Recent Research in Genetics and Genomics 2021; 3(1): 1-18

Original article:

The protective role of pomegranate juice with and without valproic acid on epileptic rats "Protective role of pomegranate juice"

Mohamed F. F. Bayomy[1*], Ibrahim A. El Elaimy[1], Ahmed M. Shehata[2], Omar A. Ahmed-Farid[2] and Omar H. Hassanein[1]

[1]Zoology Department, Faculty of Science, Menoufia University, Shebin El Kom, Egypt;

[2]Physiology department, NODCAR, Egypt[*]: Correspondence to: mffbayomy@yahoo.com

DOI: 10.21608/RRGG.2021.177752. Received: 16 May 2021; accepted: 08 June 2021; published 15 June 2021

Abstract

Epilepsy is one of the greatest common neurological disorders characterized by epileptic seizures. The present study aims to investigate the protective effects of pomegranate juice with and without valproic acid on epileptic rats. Pomegranate juice (PJ) used in popular folk medicine for the treatment of various diseases due to its antioxidants content. Valproic acid (VPA) is a well-established anticonvulsant drug used in the treatment of many forms of generalized epilepsy and psychiatric disorders to control epileptic seizures. Pentylenetetrazol (PTZ) is a central nervous system convulsant which induce seizures and used as a routine test for screening of anticonvulsants. In the present study 35 rats were divided into 7 groups: control group, PTZ group, PJ group, VPA group, VPA+PTZ group, PJ+PTZ group, and PJ+VPA+PTZ group. Groups treated with pomegranate juice received daily oral doses of 10 µL/g of body weight for 28 days. Groups treated with valproic acid received daily intraperitoneal (IP) doses of 500 mg/kg body weight for 14 days. At the last day of the experiment groups treated with PTZ were injected with a single IP dose of PTZ (60 mg/kg B.W), while other groups without PTZ received the same dose of sterile isotonic saline solution. After 30 minutes animals were decapitated. Injection of rats with PTZ or VPA resulted in significant increase in liver functions and lipid profile except HDL-c. Results showed significant decrease in antioxidants while treatment with pomegranate juice showed significant decrease in liver functions and lipid profile and significant increase in antioxidants.

Keywords: Epilepsy; Pomegranate juice; valproic acid; pentylenetetrazol.

1. Introduction

Epilepsy is a disorder of brain electrical activity that results in recurrent seizures (Gaby, 2007). Epilepsy was defined conceptually in 2005 as a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures. This definition is usually practically applied as having two unprovoked seizures >24 h apart (Fisher et al., 2014). The rate of epilepsy in 2013 is estimated at about 22 million (Global Burden of Disease Study, 2013). Epilepsy and most antiepileptic drugs may cause oxidative stress due to increase in reactive oxygen species (ROS). Oxidative stress is defined as a disturbance in the balance between antioxidants and pro-oxidants (free radicals and other reactive species) with increased levels of pro-oxidants leading to potential damage. These conditions involved in the initiation and progression of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and epilepsy (Migliore et al., 2005; Ashrafi et al., 2007; Shin et al., 2011). Reactive oxygen species (ROS) include oxygen radical's superoxide radicals (O2 • −), hydroxyl (•OH), peroxyl (RO2 •) and alkoxyl (RO•) as well as certain non-radicals that are either oxidizing agents and/or are easily converted into radicals, such as Hypochlorous acid HOCl, ozone (O3), peroxynitrite (ONOO−), singlet oxygen (^{1}O2) and hydrogen peroxide (H2O2) (Wiseman and Halliwell, 1996). The production of superoxide occurs mostly within the mitochondria of cells. During energy transduction through the mitochondrial electron transport chain, a small number of electrons “leak” to oxygen prematurely, forming the oxygen free radical superoxide, which has been implicated in the patho-physiology of a variety of diseases (Valko et al., 2004; Kovacic et al., 2005). Endogenous protection of oxidative stress can be scavenged by enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) or non-enzymatic such as glutathione (GSH), α- tocopherol, vitamin E and vitamin C. The maintenance of low ROS levels is critical to normal cell function, and therefore, prolonged increases in ROS carry an inherent risk of increasing neurodegeneration such as that disorder which is seen in epilepsy. Despite the increasing number and variety of anti-epileptic drugs, more than 30% of cases of epilepsy are medically intractable (Kwan and Brodie, 2000). Anticonvulsant drugs which are used to treat epilepsy such as carbamazepine, phenytoin, valproate, phenobarbital and lamotrigine have some side effects. Valproic acid (VPA) which is the active ingredient in many antiepileptic drugs increased intracellular ROS levels in several tissues, including liver, brain and small intestine (Ustundag et al., 2016). One of the most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzymes such as AST, ALT after VPA administration. The elevated activities

of these enzymes indicated hepatocellular damage (Bansal et al., 2005). Clinical records have reported that more than 40% of patients who received VPA also developed unexpected obesity and fatty liver disease (Saleh et al., 2012). Administration of VPA produced many metabolic and morphologic aberrations in the liver (Abdou et al., 2014). In earlier studies, pre- and post-treatment with *Ginkgo biloba* leaf extracts (GLE) and L-carnitine improved the biochemical, histological and immunohistochemical alterations in spleen and cerebral cortex of rats treated with pentylenetetrazol (PTZ) (Bayomy et al. 2018 and Tousson et al., 2015).

