was carried on 70 adult albino rats, each weighing 150-200 g. The animals were obtained from Animal House of the Faculty of Veterinary Medicine, Zagazig University. The study had been designed in the Faculty of Medicine, Zagazig University.

Study design

The rats were divided into 7 groups each of 10 rats:

- (i) Group I (control group) (10 rats): Each rat will receive only regular diet of *ad libitum* and distilled water to determine the basic values of performance.
- (ii) Group II (L- arginine group) (10 rats): Each rat will be gavaged orally with 300mg/kg body weight L -arginine once daily dissolved in distilled water (Mansour et al., 2002).
- (iii) Group III (Omega-3 fatty acid group) (10 rats): Each rat will be gavaged orally (300 mg/kg body weight omega-3 fatty acid dissolved in 1 ml of distilled water once daily (Davood et al., 2017).
- (iv) Group IV (Sodium Valproate group) (10 rats): Each rat will be gavaged orally with 400 mg/kg body weight of sodium valproate dissolved in 1ml of distilled water once daily (Elwakkad et al., 2008).
- (v) Group V (Sodium Valproate and L- arginine group) (10 rats): Each rat will be gavaged orally with (300mg/kg body weight L – arginine) 15 min before (400 mg/kg body weight sodium valproate) once daily.

(vi) Group VI (Sodium Valproate and Omega-3 fatty acid) (10 rats): Each rat will be gavaged orally with (300 mg/kg body weight omega-3 fatty acid) 15 min before oral gavage with (400 mg/kg body weight sodium valproate) once daily.

(vii) Group VII (Sodium Valproate, L- arginine and Omega-3 fatty acid group) (10 rats): Each rat will be gavaged orally with (300 mg/kg body weight omega-3 fatty acid + 300 mg/kg body weight L – arginine + 400 mg/kg body weight sodium valproate) once daily.

On the end of 12th week of the study period, rats from each group will be under light ether anesthesia as described by Nemzek et al. (2001) subjected to blood sample collection from the retro-orbital plexuses. The animal was held in the left hand and grasped from the back. While enclosing the neck and exerting slight pressure by the thumb and index fingers. The blood samples will be used for estimating the following tests: Liver function tests {serum aspartame aminotransferase (AST), serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) total bilirubin levels (TBL)}, Then the anesthetized rats by ether were sacrificed by transection of major blood vessels. Specimens from the liver were taken for determination of oxidative stress markers {malondaidehyde (MDA) and reduced glutathione (GSH)}, histopathological and immunohistochemical studies.

Liver enzymes

Serum alanine aminotransferase (ALT) (IU/L)

ALT was assayed according to the method proposed by Reitman and Frankel (1957). Alanine aminotransferase (ALT) or Glutamate pyruvate transaminase (GPT) catalyses the reversible transfer of an amino group from alanine to α -ketoglutarate forming glutamate and pyruvate. The rate of decrease in concentration of NADH, measured photo metrically, is proportional to the catalytic concentration of ALT present in the sample.

Serum aspartate aminotransferase (AST) (IU/L)

AST was assayed according to the method proposed by Reitman and Frankel (1957). Oxaloacetate formed is measured by monitoring the concentration of oxaloacetate hydrazones formed with 2, 4-dinitrophenylhydrazine.

Serum Alkaline phosphatase (ALP) (IU/L)

Estimation: ALP was assayed according to the method proposed by Reitman and Frankel (1957). Individual data were gained from the reference.

Total bilirubin levels (TBL)

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically by (ErbaChem 7) (Kaplan et al., 1984).

Tissue sampling

The anesthetized rats by ether were sacrificed by transection of major blood vessels. The liver were dissected and divided into three pieces for:

- i) Estimation of tissue oxidative stress markers (Malondialdehyde and reduced Glutathione)
- ii) Histopathological studies.
- iii) Immunohistochemical studies.

Malondialdehyde (MDA) by Thiobarbituric acid assay

The principle of the method is based on that malondialdehyde MDA can react with Thiobarbituric acid (TBA) and give pink colored trimethine complex. The reaction should be performed at pH 2.3 and at 90-100°C for 10-15 min. Tissue MDA content was gained from the absorbance on (ErbaChem 7) spectrophotometer at 532 nm (Giuffrida et al., 2014).

Estimation of reduced Glutathione (GSH) by 5, 5'-dithiobis nitro benzoic acid assay

The principle of the method is based on that reduced Glutathione GSH on reaction with 5, 5'-dithiobis nitro benzoic acid (DTNB) produces a yellow colored product. Tissue GSH content was gained from the absorbance on (ErbaChem 7) spectrophotometer at 412 nm (Moron et al., 1979).

Histopathological studies

Samples from the liver were fixed in 10% formalin. After fixation, liver and kidney were embedded in paraffin blocks as usual and processed for the preparation of 5 μ thick sections. Sections were stained with haematoxylin and eosin and Periodic acid Schiff (PAS) and examined by light microscope (Bancroft and Stevens, 1996).

Immunohistochemical studies

Immunohistochemical reactions were carried out on sections of liver of adult male albino rats using caspase-3 (apoptosis related cysteine peptidase). Paraffin sections (4 μ m thick) were incubated with a rabbit monoclonal caspase-3 antibody (delivered from Lab Vision Laboratories- Cat. #: 1475-1) using the avidinbiotin peroxidase method by Abdel Aal et al. (2009). Sections were examined by light microscope.

RESULTS

Liver enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and total bilirubin (TBL)

Group I (control group), group II (L-arginin group), group III (omega-3 group), group IV (sodium valproate group), group V (sodium valproate and L-arginin group), group VI (sodium valproate and omega-3 group) and group VII (sodium valproate, L-arginin and omega-3 group): There were no significant differences in liver enzymes (ALT, AST, ALP, TBL), in the following groups; control groups (I), L-arginin group (II), and omega-3 group (III), ($P > 0.05$). So (ALT, AST, ALP, TBL), in group (I) were used for comparison with other groups of the study (Table 1).

Table 1. A statistical comparison between control groups (control group "I", L-arginin (L-A) group "II" and omega-3 (O-3) group"III") as regard mean values of liver enzymes and total bilirubin (ALT, AST and ALP) (TBL) after 12 weeks in adult albino rats by ANOVA test:

Test	T	L-arginin "II" Mean \pm SD	Omega-3 "III" Mean \pm SD	P
ALT (U/L)/ AST (U/L)	24.5 \pm 1.5 43.3 \pm 7.9	24 \pm 3.4 44.1 \pm 5.8	29 \pm 5.3 40 \pm 6.3	0.067 0.548
ALP(U/L)	60 \pm 4.1	57 \pm 4	55.7 \pm 3.3	0.169
TBL(mg/dL)	0.36 \pm 0.12	0.35 \pm 0.9	0.36 \pm 0.12	0.97
MDA liver (μ mol/gm)	55.31 \pm 5.6	54.97 \pm 5.6	52 \pm 1.76	0.99
GSH liver (ngm/gm)	147.8 \pm 4.2	147.33 \pm 3.6	148.05 \pm 4.53	0.95

SD = Standard Deviation.

p>0.05 = non significant.

Table 2. A statistical comparison between –vet control group "I" and treated group VP "IV", VP+L-A "V", VP +O-3"VI", VP+L-A+O-3 "VII" as regard mean values of liver enzymes and total bilirubin (ALT, AST and ALP) (TBL) after 12 weeks adult albino rats along the period of the study by ANOVA test.