Pomegranate is used in popular folk medicine for the treatment of various diseases (Ajaikumar et al., 2005). The Pomegranate fruit has an ancient history and is mentioned in many Holy Scriptures such as the Torah, the Bible, and the Holy Quran (Langley, 2000; Longtin, 2003). Many studies were carried out to identify the several benefits of pomegranate juice such as a chemopreventive, chemotherapeutic, antiatherosclerotic and anti-inflammatory agent (Faria et al., 2007). Fyiad et al. (2012) studied the effect of pomegranate juice on nucleic acids alterations and oxidative stress in rats with experimentally hepatitis. Results revealed that the pretreatment with pomegranate juice protects against hepatic damage via suppression of oxidative stress. Khedr et al., (2015) studied the effect of pomegranate juice against hepatotoxicity where their results revealed that pomegranate juice increased the total antioxidants and vitamin C as well as improved the levels of liver functions in serum of rats.

The aim of this study was to evaluate the protective effects of pomegranate juice with and without valproic acid on liver functions and oxidative stress after induction of epilepsy in rats by PTZ.

2. Materials and Methods

2.1 Materials

Pentylenetetrazol (PTZ) and valproic acid (VPA) purchased from Sigma–Aldrich (3050 Spruce Street, Saint Louis, MO 63103, USA). Kits for liver functions determination purchased from Egyptian Company for Biotechnology, Egypt. Kits for determination of lipid profile parameters purchased from Spinreact, Spain. Kits for determination of (GPx, MDA, and TAC) purchased from Biodiagnostic, Egypt.

2.2 Preparation of pomegranate juice:

The fresh pomegranate fruit (*Punica granatum*), free from blemishes or obvious defects was purchased from Shiben El-Kom, Minufia, Egypt. The fruit was peeled and the seeds removed manually. The seeds were blended in a Braun blender (Model No. 4005, Hungary) at a maximum speed for 10 minute. The juice was filtrated through cheesecloth. The filtrated juice was stored at -20 ºC until used.

2.3 Animals

Thirty five adult male Sprague Dawley rats, with an average B.W of 195 - 200 g were purchased from the Veterinary Medicine Institute, Cairo, Egypt. Animals were maintained under normal laboratory conditions, with 12-hours light-dark cycle at 25 ± 1°C. Rats were housed in cylindrical wire cages with wire bottoms. The diet was introduced to rats in special food cups to avoid scattering of food. Also, water was provided to the rats by glass tube projection through the wire cage. Food and water provided and checked daily. Rats were fed standard diet. All animals received care in compliance with the Egyptian rules for animal protection. The rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment.

2.4 Experimental groups

The rats were equally divided into seven groups (5 rats each). The 1st group served as control group in which rats never received any treatments. Rats of the 2nd group were kept on normal standard diet and treated only with a single intraperitoneal dose of PTZ (60 mg/kg of body weight), which was dissolved in sterile isotonic saline solution (Rodrigues et al., 2013). Animals of the 3rd group were kept on normal standard diet and treated IP with Sodium valproate for 14 days with a high dose (500 mg/kg) according to Tong et al., (2005). Rats of the 4th group were kept on normal standard diet and treated with Pomegranate juice orally for 28days (10 µL/g) according to Khedr et al., (2015). Animals of the 5th group were kept on normal standard diet and treated IP with sodium valproate (500mg/kg) for 14 days and a single IP dose of PTZ (60mg/kg) at the last day of the experiment. Rats of the 6th group were kept on normal standard diet and treated orally with pomegranate juice (10µL/g) for 28 days and a single IP dose of PTZ (60 mg/kg) at the last day of the experiment. Animals of the 7th group were kept on normal standard diet and treated orally with pomegranate juice (10µL/g) for 28 days, sodium valproate IP (500mg/kg) for the last 14 days and a single IP dose of PTZ (60 mg/kg) at the last day of the experiment. Groups without PTZ were sacrificed at the last day of the experiment, whereas groups treated with PTZ were sacrificed after 30 minutes of PTZ injection, Blood obtained from slaughter collected in clean and dry test tubes and were then centrifuged at 3000 rpm for 15 minutes. The clear supernatant sera were separated and stored at -20°C until assayed. Animals were dissected and the liver tissues were directly removed, liver was embedded in 10% formalin for histopathological examination.

2.5 Biochemical assays

Kits from the Egyptian Company for Biotechnology were used to undertake liver function tests according to the manufacturer's instructions. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed

according to the method described by Thefeld et al., (1974). Alkaline Phosphatase (ALP) and gamma-Glutamine transferase (GGT) were assayed according to the method described by Moss et al., (1987). Total Protein (TP) and albumin (alb) were assayed according to the method described by Young. (1995). Kits (Spinreact, Spain) were used to determine lipid profile parameters (TC, TG, HDL-c and LDL-c) according to the manufacturer's instructions. Total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were assayed according to the method described by Young. (1995). Low density lipoprotein cholesterol was assayed according to the method described by Fridewald et al., (1972). Kits from Biodiagnostic, Egypt, were used to determine glutathione peroxidase (GPx), malondialdehyde (MDA) and total antioxidant capacity (TAC) according to the manufacturer's instructions. Glutathione peroxidase (GPx) was assayed according to the method described by Paglia and Valentine, (1967). Malondialdehyde (MDA) was assayed according to the method described by Ohkawa et al., (1979). Total antioxidant capacity (TAC) was assayed according to the method described by Koracevic et al., (2001).

2.6 Histopathological studies

Liver tissues were carefully fixed in neutral formalin solution (10%), dehydrated in ascending grades of ethanol, cleared in xylene, embedded in a paraffin wax, sectioned at 5-7 µm and stained with hematoxylin and eosin. The stained sections were examined and photographed under a light microscope to detect histopathological changes (Drury et al., 1976).

2.7 Statistical analysis

Data were analyzed using a Statistical Analysis System SPSS for windows Version 22 and recorded as means ± standard error (SE). Analysis of variance among groups was performed using one-way ANOVA test followed by Duncan's multiple range test at a significance level of $P < 0.05$.

3. Results

3.1 Biochemical analysis

Data in table 1 showed the effect of PJ, VPA and their combination on the liver functions in serum of rats. Rats treated only with PTZ showed significant impairment in liver functions ($P<0.05$) when compared with normal control rats, rats treated with PJ or rats treated with VPA. Enzymes ALT and AST of rats treated with PJ alone were similar ($P>0.05$) to those of normal control rats, whereas enzymes of rats administrated PJ and injected with PTZ were higher ($p <0.05$) than rats supplemented with PJ alone and lower ($P<0.05$) than those of normal control rats injected with (PTZ). Enzymes (ALT and AST) of rats treated with VPA alone showed significant increase ($P<0.05$) compared with those of the normal control group, while they showed

significant decrease compared with rats injected with PTZ alone. These enzymes of rats administrated VPA and injected with (PTZ) showed significant increase (P<0.05) compared with normal control group and those of rats treated with PTZ alone. Group treated with PJ and VPA before PTZ injection showed significant increase (P<0.05) in their enzymes compared with normal control group. Enzyme GGT showed similar pattern of changes in nearly all experimental groups except when it compared with that of rats treated with PTZ alone.

Data in table 2 showed the effect of PJ, VPA and their combination on albumin and total protein in serum of rats. Rats treated only with PTZ showed significant decrease in serum albumin and total protein (P<0.05) when compared with normal control rats, rats treated with PJ or rats treated with VPA. Animals treated with PJ alone showed non-significant decrease in albumin and total protein compared to normal control group. Rats administrated PJ and injected with PTZ showed significant increase in albumin and total protein compared to rats treated with PTZ only, while they showed significant decrease in those parameters compared to those of rats treated with PJ alone or normal control group. Rats treated with VPA alone showed significant decrease in albumin and total protein (P<0.05) compared with normal control group, while they showed significant increase compared with rats injected with PTZ alone. Rats administrated VPA and injected with PTZ showed significant decrease (P<0.05) in these parameters compared with normal control group and rats treated with VPA alone, while they showed non-significant decrease in albumin and significant decrease in total protein compared with rats treated with PTZ alone. Group treated with PJ and VPA before PTZ injection showed significant increase in albumin and total protein compared with rats treated with PTZ alone, while they showed significant decrease in albumin and total protein compared with that of the normal control group.

Data in table 3 showed the effect of PJ, VPA and their combination on antioxidants in serum of rats. Rats treated only with PTZ showed significant increase in serum malondialdehyde (MDA), while showed significant decrease in glutathione peroxidase (GPx) and total antioxidant capacity (TAC) compared to normal control group. Rats treated with PJ alone showed non-significant decrease in serum MDA, non-significant increase in GPx and significant increase in TAC compared to normal control group. Rats administrated PJ and injected with PTZ showed significant decrease in serum MDA and significant increase in GPx and TAC compared to rats treated with PTZ alone, while they showed non-significant increase in serum MDA and significant decrease in GPx and TAC compared to rats treated with PJ alone and normal control group. Rats treated with VPA alone showed significant increase in serum MDA and

significant decrease in GPx and TAC compared with normal control group, while they showed non-significant decrease in serum MDA, significant increase in GPx and non-significant increase in TAC compared with rats injected with PTZ alone. Rats administrated VPA and injected with PTZ showed significant increase in serum MDA, GPx and non-significant increase in TAC compared with rats injected with PTZ alone, while they showed significant increase in serum MDA and significant decrease in GPx and TAC compared with normal control group. Group treated with PJ and VPA before PTZ injection showed significant increase in serum MDA and significant decrease in GPx and TAC compared with normal control group, while they showed significant decrease in serum MDA and significant increase in GPx and TAC compared with rats treated with PTZ alone.

Data in table 4 showed the effect of PJ, VPA and their combination on lipid profile of rats. Rats treated only with PTZ showed significant increase in total Cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL-c), while they showed significant decrease in high density lipoprotein (HDL-c) compared with normal control group. Rats treated with PJ alone showed significant decrease in TC, non-significant decrease in TG and LDL-c, while they showed non-significant increase in HDL-c compared with normal control group. Rats administrated PJ and injected with PTZ showed significant increase in TC, TG, LDL-c and showed significant decrease in HDL-c compared with those of the normal control group, while this group showed significant decrease in TC, LDL-c and non-significant decrease in TG and HDL -c compared with rats treated with PTZ alone. Rats treated with VPA alone showed significant increase in TC, TG, LDL-c and showed significant decrease in HDL-c compared with normal control group. Rats administrated VPA and injected with PTZ showed significant increase in TC, TG, LDL-c and showed significant decrease in HDL-c compared with normal control group and rats treated with PTZ alone. Group treated with PJ and VPA before PTZ injection showed non-significant increase in TC, significant increase in TG and non-significant decrease in HDL-c and LDL-c, while they showed significant decrease in TC, TG, LDL-c and significant increase in HDL-c compared with rats treated with PTZ alone.

3.2 Histopathological examinations

Figure 1 (A-G) show the effect of PJ, VPA and their combination on histology of liver. (A) Shows photomicrograph of liver tissue from control group showing normal hepatocytes, vesicular nuclei and central vein. (B) Shows Photomicrograph of liver tissue from PTZ group showing loss of normal architecture exhibiting severe vacuolation, periportal inflammation, bile duct and dilated congested blood vessel. (C) Photomicrograph of liver tissue from PJ group

showing intact hepatocytes with vesicular nuclei. (D) Shows Photomicrograph of liver tissue from group treated with PJ+PTZ showing marked improvement in hepatocytes, normal liver architecture. (E) Shows Photomicrograph of liver tissue from VPA group showing vacuolated hepatocytes, pykanotic nuclei, and normal hepatocytes. (F) Shows Photomicrograph of liver tissue from VPA+PTZ group showing loss of normal architecture, severe vacuolation, pykanotic nuclei and dilated congested blood vessel.