Test	(I) Mean \pm SD	VP (IV) Mean \pm SD	VP- L-A (V) Mean \pm SD	VP-O-3 (VI) Mean \pm SD	VP –L-A-O-3 (VII) Mean \pm SD	P
ALT	24.5 \pm 1.5	97.33 \pm 6.71	30.50 \pm 6.15	26.00 \pm 1.75	23.83 \pm 5.91	<0.001**
AST	43.3 \pm 7.9	145.50 \pm 32.52	47.00 \pm 4.00	55.66 \pm 8.84	40.66 \pm 9.83	<0.001**
ALP	60 \pm 4.1	586.66 \pm 83.10	74.16 \pm 14.74	75.66 \pm 8.82	60.50 \pm 9.35	<0.001**
TBL	0.36 \pm 0.12	0.86 \pm 0.10	0.63 \pm 0.04	0.44 \pm 0.09	0.42 \pm 0.08	<0.001**
MDA liver (μ mol/gm)	55.31 \pm 5.6	165.95 \pm 14.3	72.8 \pm 5.7	67.34 \pm 11.6	54.5 \pm 9.5	<0.001**
GSH liver (ngm/gm)	147.8 \pm 4.2	62.49 \pm 20.1	124.02 \pm 8.6	103.2 \pm 2.79	122.5 \pm 11.3	<0.001**

SD = Standard Deviation.

N.B: ** = highly significant.

Other treated groups: (group IV (sodium valproate group), group V (sodium valproate and L-arginin group), group VI (sodium valproate and omega-3 group) and group VII (sodium valproate, L-arginin and omega-3 group): There were highly significant differences in groups by (ANOVA) study (P<0.001) as regard ALT,AST, ALP and TBL values (Table 2) (Figures 1 and 2).The least significant difference (LSD) of alanine aminotransferase (ALT) among groups I,VP (IV), L-A + VP (V), O-3 + VP (VI) and L-A +O-3+VP (VII) revealed that there was highly significant reduction in ALT level in group I when compared with other groups of the study(P<0.001). There was highly significant elevation in ALT in group VP (IV) when compared with other groups of the study (P<0.001). There was no significant difference in ALT value between groups L-A+ VP(V) and O-3 + VP (VI) groups L-A+ O-3+ VP(VII) (P>0.05) (Table 3).

The least significant difference (LSD) of aspartate (AST) among groups I, VP (IV), L-A + VP (VI), O-3 + VP (VI) and L-A+ O-3+ VP (VI) revealed that there was

highly significant reduction in AST level in group I when compared with other groups of the study(P<0.001). There was highly significant elevation in AST in group VP (IV) when compared with other groups of the study (P<0.001). There was no significant difference in AST value between groups L-A+ VP(V) and O-3 + VP (VI) groups L-A+ O-3+ VP(VII) (P>0.05) (Table 4).

The least significant difference (LSD) of aspartate (ALP) among groups I, VP (IV), L-A + VP (VI), O-3 + VP (VI) and L-A+ O-3+ VP (VI) revealed that there was highly significant reduction in ALP level in group I when compared with other groups of the study(P<0.001). There was highly significant elevation in ALP in group VP (IV) when compared with other groups of the study (P<0.001). There was no significant difference in AST value between groups L-A+ VP(V) and O-3 + VP (VI) groups L-A+ O-3+ VP(VII) (P>0.05) (Table 5).

The least significant difference (LSD) of aspartate (TBL) among groups I, VP (IV), L-A + VP (VI), O-3 + VP (VI) and L-A+ O-3+ VP (VI) revealed that there was

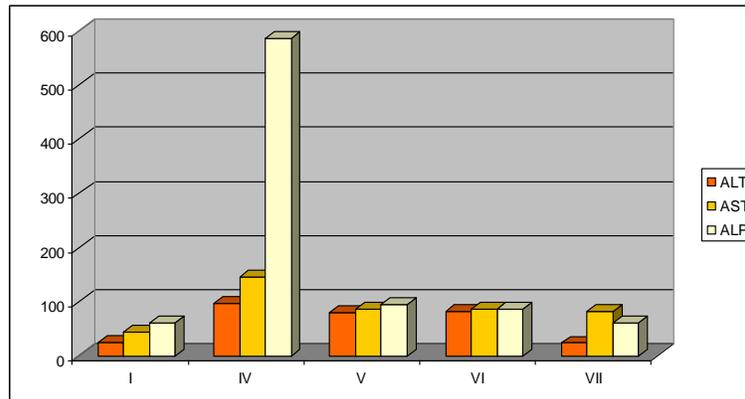


Figure 1. Bar chart showing comparative magnitude of mean values of liver enzymes (ALT, AST, ALP) at different groups control group (I), sodium valproate treated group (IV), L-arginin and sodium valproate group (V), omega-3 and sodium valproate group (VI) and L-arginin and omega-3 and sodium valproate group (VII) after 12 weeks of the study.

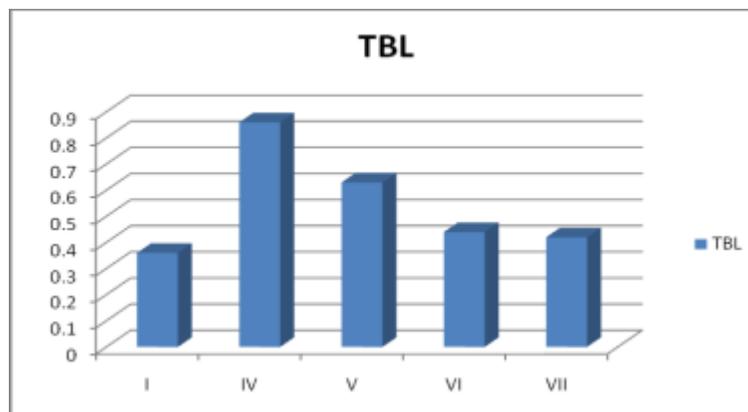


Figure 2. Bar chart showing comparative magnitude of mean values of total bilirubin (TBL) at different groups control group (I), sodium valproate treated group (IV), L-arginin and sodium valproate group (V), omega-3 and sodium valproate group (VI) and L-arginin and omega-3 and sodium valproate group (VII) after 12 weeks of the study.

highly significant reduction in TBL level in group I when compared with other groups of the study ($P < 0.001$). There was highly significant elevation in TBL in group VP (IV) when compared with other groups of the study ($P < 0.001$). There was no significant difference in TBL value between groups L-A+ VP(V) and O-3 + VP (VI) groups L-A+ O-3+ VP(VII) ($P > 0.05$) (Table 6).

Liver oxidative stress markers (malondialdehyde (MDA) and reduced glutathione (GSH))

Group I (control group), group II (L-arginin group), group

III (omega-3 group), group IV (sodium valproate group), group V (sodium valproate and L-arginin group), group VI (sodium valproate and omega-3 group) and group VII (sodium valproate, L-arginin and omega-3 group): There were no significant differences in liver oxidative stress markers (MDA, GSH), in the following groups; control groups ((I), L-arginin group (II), and omega-3 group (III), ($P > 0.05$)). So (MDA, GSH), in group (I) were used for comparison with other groups of the study (Table 1). Other treated groups: (group IV (sodium valproate group), group V (sodium valproate and L-arginin group), group VI (sodium valproate and omega-3 group) and group VII (sodium valproate, L-arginin and omega-3

Table 3. Least significance difference (LSD) among the negative control (I), (VP) IV, (VP+ L-A) V, (VP+ O-3) VI and (VP+ L-A+ O-3) VII groups as regard alanine aminotransferase enzyme ALT values after 12 weeks of the study.

ALT (IU/L)	I	(VP) IV	V(VP-L-A)	VI (VP-O-3)	VII(VP-L-A-O-3)
	Mean ± SD				
	24.5±1.5	97.33±6.71	30.50±6.15	26.00±1.75	23.83±5.91#
I		0.00**	0.43#	0.14#	0.79#
IV			0.00**	0.00**	0.00**
V				0.11#	0.08#
VI					0.40#

** = highly significant (P<0.001)

#= Non-significance (P>0.05).

Table 4. Least significance difference (LSD) among the negative control (I), (VP) IV, (VP+ L-A) V, (VP+ O-3) VI and (VP+ L-A+ O-3) VII groups as regard aspartate aminotransferase enzyme AST values after 12 weeks of the study.

AST (IU/L)	I	(VP) IV	V(VP-L-A)	VI (VP-O-3)	VII(VP-L-A-O-3)
	Mean ± SD				
	43.3 ± 7.9	145.50±32.52	47.00±4.00	55.66±8.84	40.66±9.83
I		0.00**	0.33#	0.29#	0.61#
IV			0.00**	0.00**	0.00**
V				0.054#	0.17#
VI					0.19#

** = highly significant (P<0.001)

#= Non-significance (P>0.05).

Table 5. Least significance difference (LSD) among the negative control (I), (VP) IV, (VP+ L-A) V, (VP+ O-3) VI and (VP+ L-A+ O-3) VII groups as regard alkaline phosphatase enzyme ALP values after 12 weeks of the study.

ALP (IU/L)	I	(VP) IV	V(VP-L-A)	VI (VP-O-3)	VII(VP-L-A-O-3)
	Mean ± SD				
	60 ± 4.1	586.66±83.10	74.16±14.74	75.66±8.82	60.50±9.35
I		0.00**	0.47#	0.30#	0.50#
IV			0.00**	0.00**	0.00**
V				0.83#	0.08#
VI					0.16#

** = highly significant (P<0.001)

#= Non-significance (P>0.05).

group): There were highly significant differences in groups by (ANOVA) study (P<0.001) as regard MDA and GSH values (Table 2).

The least significant difference (LSD) of malondaldehyde (MDA) among groups I, VP (IV), L-A + VP (V), O-3 + VP (VI) and L-A + O-3 + VP (VII) revealed

that there was highly significant reduction in MDA level in group I when compared with other groups of the study (P<0.001). There was highly significant elevation in MDA in group VP (IV) when compared with other groups of the study (P<0.001). There was no significant difference in MDA value between groups L-A + VP (V) and

Table 6. Least significance difference (LSD) among the negative control (I), (VP) IV, (VP+ L-A) V, (VP+ O-3) VI and (VP+ L-A+ O-3) VII groups as regard total bilirubin TBL values after 12 weeks of the study.

TBL (mg/dL)	I	(VP) IV	V(VP-L-A)	VI (VP-O-3)	VII(VP-L-A-O-3)
	Mean ± SD				
	0.36±0.12	0.86 ±0.10	0.63 ± 0.04	0.44±0.09	0.42±0.08
I		0.00**	0.57#	0.40#	0.72#
IV			0.00**	0.00**	0.00**
V				0.93#	0.1#
VI					0.18#

** = highly significant (P<0.001)

#= Non-significance (P>0.05).

Table 7. Least significance difference (LSD) among the negative control (I), (VP) IV, (VP+ L-A) V, (VP+ O-3) VI and (VP+ L-A+ O-3) VII groups as regard liver malondaidehyde (MDA) values after 12 weeks of the study.

MDA liver (µmol/gm)	I	(VP) IV	V(VP-L-A)	VI (VP-O-3)	VII(VP-L-A-O-3)
	Mean ± SD				
	16.00±1.26	61.66±7.60	58.16±4.79	48.83±9.34	17.50±2.42
I		0.00**	0.00**	0.00**	0.66#
IV			0.31#	0.070#	0.00**
V				0.11#	0.00**
VI					0.00**

Table 8. Least significance difference (LSD) among the negative control (I), (VP) IV, (VP+ L-A) V, (VP+ O-3) VI and (VP+ L-A+ O-3) VII groups as regard liver reduced glutathione (GSH) values after 12 weeks of the study.

GSH liver (ngm/gm)	I	(VP) IV	V(VP-L-A)	VI (VP-O-3)	VII(VP-L-A-O-3)
	Mean ± SD				
	15.00±0.16	41.16±1.16	45.66±5.68	41.61±6.33	26.83±2.92
I		0.00**	0.00**	0.00**	0.46#
IV			0.10#	1.00#	0.00**
V				0.10#	0.00**
VI					0.00**

O-3 + VP (VI) groups L-A+ O-3+ VP(VII) (P>0.05) (Table 7). The least significant difference (LSD) of reduced glutathione (GSH) among groups I, VP (IV), L-A + VP (V), O-3 + VP (VI) and L-A +O-3+VP (VII) revealed that there was highly significant reduction in GSH level in group I when compared with other groups of the study(P<0.001). There was highly significant elevation in GSH in group VP (IV) when compared with other groups of the study (P<0.001). There was no significant difference in GSH value between groups L-A+ VP(V) and O-3 + VP (VI) groups L-A+ O-3+ VP(VII) (P>0.05) (Table 8).

Histopathological changes of the liver

Light microscopic examination of H&E stained sections from the livers of the control groups (Groups I, II and III) revealed that; the liver showed multiple polygonal classic hepatic lobules with tightly packed cords of hepatocytes radiating from central veins toward the periphery of the lobules where the portal area is noticed. It contains the pre terminal branches of the portal vein, hepatic artery, and bile duct. Polygonal hepatocytes with rounded vesicular nuclei and acidophilic cytoplasm. Some cells

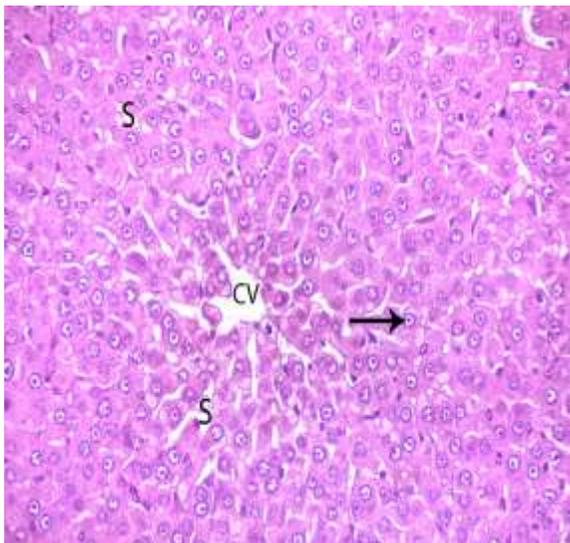


Figure 3. A photomicrograph of a section in the liver from a rat of the control group showing normal lobular architecture with central vein (CV) and radiating cords of hepatocytes, separated by blood sinusoids (s). Hepatocytes are polyhedral with acidophilic cytoplasm and the nucleus is rounded vesicular with prominent nucleolus (arrow) (H&Ex400).