Table 1: Effect of administration of pomegranate juice, valproic acid, pentylenetetrazol, and their combiation on aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphates (ALP), gamma glutamine transferase (GGT).

Group	Parameter			
	AST (UL/I)	ALT (UL/I)	ALP (UL/I)	GGT (UL/I)
Control	54.05±1.20^{d}	41.47±0.78^{g}	40.82±0.63^{f}	11.06±.09^{e}
PJ	52.15±1.13^{d}	41.53±.65^{g}	41.27±0.65^{f}	11.52±.16de
VPA	116.72±2.82^{c}	61.43±4.22^{d}	168.73±7.21^{c}	12.65±.23^{cd}
PTZ	143.92±11.12^{b}	126.59±1.79^{b}	213.76±11.91^{b}	16.58±.88^{a}
PJ+PTZ	69.21±3.21^{d}	56.96±4.28^{de}	108.79±2.45^{de}	12.28±.22^{cde}
VPA+PTZ	247.46±4.19^{a}	137.68±3.37^{a}	263.82±15.51^{a}	17.36±.54^{a}
PJ+VPA+PTZ	131.79±4.45^{bc}	70.84±3.59^{c}	110.07±6.95^{de}	12.07±.23^{cde}

- Results were represented as means ± SE (n=5 for each group).
- Means in the same column with different letters are significantly different (P<0.05).
- (PTZ): pentylenetetrazol, (VPA): valproic acid, (PJ): pomegranate juice.

Table 2: Effect of administration of pomegranate juice, taurine, valproic acid, pentylenetetrazol, and their combination on albumin (Alb), total protein (TP), and albumin to globulin ratio (A/G).

Group	Parameter		
	Alb (g/dl)	TP (g/dl)	A/G
Control	3.81±.06[a]	7.66±.36[a]	1.03±.12[c]
PJ	3.61±.09[ab]	7.60±.23[a]	.91±.05[cd]
VPA	3.00±.12[de]	6.37±.22[c]	.82±.01[cd]
PTZ	2.76±.13[ef]	5.69±.30[d]	.88±.07[cd]
PJ+PTZ	3.15±.08[cd]	6.36±.15[c]	1.00±.07[c]
VPA+PTZ	2.74±.11[ef]	4.20±.15[f]	1.73±.10[b]
PJ+VPA+PTZ	3.28±.08[c]	6.94±.20[bc]	.89±.03[cd]

- Results were represented as means ± SE (n=5 for each group).
- Means in the same column with different letters are significantly different (P<0.05).
- (PTZ): pentylenetetrazol, (VPA): valproic acid, (PJ): pomegranate juice.

Table 3: Effect of administration of pomegranate juice, valproic acid, pentylenetetrazol, and their combination on malondialdehyde (MDA), glutathione peroxidase (GPx), and total antioxidant capacity (TAC).

Group	Parameter		
	MDA (nmol)	GPx (U/L)	TAC (mmol/L)
Control	2.58±.11[ef]	1914.80±39.73[a]	6.48±.25[b]
PJ	2.44±.14[f]	2028.20±51.59[a]	7.27±.22[a]
VPA	3.89±.05[bc]	1181.40±32.40[e]	3.04±.09[ef]
PTZ	4.18±.10[b]	1069.40±30.53[f]	2.62±.14[f]
PJ+PTZ	2.72±.07[ef]	1239.60±34.99[de]	4.00±.17[d]
VPA+PTZ	4.89±.13[a]	1204.80±45.99[e]	2.84±.07[ef]
PJ+VPA+PTZ	3.51±.20[cd]	1567.80±29.98[b]	4.80±.14[c]

- Results were represented as means ± SE (n=5 for each group).
- Means in the same column with different letters are significantly different (P<0.05).
- (PTZ): pentylenetetrazol, (VPA): valproic acid, (PJ): pomegranate juice.

Table 4: Effect of administration of pomegranate juice, valproic acid, pentylenetetrazol, and their combination on serum total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL-c) and low density lipoproteins (LDL-c).

Group	Parameter			
	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Control	61.5±1.53^{fg}	43.26±.97^{h}	21.96±.57^{a}	30.88±.82^{fgh}
PJ	56.77±2.54^{g}	42.22±.40^{h}	22.09±.86b^{a}	26.23±2.62^{h}
VPA	83.02±1.77^{c}	100.26±2.56^{b}	16.1±.92^{cd}	46.04±2.28^{c}
PTZ	105.28±2.1^{b}	86.28±1.14^{c}	18.06±.4d^{bc}	69.96±1.63^{b}
PJ+PTZ	76.02±3.40^{cde}	80.62±2.07^{cd}	17.36±.82^{bc}	40.98±2.40^{cde}
VPA+PTZ	126.40±1.69^{a}	152.71±4.59^{a}	14.57±1.4^{d}	83.52±1.78^{a}
PJ+VPA+PTZ	64.44±4.33^{fg}	57.72±2.92^{f}	20.95±.51^{a}	30.26±1.97^{gh}

- Results were represented as means ± SE (n=5 for each group).
- Means in the same column with different letters are significantly different ($p < 0.05$).
- (PTZ): pentylenetetrazol, (VPA): valproic acid, (PJ): pomegranate juice, (TG): triglycerides, (HDL-C): high density lipoproteins, (LDL-C): low density lipoproteins.