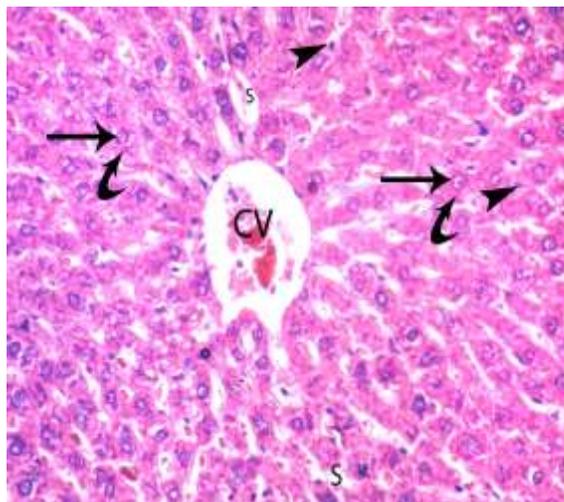


Figure 5. A photomicrograph of a section in the liver of a rat receiving Sodium valproate +L- arginine (groupV) same result for that administrated Omega-3 after sodium valproate (group VI) treated for 12 weeks showing mild dilated central vein (CV). Some hepatocytes appear with shrunken darkly stained nuclei (arrow head). There are areas of sinusoidal dilatation (S). Other hepatocytes are polyhedral with acidophilic cytoplasm (curved arrow) with rounded vesicular nucleus and prominent nucleolus (arrow) (H&Ex 400).

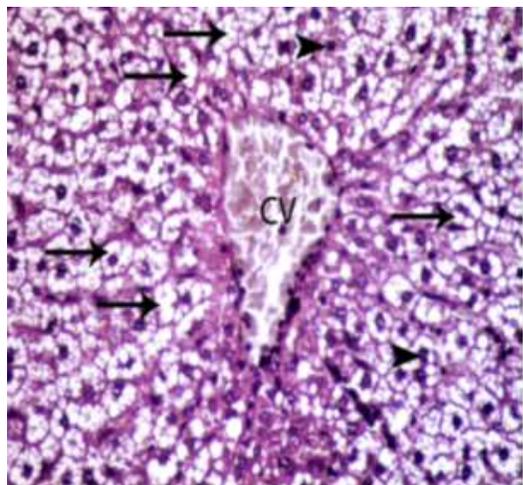


Figure 4. A photomicrograph of a section in the liver of a rat gavaged orally Sodium valproate (groupIV) treated for 12 weeks showing dilated congested central vein (CV). There are wide spread cytoplasmic vacuolation of hepatocytes (arrow). The nuclei are pyknotic in the majority of cells (arrow head) (H&Ex400).

are binucleated. Blood sinusoids and their lining of endothelial and kupffer cells are observed between hepatocyte cords (Figure 3).

In sodium valproate treated group the sections showed disturbed hepatic lobular architecture with hepatocytes showed vacuolated cytoplasm, focal necrosis, aggregates of chronic inflammatory cells, dilated congested central vein, enlarged portal tract with dilated congested blood vessels, bile duct proliferation and areas of hemorrhage (Figure 4). Examination of H&E stained sections of the liver specimens of the rats treated with L-arginin or omega-3 alone showed partial improvement of the changes that occurred after sodium valproate administration with almost normal lobular architecture and normal hepatocytes with average sized nuclei (Figure 5). While microscopic examination of the liver specimens of the rats treated with combined L-arginin and omega-3 showed near complete improvement of the changes that occurred after sodium nitrate administration with almost normal lobular architecture. Congested blood vessels still present (Figure 6).

Immunohistochemistry examination for detection of Caspase -3 apoptotic markers that appear as a cytoplasmic reaction brown in color. The stained sections from the livers of the Groups I, II and III showed no immunoreactivity within hepatocytes. Central vein (CV) and sinusoids (S) were noticed (Figure 7).

In sodium valproate treated group the sections showed most hepatocytes with granular strong +ve brown reaction within cytoplasm (arrows) around central vein

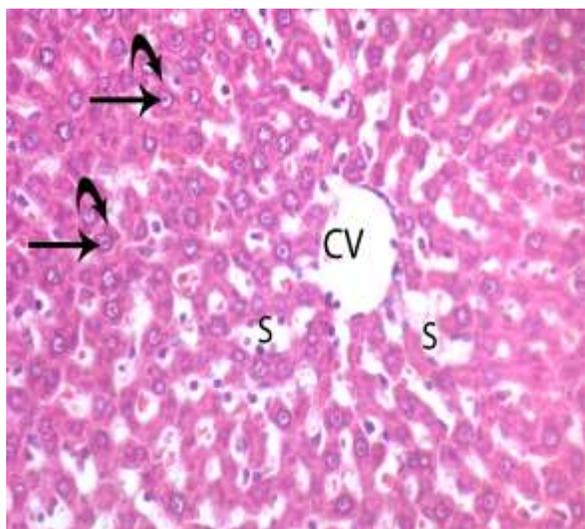


Figure 6. A photomicrograph of a section in the liver of a rat receiving Sodium valproate +L arginine and omega-3 (for 12 weeks showing sinusoidal dilatation (S). Most of the hepatocytes are polyhedral with acidophilic cytoplasm (curved arrow) with rounded vesicular nucleus and prominent nucleolus(arrow) (H&Ex400).

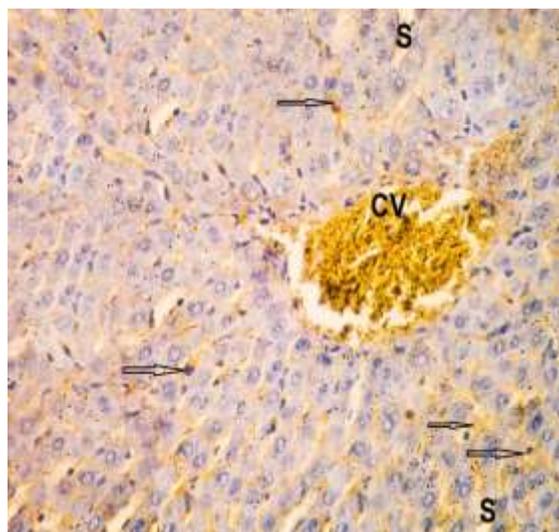


Figure 8. A photomicrograph of a section in the liver of a rat from the Group IV (sodium valproate) treated for 12 weeks showing dilated central vein (CV) and hepatocytes. There is strong caspase 3 positive immune reaction in the nuclei of hepatocytes (arrow) (caspase 3 X400).

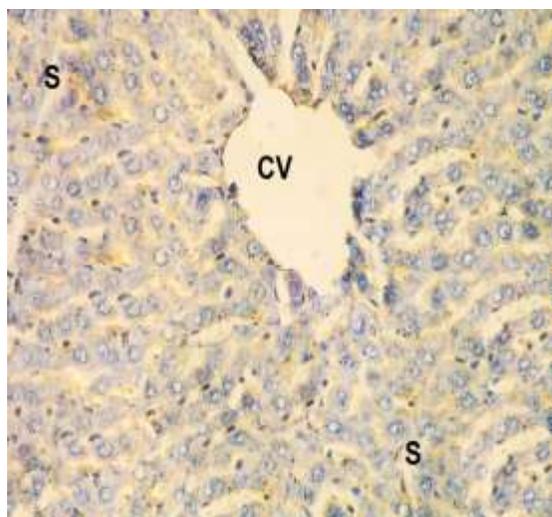


Figure 7. A photomicrograph of a section in the liver of a rat from the control group (group I) showing no caspase 3 negative immune reaction in the nuclei of hepatocytes (arrow) (caspase 3 x400).