4. Discussion

The present study indicated impairment in liver functions of rats injected with PTZ. Khedr et al., (2015) reported that PTZ injection caused poor liver functions. Rodrigues et al., (2013) reported that PTZ injection induced an increase in protein damage as expressed by carbonyl protein content, and NOx levels, in both the liver and serum of rats. These results are in well agreement with results of the present study. Dangerous effects after PTZ injection might be mediated by activation of membrane phospholipases, proteases and nucleases. These changes in membrane phospholipid lead to an increase of reactive oxygen species (ROS) and lipid peroxidation (Yegin et al., 2002). Rats treated with pomegranate juice alone showed similar results with control group, whereas rats treated with valproic acid showed significant liver hypo-function. Other studies are in agreement with the present study, where Hamza et al. (2015) and Niaraki et al. (2013) reported elevation in serum liver enzymes (ALT and AST) after treatment with valproic acid. The mechanism of hepatic injury has been extensively studied but it is still unclear. Some authors hypothesized that VPA causes aberration in metabolism with the formation of toxic metabolites. Meanwhile, mediation of lipid peroxidation might, also, be underlying mechanism of serious hepatic reactions (Khan et al., 2005; Raza et al., 1999). These effects of valproic acid were associated with increased ROS formation.

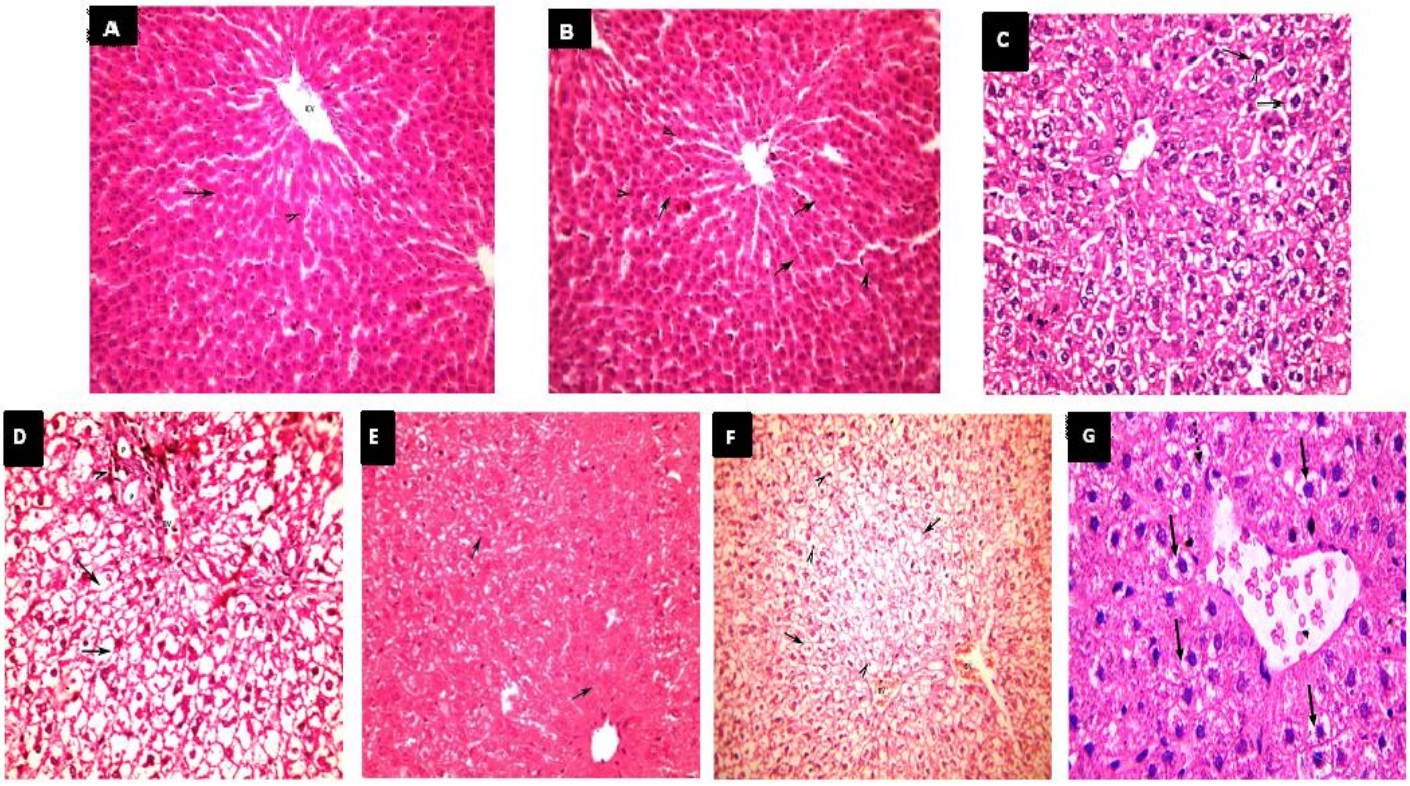

Figure1: Photomicrographs of rat liver sections. **(A)**: photomicrograph of liver tissue from control group showing normal hepatocytes (arrow), vesicular nuclei (arrow head) and central vein (CV) (H&E, X: 200). **(B)**: Photomicrograph of liver tissue from PJ group showing intact hepatocytes (arrow) with vesicular nuclei (arrow head) (H&E, X: 200). **(C)**: Photomicrograph of liver tissue from VPA group showing vacuolated hepatocytes (arrow), pykanotic nuclei (arrow head), and normal hepatocytes (H) (H&E, X: 400). **(D)**: Photomicrograph of liver tissue from PTZ group showing loss of architecture, sever vacuolation (arrow), periportal inflammation (arrow head), bile duct (P) and dilated congested blood vessel (BV) (H&E, X: 400). **(E)**: Photomicrograph of liver tissue from group treated with (PJ+PTZ) showing marked improvement in hepatocytes (arrow), normal liver architecture (H&E, X: 400). **(F)**: Photomicrograph of liver tissue from (VPA+PTZ) group showing loss of architecture, sever vacuolation (arrow), pykanotic nuclei (arrow head) and dilated congested blood vessel (BV) (H&E, X:400). **(G)**: Photomicrograph of liver tissue from (PJ+VPA+PTZ) group showing fatty liver and lipid accumulated in hepatocytres (arrow) and shrinking in hepatocytes nuclei (discrete arrow) (H&E, X: 400).

Pretreatment juice improved liver injury and decreased liver enzymes. Fyiad et al. (2012) and Khedr et al. (2015) reported the protective effects of pomegranate juice on liver and their results showed significant decrease in liver enzymes, which are in agreement with this study. The decrease of liver enzymes after treatment with pomegranate juice is related to the antioxidant and anti-inflammatory properties of pomegranate juice due to its high content of flavonoids.

The present study indicated significant decrease in albumin and total proteins of rats injected with PTZ. Other studies indicated the dramatic effects of PTZ such as those of Rodrigues et al., (2013), and Akbas et al., (2005), who reported that PTZ led to an increase in lipid and protein damage. Proteolysis and the release of ROS can lead to lipid and protein damage in one hand and a decrease in antioxidant defenses and sulfhydryl protein content on the other hand (Militão et al. 2010; Dilliogluglil et al. 2010). Rats treated with valproic acid showed a significant decrease in albumin and total proteins which are in accordance with results of Hamza et al., (2015), who reported that treatment with sodium valproate reduced total proteins in serum of rats; these effects of valproic acid might be due to either direct hepatocyte damage or due to oxidative stress leading to apoptosis of hepatocytes. Treatment with pomegranate juice improved the effects of PTZ and valproic acid. Abd Elmonem, (2014), reported that rats treated with pomegranate improved the effects of Diazinon and restored the concentration of albumin and total protein to normal level; these effects of pomegranate juice may be due to its antioxidant and anti-inflammatory properties.

Injection of PTZ resulted in significant decrease in antioxidants. Khedr et al., (2015), indicated that rats injected with PTZ showed significant decrease in antioxidants in serum and liver of rats; the present results are in agreement with results of their study. PTZ may trigger a variety of biochemical processes including the activation of membrane phospholipases, proteases, and nucleases (Costa, 1994). Accordingly, marked alterations in membrane phospholipid metabolism results in the liberation of free fatty acids (FFAs), diacylglycerols, eicosanoids, lipid peroxides and free radicals which are the direct causes of antioxidants decrease (Costa, 1994).

Treatment with valproic acid resulted in significant decrease in antioxidants under the present experimental conditions. Results of this study are in agreement with other studies which indicated nearly the same results (Chaudhary and Parvez (2012) and Hamza et al. (2015). Many studies indicated that treatment with valproic acid stimulated the generation of ROS (Tong et al., 2005; Raza et al., 1997) which may be the direct factor in depletion of antioxidants and increase in lipid peroxidation.

Pretreatment with pomegranate juice improved the antioxidants and decreased MDA in both groups PJ+PTZ and PJ+VPA+PTZ. Dkhil et al. (2013) reported that supplementation with PJ and methanol extract of pomegranate peel (MPPE) markedly enhanced the activities of SOD and CAT enzymes and reduced the elevated levels of MDA and nitric oxide (NO) in testes of rats. Tu¨rk et al. (2013) reported that PJ administration to CCl4-treated rats significantly decreased the CCl4-induced increment in MDA level. Pomegranate juice counteracted the oxidative stress through its antioxidant properties.

The present study indicated an elevation in total cholesterol, triglycerides, low density lipoprotein and significant decrease in high density lipoprotein cholesterol of rats injected with PTZ. Rats treated with either valproic acid or valproic acid and PTZ showed significant increase in TC, TG, LDL-c and significant decrease in HDL-c. Hamza et al., (2015), indicated that the administration of different doses of sodium valproate (SV) caused a significant increase in the levels of lipid profile parameters (TC, TG, LDL-c) and decrease of HDL-c. Lahneche et al., (2017), reported that the VPA-treated rats exhibited significantly higher cholesterol and triglycerides levels than the control rats. Liver plays an important role in lipid metabolism, lipid synthesis and transportation. Therefore, it is reasonable to expect an abnormal lipid profile in those with severe liver dysfunction (Verrotti et al., 2002); so, the serious effects of valproic acid on liver are the main cause in elevation of lipid profile (TC, TG, LDL-c) and the decrease in HDL-c. Al-Moraie et al., (2013), demonstrated the effect of PJ on lipid profile in hypercholesterolemic rats and reported that pomegranate juice at different doses reduced the elevation of lipid profile and restored its concentrations to normal in hypercholesterolemic rats.

Cross sections of normal liver of control rats showed that the hepatocytes arranged in cords or plates, one or two cell thick, forming the normal liver strands radiating from the central vein. The spaces lying between the hepatic cords constitute hepatic sinusoids, which are lined with flattened endothelial cells and few phagocytic cells, mainly Kupffer cells. The nuclei of the later are spindle shaped. The cytoplasm of the hepatic cells is pink in color with scattered basophilic granules. The nuclei of the hepatic cell are rounded in shape with granular chromatin material, some hepatocytes are binucleated. Microscopical examination of liver tissue obtained from PTZ treated group revealed severe vacuolar degenerative changes in hepatocytes with pykanotic nuclei. Dilated congested blood vessel could be detected. Periportal inflammation and bile duct proliferation were seen. Sections of animals treated with valproic acid showed moderated vacuolation in hepatocytes with pykanotic nuclei. Some

hepatocytes appeared with normal nuclei. These results are in agreement with those of Shaalan et al., (2015), who reported that sections of liver in rats treated with valproic acid showed massive vacuolar degeneration of hepatocytes together with dilated and congested blood sinusoids. Examination of liver tissue from group treated with pomegranate revealed normal hepatocytes with vesicular nuclei where no pathological alterations were observed. Marked improvement in liver tissue could be seen after treatment of induced animals with pomegranate juice; these histological findings are in agreement with those of Al-Moraie et al. (2013) and Fyiad et al. (2012). Histopathological studies of the liver of different groups, also, supported the protective effects exhibited by pomegranate juice through restoring the normal hepatic architecture.