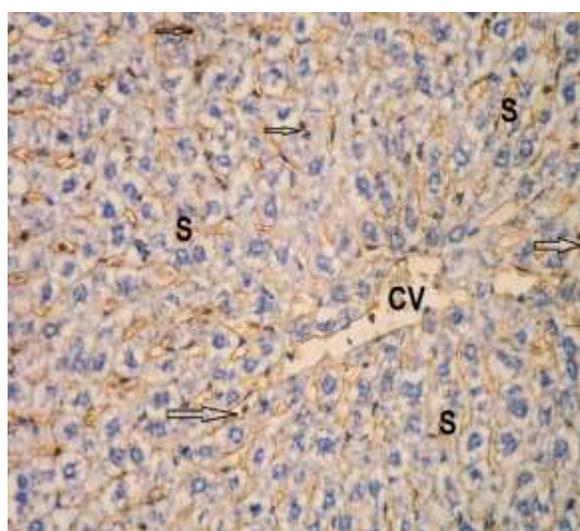


Figure 9. A photomicrograph of a section of liver from adult albino rat orally gavaged by sodium valproate followed by L-arginin same result for that administrated omega-3 after sodium valproate for 12 weeks showing weak positive caspase 3 immune reaction in the nuclei of hepatocytes (arrow) (caspase 3 X400).

(CV) (Figure 8). Examination of sections of the liver specimens of the rats treated with L-arginin or omega-3 alone showed little restoration of the normal cellular structure. Most hepatocytes had granular nucleus and granular moderate brown reaction within cytoplasm (arrows) around central vein (CV) (Figure 9). While

microscopic examination of the liver specimens of the rats treated with combined L-arginin and omega-3 showed restoration of the normal cellular structure. Most hepatocytes had granular nucleus and granular negative

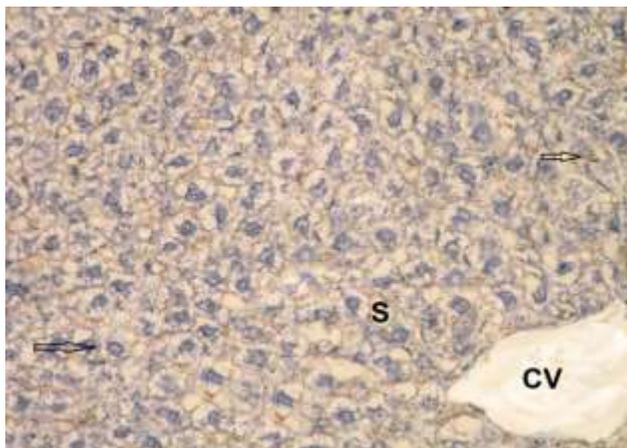


Figure 10. A photomicrograph of a section in the liver of a rat from the group orally gavaged by sodium valproate followed by L-arginin and Omega-3 showing negative caspase 3 immune reaction with normal vesicular nuclei of hepatocytes (arrow) (caspase 3 X400).

reaction within cytoplasm (arrows) around central vein (CV) (Figure 10).

DISCUSSION

The sodium valproate is the mostly prescribed anticonvulsant drug used in epilepsy. Epilepsy is a relatively common neurological disorder and medical therapy is presently the most common form of epilepsy treatment. Several side effects are associated with sodium valproate treatment such as hepatotoxicity, thrombocytopenia, platelet aggregation and pancreatitis.

In the present study, there was a significant increase in AST, ALT, ALP and total bilirubin mean values in sodium valproate treated group as compared to their corresponding values in control group. These results were in accordance with reports of Lee et al. (2008) who stated that high serum concentrations of ALT, AST indicated cellular leakage due to disintegration of cell membrane in liver. The increase in transaminase activities is probably due to the sodium valproate-induced pathological changes in liver and is an indication of liver damage. The liver is the primary organ for drug metabolism and elimination for many antiepileptic drugs (AEDs) and thus is subjected to drug-induced toxicity (Arroyo and De la Morena, 2001). ALT activity is related to general hepatocellular and AST to mitochondrial damage, leading to leakage of these enzymes into general circulation (Goulet, 2015). Sodium valproate (VPA) is a simple fatty acid largely interacts with cell membranes, extensively metabolized by the liver via glucuronic acid conjugation, mitochondrial β - and cytosolic omega oxidation to produce multiple metabolites that

involved in its toxicity (Salama et al., 2013). Up to 44% of patients chronic dosing with VPA may be associated with elevation in transaminases during the first months of therapy (Shaaban et al., 2014). Plasma transaminases are sensitive indicators of liver cell injury (Natarajan et al., 2006). These results were in accordance with reports of Babcock et al. (2000) who stated that sodium valproate induced toxicity manifested its activity by elevated ALP s in serum of sodium valproate intoxicated rats. Elevated ALP activity in serum is increased in hepatobiliary diseases characterized by some degree of cholestasis.

Biochemical assays done in the present study showed that administration of L-arginin and omega-3 individually or together with sodium valproate result in improvement of enzymes levels. These findings are in agreement with study of El-Banna et al. (2009) who estimated the role of L-arginin and omega-3 as an antioxidant that preventing the accumulation of toxins within the cell and promote the production of energy improvement of antioxidants enzymes levels with reduced Thiobarbituric acid-reactive substances (TBARS) levels which promotes the apoptosis.

However, hepatoprotective effect of L-arginin tends to increase synergistically when co administered with omega-3 can modulate the oxidative stress and improve the antioxidant system through the direct cytoprotective effect radical scavenger, thereby protecting membrane permeability.

Lee et al. (2008) determined that omega-3 fatty acid can repair hepatocellular damage caused by obstruction of the bile ducts improving hepatic injuries. The omega-3 fatty acid improves the hepatic inflammatory responses by suppressing inflammatory cytokine production in hepatocytes. In the present study, there was a significant increase in liver MDA and decrease in reduced glutathione mean values in sodium valproate treated group as compared to their corresponding values in control group. These results were in accordance with reports of Akande and Akinyinka (2005) who stated that lipid peroxides derived from polyunsaturated fatty acids are unstable and can be decomposed to form a complex series of compounds. These include reactive carbonyl compound, which is the most abundant malondialdehyde (MDA). The measurement of MDA is widely used as an indicator of lipid per oxidation and increased levels of the per oxidation products (Schulpis et al., 2006). Administration of VPA to the rats produced a time related hepatotoxicity disorder characterized by MDA production. Several studies suggest that lipid per oxidation plays a role in VPA toxicity (Henshall and Simon, 2005).