5. Conclusion

From the above results, it could be concluded that the pomegranate juice potentiated antioxidants defense systems and reduced lipid oxidation and improved liver functions and lipid profile in epileptic rats induced by PTZ, therefore, it can be used as a co-medicine with, e.g, valproic acid or other antiepileptic drugs for its protective effects.

6. Acknowledgment

This research was supported by Faculty of Science, Menoufia Uinversity, Shibin El-Kom, Egypt.

7. References

Abd Elmonem, H.A. (2014): Assessment the effect of pomegranate molasses against diazinon toxicity in male rats. IOSR J. Environ. Sci. Toxicol. Food Technol., 8(2): 135-141.

Abdou, S.F.; El-Wakeel, M. and El-Saied, M.H. (2014): Protective effect of silymarin and vitamin E in hepatotoxicity induced by valproic acid in albino rats. J. Med. Sci., 14(6-8): 290-296.

Ajaikumar, K.B.; Asheef, M.; Babu, B.H. and Padikkala, J. (2005): The inhibition of gastric mucosal injury by Punica granatum L. (pomegranate) methanolic extract. J. Ethnopharm, 96(1-2): 171-176.

Akbas, S.H.; Yegin, A. and Ozben, T. (2005): Effect of pentylenetetrazol-induced epileptic seizure on the antioxidant enzyme activities, glutathione and lipid peroxidation levels in rat erythrocytes and liver tissues. Clin. Biochem, 38:1009–1014.

Al-Moraie, M.M.D.; Arafat, R.A. and Al-Rasheedi, A.A. (2013): Effect of pomegranate juice on lipid profile and antioxidant enzymes in hypercholesterolemic rats. Life Sci. J., 10(3): 2717-2728.

Ashrafi, M.R.; Shams, S.; Nouri, M.; Mohseni, M.; Shabanian, R.; Yekaninejad, M.S.; Chegini, N.; Khodadad, A. and Safaralizadeh, R. (2007): A probable causative factor for an old problem: Selenium and glutathione peroxidase appear to play important roles in

epilepsy pathogenesis. Epilepsia, 4(9): 1750-1755.

Bansal, A.K.; Bansal, M.; Soni, G. and Bhatnagar, D. (2005): Protective role of vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. Chem. Biol. Interact., 156(2-3): 101-111.

Bayomy, M.F.F; Tousson, E. and El-sendiony, B.F. (2018): Effects of L-Carnitine and Ginkgo Biloba on Spleen in Experimentally-Induced Epileptic Seizures Disease in Rats. Ann Epilepsy Seizure, 1(1): 1003.

Chaudhary, S. and Parvez, S. (2012): An in vitro approach to assess the neurotoxicity of valproic acid-induced oxidative stress in cerebellum and cerebral cortex of young rats. Neuroscience, 225: 258-268.

Costa, L.G. (1994): Cell signaling and neurotoxic events. In: Chang, L.W. (Ed.), Principles of Neurotoxicology. Marcel Dekker, New York, 475:493.

Dillioglugil, M.O.; Kir, H.M.; Demir, C.; Ilbay, G.; Sahin, D.; Dillioglugil, O.; Bambal, G.; Mekik, H. and Ates, N. (2010): Effect of pentylenetetrazole and sound stimulation induced single and repeated convulsive seizures on the MDA, GSH and NO levels, and SOD activities in rat liver and kidney tissues. Brain Res. Bull., 83:356–359.

Dkhil, M.A.; Al-Quraishy, S. and Abdel Moneim, A.E. (2013): Effect of pomegranate (*Punica granatum L.*) juice and methanolic peel extract on testis of male rats. Pak. J. Zool., 45(5): 1343-1349.

Drury, R. A.; Wallington, E. A. and Cancerson, R. Carlton's histopathological techniques, 1976; 4th ed. Oxford university, press, oxford.

Faria, A.; Monteiro, R.; Mateus, N.; Azevedo, I. and Calhau, C. (2007): Effect of pomegranate (*Punica granatum*) juice intake on hepatic oxidative stress. Eur. J. Nutr., 46(5): 271-278.

Fisher, R.S.; Acevedo, C.; Arzimanoglou, A.; Bogacz, A.; Cross, J.H.; Elger, C.E. and Wiebe, S. (2014): ILAE Official Report: A practical clinical definition of epilepsy. Epilepsia, 55(4): 475-82.

Fridewald, W.T.; Leve, R. I. and Fredrickson, D. S. (1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem., 18(6): 499-502.

Fyiad, A.A.; Abd El-Kader, M.A. and Abd El-Haleem, A.H. (2012): Modulatory effects of Pomegranate Juice on Nucleic Acids Alterations and Oxidative Stress in Experimentaly Hepatitis Rats. Life Sci. J., 9(3): 676-82.

Gaby, A.R. (2007): Natural Approaches to Epilepsy. Altern. Med. Rev., 12(1): 9-24.

GBD 2013 Mortality and Causes of Death Collaborators, (2015): Global, regional, and national age sex specific all-cause and causes pecific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study. Lancet, 385(9963): 117-171.

Hamza, R.Z.; Ismail, H.A.A.; Khaled, H.E. and El-Shenawy, N.S. (2015): Antioxidant effect of L-cysteine on sodium-valproate-induced oxidative stress in rat liver: Biochemical and ultrastructural approaches. Toxicol. Environ. Chem., 97(8): 1041-1056.

Khan, S.K; Abdul-Shakoor; Jan, M.A.; Khattak, A.M. and Shah, S.H. (2005): Study of histopathologic changes in the liver of albino rats, induced by toxic doses of valproic acid. Gomal J. Med. Sci., 3:15-18.