The increased level of liver MDA observed in this study could be explained by the cytotoxic activity of VPA is the result of generation of hydrogen peroxide and production of highly reactive hydroxyl radical (ALshafei et al., 2013). Some investigators, observed significantly increased lipid per oxidation in epileptic patients treated with VPA

(Owens and Nemeroff, 2003). The present work also showed that the increase in MDA level was accompanied by a concomitant decrease reduced glutathione. This is explained by reduced activities of antioxidant enzymes, SOD and CAT. Oxidative stress may be an important factor in VPA-induced hepatotoxicity. VPA administration leads to an increase in reactive oxygen species (ROS). In addition, long-term use of antiepileptic drugs has been shown to increase free radical formation and cause oxidative damage (Kim et al., 2013).

In the present study administration of L-arginin and omega-3 individually or together with sodium valproate protect the liver resulting in improvement of oxidative stress markers levels. These results are in accordance with Popescu et al. (2013) who stated that omega-3 can attenuate hepatic damage caused by ischemia and tissue reperfusion via reduction of NF-Kb activity. Other studies have shown that fish oil diet prevents hepatocyte cancer in B6C3F1 mice (Zhou et al., 2011). Similarly, omega-3-rich fish oil improves liver damage caused by LPS through the inhibition of TLR4 signaling pathway and NOD (Chen et al., 2013).

De Meijer et al. (2009) showed that emulsion based on fish oil prevents parenteral nutrition-associated liver disease. Also, Khan et al. (2016) demonstrated that fish oil can protect against apoptosis, tissue damage and hepatotoxicity induced by nitric oxide; it can reduce lipid per oxidation and improve body's antioxidant system (Khan et al., 2016). Blocking of MCP-1 and mitochondrial functional response as well as reducing DNA fragmentation (Salama et al., 2013). Omega-3 fatty acids down regulate the TNF-a response to lip polysaccharide insult, as would be seen in sepsis and this may have a direct role in Hepatoprotection (Udristioiu et al., 2014).

Pekarova and Lojek (2015) demonstrated that L-arginine serves as a precursor in protein synthesis, and it is a substrate for a number of enzymes, including nitric oxide synthase (NOS), arginase, arginine glycine aminotransferase, and arginine decarboxylase, yielding nitric oxide (NO) and citrulline, ornithine and urea, creatine, and agmatine, respectively. These findings were in line with other study done by Lai et al. (2015) supposing that oxidative stress play a crucial role in the pathogenesis of VPA induced hepatotoxicity.

Mansour et al. (2012) founded that Pre-treatment of rats with L-arginine before the administration of liver prevented the significant increase in liver malondialdehyde (MDA). The protective effect of L-arginine on the liver was also studied by Nanji et al. (2001) who observed that animals with alcohol-induced liver injury treated with L-arginine had an approximately 50% decrease in the level of lipid per oxidation.

Saad (2012) studied the curative and protective effects of L-arginine on carbon tetrachloride (CCl₄)-induced hepatotoxicity in mice and found that pre treatment or post-treatment with L-arginine significantly increased

reduced glutathione (GSH) level and decreased the MDA content compared with untreated CCl₄-intoxicated mice. The mechanism for the decreased lipid per oxidation by L-arginine administration might be related to NO ability, due to the presence of an unpaired electron, which could accept other electrons and function as scavenger, or it could be related to the antioxidant effects of L-arginine itself (Al-Dalaen et al., 2016).

Sodium valproate increases ROS production in many cellular models, modifying the oxidative balance, decreasing antioxidant levels and inducing lipid per oxidation. Glutathione plays a unique role in the cellular defense system, and is known for its ability to repair membrane lipid peroxides affected by toxic chemicals of endogenous and exogenous origin. Depletion of GSH increases vulnerability to free radical induced damage (Martinc et al., 2012).

In the present work, treatment of rats with sodium valproate resulted in obvious changes in the general architecture of the liver which generally, exhibited per portal inflammatory cells infiltration and blood vessels dilatation and congestion. The most striking histological feature recorded in rats of sodium valproate-treatment, was vacuolar degeneration in the cytoplasm of hepatocytes, pyknotic nuclei and infiltration with mononuclear leukocytes. Bile duct proliferation was observed. The histopathological changes in the liver of rats exposed to sodium valproate agree with those published by Hussein et al. (2013) who reported that a single dose of 62.5 mg/kg of cypermethrin administered orally to adult Albino rats produced necrotic areas in hepatocytes and cell swelling, cytoplasmic hypertrophy and intracytoplasmic vacuoles were seen.

Also Bryant and Dreifuss (2016) reported that sodium valproate administration produced congestion, marked degenerative changes of hepatocytes, these histopathological changes in the liver may be explained by the fact that sodium valproate induced oxidative stress may account for the degenerative changes in various organs such as liver, lung, heart and kidneys. These results were in accordance with Tong et al. (2015) who stated that, liver parenchyma in treated animals exhibited vacuolar degeneration and moderate proliferation of bile ducts. The later is mostly obvious in cholestatic injuries. Pathologic changes in hepatocytes due to cypermethrin can be related to its inhibitory effect on total adenine tri-phosphate activity in the liver, which may disturb active transport of Na⁺, K⁺ and Ca²⁺ ions, thus injuring hepatocytes. The cytoplasmic vacuolization that were observed in hepatocytes of sodium valproate treated animals reflect a form of cell injury as these vacuoles develop due to accumulation of ions and water in cytosol and rapidly pass through leaky membranes of cell organelles. Massive accumulation of fluids in the vacuoles may finally lead to cell lysis (Schulpis et al., 2016).

Henshall and Simon (2015) attributed these vacuoles to the free radicals that facilitate the release of lysosomal enzymes into the cytosol with subsequent oxidation of the protein architecture of the cells causing their fragmentation. Azeez et al. (2011) stated that the observed vascular dilatation may represent an adaptive process as an attempt to overcome oxygen deficiency, when prolonged, may be the cause of atrophic cells formation. Atrophied liver cells probably later, results in the presence of necrotic patches.

In the present study, upon supplementation with L-arginin or omega-3 individually or together to sodium valproate treated rats there was a significant improvement in histopathological changes; almost normal lobular architecture and normal hepatocytes with average sized nuclei except of few central veins and sinusoids that showed congestion. The immunohistochemistry examination showed hepatocyte with decreased to negative immune reactivity.

In general the results of Yiksel et al. (2017) who reported that the oral administration of fish oil omega-3 supplement has protective effect on thioacetamide induced liver toxicity by neutralizing free radicals, stimulating the activity of antioxidant enzymes, and reducing the production of inflammatory cytokinin. As no similar study on the protective effects of fish oil omega-3 supplement on hepatic enzymes and histological changes could be found, it was not possible do a comparative study in this respect. Anyhow, more studies should be conducted to examine the hepatic antioxidant enzymes and molecular changes inducing apoptosis so that the effects of fish oil omega-3 supplement on healing liver toxicity can be determined with higher certainty (Yiksel et al., 2000).

In general, the results of the present study showed that fish oil omega-3 supplement in rat model with hepatic malfunction can cause desirable improvements. Thus, if supported by more experiments, it is possible to add fish oil omega-3 supplement to the diet of patients with liver malfunction (Al-Rouby and Gawish, 2013). The involvement of ROS in VAP toxicity was confirmed by the fact that many antioxidants and free radical scavengers provided marked functional and histopathological protection against VPA toxicity (Martinez-Ballesteros et al., 2013).

Free radicals, known to cause oxidative stress, can be prevented or reduced by dietary natural antioxidants through their capacity to scavenge these products. L-arginin or omega-3 may protect lipids and lipoproteins in cellular membranes against this oxidative damage, thus may prevent certain types of hepatic cellular damage (ALshafei et al., 2013).

RECOMMENDATIONS

Improvement of health education programs for the

purpose of increasing public awareness regarding the health side effects of sodium valproate of its long run use. Administration of L-arginin or omega-3 may be of immense prophylactic and therapeutic values in exposed individuals.

Conclusion

Sodium valproate has hepatic damaging effects and administration of L-arginin and O-mega 3 has protective effects against its side effects.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support and help provided by all the staff of Department of animal house, Zagazig University Hospitals. Commercial or institutional resources did not sponsor this article and author received no financial support.

REFERENCES

- Abdel AaL KM, Mubark HA, Abdel Hady RH, Abdel Moneim WM, Hussein AR (2009). Protective Effect of Aminoguanidine Against Cyclosporine Induced Nephrotoxicity and Hepatotoxicity in Rats: Histological and Biochemical Study. *Medical Journal of Cairo University* 77(1):181-191.
- Akande AA, Akinyinka AO (2005). Serum malondialdehyde levels during menstrual cycle. *African Journal of Biotechnology* 4(11).
- Al-Dalaen S, Alzyoud J, Al-Qtaitat A (2016). The Effects of L-Arginine in Modulating Liver Antioxidant Biomarkers Within Carbon Tetrachloride Induced Hepatotoxicity: Experimental Study in Rats. *Biomedical and Pharmacology Journal* 9(1):293-298.
- Al-Rouby NM, Gawish SM (2013). Histological study on the possible protective effect of nigella sativa oil on experimentally induced hepatotoxicity in albino rats treated with sodium valproate. *Global Advanced Research Journal of Medicine Science* 2:90-99.
- ALshafei MM, Kassem SS, Kader MMA (2013). Effect of long term treatment with antiepileptic drugs on oxidant status, Zinc and magnesium in epileptic patients. *World Applied Sciences Journal* 28:316-323.
- Arroyo S , De la Morena A (2001). Life-threatening adverse events of antiepileptic drugs. *Epilepsy Research* 47:155-174.
- Azeez OH, Mahmood MB, Hassan JS (2011). Effect of nitrate poisoning on some biochemical parameters in rats. *Iraqi Journal of Veterinary Sciences* 25(2):47-50.
- Babcock T, Helton WS , Espat NJ (2000). Eicosapentaenoic acid (EPA): an antiinflammatory omega-3 fat with potential clinical applications. *Nutrition* 16(11-12):1116-1118.
- Bancroft JD, Stevens A (1996). *Theory and Practice of Histological Techniques*. (4th ed.). Churchill Livingstone, Edinburgh.
- Bryant AE, Dreifuss FE (2016). Valproic acid hepatic fatalities. III. US experience since 1986. *Neurology* 46:465-469.
- Chen F, Liu Y, Zhu H, Hong Y, Wu Z, Hou Y (2013). Fish oil attenuates liver injury caused by LPS in weaned pigs associated with inhibition

- of TLR4 and nucleotide-binding oligomerization domain protein signaling pathways. *Innate Immunity* 19(5):504-515.
- Cuschieri A, Backer P (1977). *Introduction to Research in Medical Sciences* (1st ed.). By: Cuschieri, A. and Backer, P.P. (eds.). Churchill Livingstone, Edinburgh. pp: 112-120.
- Davood M, Mokhtar M, Saeed K (2017). Protective Effects of Omega Supplement on Induced Hepatic Mal-Function by Thioacetamide in Male Rats Zahedan. *Journal of Research in Medical Sciences* 19(3).
- de Meijer VE, Gura KM, Le HD, Meisel JA, Puder M (2009). Fish oil-based lipid emulsions prevent and reverse parenteral nutrition-associated liver disease: the Boston experience. *Journal of Parenteral and Enteral Nutrition* 33(5):541-547.
- Defoort EN, Kim PM, Winn LM (2006). Valproic acid increases conservative homologous recombination frequency and reactive oxygen species formation: a potential mechanism for valproic acid-induced neural tube defects. *Molecular Pharmacology* 69:1304-10.
- El-Banna Sabah G, Attia M, Hafez M, El-Kazaz M (2009). Effect of garlic consumption on blood lipid and oxidant/antioxidant parameters in rat males exposed to chlorpyrifos. *Slovakian Journal of Animal Science* 42(3):111-117.
- Elwakkad AS, El EShamy KA, Sibaii H (2008). Fish liver oil and propolis as protective natural products against the effect of the anti-epileptic drug valproate on immunological markers of bone formation in rats. *Epilepsy Research* 80:47-56.
- Giuffrida F, Destailats F, Skibsted L, Dionisi F (2014). Structural analysis of hydroperoxy- and epoxy-triacylglycerols by liquid chromatography mass spectrometry. *Chemistry and Physics of Lipids* 131(1):41-49.
- Goulet OJ (2015). Intestinal failure-associated liver disease and the use of fish oil-based lipid emulsions. *World Rev. Nutr. Diet.* 112:90-114.
- Haast RA, Kiliaan AJ (2015). Impact of fatty acids on brain circulation, structure and function. *Prostaglandins Leukot Essent Fatty Acids.* 92:3-14.
- Henshall DC, Simon RP (2005). Epilepsy and apoptosis pathways. *Journal of Cerebral Blood Flow Metabolism* 25:1557-1572.
- Henshall DC, Simon RP (2015). Epilepsy and apoptosis pathways. *Journal of Cerebral Blood Flow Metabolism* 25:1557-1572.
- Hussein RR, Soliman RH, Abdelhaleem AM, Tawfeik MH, Abdelrahim ME (2013). Effect of antiepileptic drugs on liver enzymes. *Beni-Suef University Journal of Basic and Applied Sciences* 2:14-9.
- Institute of Laboratory Animal Resources (1996). *Guide for the Care and Use of Laboratory Animals*. (7th ed.). National Academy Press P 125.
- Ivanova S, Pankova S, Dimitrov M, Petkova MV (2014). Food additives with beneficial effects in the treatment of erectile dysfunction, containing larginine, pycnogenol and ginseng extract. *World journal pharmacy and pharmaceutical science* 3(11):234-245.
- Kaplan A, Rubatelli FF, Hammerman C, Vilei M, Leiter C, Abramov A (1984). Bilirubin. In: *Clinical Chemistry* (1st ed.). By: Kaplan, A. and Peace, AL. (eds.). The C.V. Mosby Co., St Louis, Toronto, Princeton. Chap: 5. pp. 1238-1241.
- Khan MW, Priyamvada S, Khan SA, Khan S, Gangopadhyay A, Yusufi AN (2016). Fish/flaxseed oil protect against nitric oxide-induced hepatotoxicity and cell death in the rat liver. *Human and Experimental Toxicology* 35(3):302-311.
- Khan SK, Tasleem S, Ismail N, Khan P, Shakoor KA (2013). Prevention of hepatotoxicity of valproic acid with concomitant supplement of carnitine: an experimental study in albino rats. *Gomal Journal of Medical Sciences* 11:227-229.
- Kim EH, Bae JS, Hahm KB, Cha JY (2012). Endogenously synthesized n-3 polyunsaturated fatty acids in fat-1 mice ameliorate high-fat diet-induced non-alcoholic fatty liver disease. *Biochemical Pharmacology* 84(10):1359-1365.
- Kim K, Jung N, Lee K, Choi J, Kim S, Jun J (2013). Dietary omega-3 polyunsaturated fatty acids attenuate hepatic ischemia/reperfusion injury in rats by modulating toll-like receptor recruitment into lipid rafts. *Clinical Nutrition* 32(5):855-862.
- Lai Q, Wei J, Zhang C, Quan S, Li T, Yu Y (2015). Pharmacokinetic and nephroprotective benefits of using *Schisandra chinensis* extracts in a cyclosporine A-based immune-suppressive regime. *Drug Design, Development and Therapy* 9:4997-5018.
- Lee S, Kim S, Le HD, Meisel J, Strijbosch RA, Nose V (2008). Reduction of hepatocellular injury after common bile duct ligation using omega-3 fatty acids. *Journal of Pediatric Surgery* 43(11):2010-2015.
- Mansour M, Daba MH, Gad, A, Al Rikabi A, Al-Majed A (2002). Protective effect of L-arginine against nephrotoxicity induced by cyclosporine in normal rats. *Pharmacological research* 45(6):441-446.
- Martinc B, Grabnar I, Vovk T (2012). The role of reactive species in epileptogenesis and influence of antiepileptic drug therapy on oxidative stress. *Current Neuropharmacology* 10(4):328-343.
- Martinez-Ballesteros C, Pita-Calandre E, Sanchez-Gonzalez Y, Rodriguez-Lopez C, Agil A (2013). Lipid peroxidation in adult epileptic patients treated with valproic acid. *Revue Neurologique* 38:101-106.
- Morita M, Hayashi T, Ochiai M, Maeda M, Yamaguchi T, Ina K, Kuzuya M (2014). Oral supplementation with a combination of L-citrulline and L-arginine rapidly increases plasma L-arginine concentration and enhances NO bioavailability. *Biochemical and Biophysical Research Communications* 454(1):53-57.
- Moron MS, DePierre JN, Manervik V (1979). Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. *Biochimica. et Biophysica* 582(1):67-68.
- Nanji AA, Jokelainen K, Lau GKK, Rahemtulla A, Tipoe GL, Polavarapu R, Lalani E (2001). Arginine reverses ethanol-induced inflammatory and fibrotic changes in liver despite continued ethanol administration. *Journal of Pharmacology and Experimental Therapeutics* 299(3):832-839.
- Natarajan SK, Thomas S, Ramamoorthy P, Basivireddy J, Pulimood AB, Ramachandran A (2006). Oxidative stress in the development of liver cirrhosis: a comparison of two different experimental models. *Journal of Gastroenterology and Hepatology* 21(6):947-957.
- Nemzek JA, Bolgos GL, Williams BA, Remick DG (2001). Differences in normal values for murine white blood cell counts and other hematological parameters based on sampling site. *Inflammation Research* 50(10):523-527.
- Ochs-Balcom H, Grant B, Muti P, Sempos C, Freudenheim J, Browne R (2006). Antioxidants, oxidative stress, and pulmonary function in individuals diagnosed with asthma or COPD. *European Journal of Clinical Nutrition* 60:991-999.
- Owens MJ, Nemeroff CB (2003). "Pharmacology of valproate". *Psychopharmacology Bulletin* 37(2):17-24.
- Pekarova M, Lojek A (2015). The crucial role of L-arginine in macrophage activation: What you need to know about it. *Life Science* 137:44-48.
- Plourde M, Cunnane SC (2007). Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Applied Physiology Nutrition and Metabolism* 32(4):619-634.
- Popescu LA, Virgolici B, Lixandru D, Miricescu D, Condruț E, Timnea O (2013). Effect of diet and omega-3 fatty acids in NAFLD. *Romanian Journal of Morphology and Embryology* 54:785-790.
- Reitman S, Frankel A (1957). colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Ame. Journal of Clinical Pathology* 28(1):56-63.
- Saad EA (2012). Curative and protective effects of L-arginine on carbon tetrachloride-induced hepatotoxicity in mice. *Biochemical and Biophysical Research Communications* 423(1):147-151.
- Salama MF, Abbas A, Darweish MM, El-Hawary AA, Al-Gayyar MM (2013). Hepatoprotective effects of cod liver oil against sodium nitrite toxicity in rats. *Pharmaceutical Biology* 51(11):1435-1443.
- Saleh D, Ismail MA, Ibrahim AM (2012). Non-alcoholic fatty liver disease, insulin resistance, dyslipidemia and atherogenic ratios in epileptic children and adolescents on long term antiepileptic drug therapy. *Pakistan Journal of Biological Sciences* 15:68-77.
- Schulpis KH, Lazaropoulou C, Regoutas S, Karikas GA, Margeli A and Tsakiris S (2016). Valproic acid monotherapy induces DNA oxidative damage. *Toxicological Sciences* 217:228-32.
- Schulpis KH, Lazaropoulou C, Regoutas S, Karikas GA, Margeli A, Tsakiris S (2006). Valproic acid monotherapy induces DNA oxidative damage. *Toxicological Research* 217:228-232.
- Semler DE (1992). The rat toxicology. In: *Animal Models in Toxicology*.

- (2nd ed.). By: Gad, S.C. and Chengelis, C.P. (eds.). Marcl Dekker, New York and Hong Kong.chap:2. pp. 21-75.
- Shaaban AA, Shaker ME, Zalata KR, El-kashef HA, Ibrahim TM (2014). Modulation of carbon tetrachloride-induced hepatic oxidative stress, injury and fibrosis by olmesartan and omega-3. *Chemico-Biological Interactions* 207:81-91.
- Tapiero H, Mathé G, Couvreur P, Tew KD (2002). Arginine. *Biomedicine & pharmacotherapy* 56(9):439-445.
- Tolou-Ghamari Z, Palizban AA (2015). Review of sodium valproate clinical and biochemical properties. *Zahedan. Journal of Research in Medical Sciences* pp. 29-34.
- Tong V, Teng XW, Chang TK, Abbott FS (2015). Valproic acid II: effects on oxidative stress, mitochondrial membrane potential, and cytotoxicity in glutathione-depleted rat hepatocytes. *Toxicological Sciences* 86:436-443.
- Udristioiu A, Iliescu RG, Cojoraru M, Udristioiu A (2014). Alkaline phosphatase isoenzymes and leukocyte alkaline phosphatase score in patients with acute and chronic disease: a brief review. *British Journal of Medicine and Medical Research* 4:340-50.
- Utagawa T (2004). Production of arginine by fermentation. *Journal of nutrition* 134(10):2854-2857.
- Wergedahl H, Gudbrandsen OA, Rost TH, Berge RK (2009). Combination of fish oil and fish protein hydrolysate reduces the plasma cholesterol level with a concurrent increase in hepatic cholesterol level in high-fat-fed Wistar rats. *Nutrition* 25(1):98-104.
- Yiiksel A, Cengiz M, Seven M, Ulutin T (2000). Erythrocyte glutathione, glutathione peroxidase, superoxide dismutase and serum lipid peroxidation in epileptic children with valproate and carbamazepine monotherapy. *Journal of Basic Clinical Physiology and Pharmacology* 11:73-81.
- Zhou GD, Zhu H, Phillips TD, Wang J, Wang SZ, Wang F (2011). Effects of dietary fish oil on the depletion of carcinogenic PAH-DNA adduct levels in the liver of B6C3F1 mouse. *PLoS One* 6(10):e26589.