Khedr, A.A.; Serag El-Din, M.F. and Yossef, H.E. (2015): The protective role of pomegranate (Punicagranatum L.) juice against hepatotoxicity induced by pentylenetetrazol in epileptic rats. Life Sci. J., 12(12): 119-124.

Koracevic, D.; Koracevic, G.; Djordjeric, V.; Andrejevic, S. and Cosic, V. Method for the measurmet of antioxidant activity in human fluids. J. Clin. Pathol., 2001; 54:356-361.

Kovacic, P.; Pozos, R.S.; Somanathan, R.; Shangari, N. and O'Brien, P.J. (2005): Mechanism of mitochondrial uncouplers, inhibitors, and toxins: Focus on electron transfer, free radicals, and structure-activity relationships. Cur. Med. Chem., 12(22): 2601-2623.

Kwan, P. and Brodie, M.J. (2000): Early identification of refractory epilepsy. N. Eng. J. Med, 342(5): 314-319.

Lahneche, A.M.; Boucheham, R.; Boubekri, N.; Bensaci, S.; Bicha, S.; Ali Bentamenne, A.; Benayache, F.; Benayache, S. and Zama, D. (2017): Sodium valproate-induced hepatic dysfunction in albino rats and protective role of n-butanol extract of *Centaurea sphaerocephala L*. Int. J. Pharmacogn. Phytochem. Res., 9(10): 1335-1343.

Langley, P. (2000): Why a pomegranate? B. M. J., 321(7269): 1153-1154.

Longtin, R. (2003): The pomegranate: Nature's power fruit? J. Natl. Cancer Inst., 95(5): 346-348.

Migliore, L.; Fontana, I.; Colognato, R.; Coppede, F.; Siciliano, G. and Murri, L. (2005): Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. Neurobiol. Aging, 26: 587–595.

Militão, G.C.; Ferreira, P.M. and de Freitas, R.M. (2010): Effects of lipoic acid on oxidative stress in rat striatum after pilocarpine-induced seizures. Neurchem Int., 56:16–20.

Moss, D.W.; Henderson, A.R. and Kachmar, J.F. (1987): Enzymes In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders, pp. 346-421.

Niaraki, M.S.; Nabavizadeh, F.; Vaezi, G.H.; Alizadeh, A.M.; Nahrevanian, H.; Moslehi, A. and Azizian, S. (2013): Protective effect of ghrelin on sodium valproate-induced liver injury in Rat. J. Stress Physiol. Biochem., 9(1): 96-105.

Ohakawa, H.; Ohishi, N. and Yagi, K. (1979): Assay of lipid peroxidation by thiobarbituric acid reaction. Anal. Biochem, 95(2): 351-358.

Paglia, D.E. and Valentine, W.N. (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70(1): 158-169.

Raza, M.; Al-Bekairi, A.M.; Ageel, A.M. and Qureshi, S. (1997): Biochemical basis of sodium valproate hepatotoxicity and renal tubular disorders: time dependence of peroxidative injury. Pharmacol. Res., 35(2): 153-157.

Raza, M.; Albekairi, A.M.; Ageel, A.M. and Qurishi, S. (1999): Biochemical basis of sodium valprovate hepatotoxicity & renal tubular disorders: time dependence of peroxidative injury. Pharmacol Res, 35: 153-7.

Rodrigues, A.D.; Scheffel, T.B.; Scola, G.; Dos Santos, M.T.; Fank, B.; Dani, C.; Vanderlinde, R.; Henriques, J.A.P.; Coitinho, A.S. and Salvador, M. (2013): Purple grape juices prevent pentylenetetrazol induced oxidative damage in the liver and serum of Wistar rats. Nutr. Res., 33(2): 120-125.

Saleh, D.A.A.; Ismail, M.A. and Ibrahim, A.M. (2012): Nonalcoholic fatty liver disease, insulin resistance, dyslipidemia and atherogenic ratios in epileptic children and adolescents on long term antiepileptic drug therapy. Pak. J. Biol. Sci., 15(2): 68-77.

Shaalan, S.; El-Wakkad, A.S.E.; Saleh, H. and Deab, A. (2015): Protective effect of L-carnitine and baker yeast saccharomyces cerevislae against hepatic toxicity induced by valproateas antiepileptic drug in rat. Int. J. Pharm. Pharm. Sci., 7(5): 89-95.

Shin, E.-J., Jeong, J.H., Chung, Y.H., Kim, W.-K., Ko, K.-H., Bach, J.-H. and Kim, H.-C. (2011): Role of oxidative stress in epileptic seizures. Neurochem. Int., 59(2): 122-137.

Thefeld, W.; Hoffmeister, H.; Busch, E.W.; Koller, P.U. and Vollmar, J. (1974): Reference values for the determinations of the transaminases GOT and GPT as well as the alkaline phosphatase in serum using

optimized standard methods. DTSH. Med. Wochenschr, 99(8): 343-351.

Tong, V.; Teng, X.W.; Chang, T.K.H. and Abbott, F.S. (2005): Valproic acid I: time course of lipid peroxidation biomarkers, liver toxicity, and valproic acid metabolite levels in rats. Toxicol Sci., 86(2): 427-435.

Tousson, E.; Bayomy. M.F.F and El-sendiony, B.F. (2015): Effects of L-carnitine and *Ginkgo biloba* on cerebral cortex in experimentally-induced epileptic seizures disease in rats. J. Biosci. Appl. Res., 1 (6):277-289.

Tu¨rk, G.; Songu¨l, C.; Eribas, I.; So¨nmez, M.; Mehmet, C.; Iftc¸ I.; Yu¨ce, A.; Gu¨venc¸, M.; Kaya, S.O.; Mehmet, C. and Aksakal, M. (2016): Ameliorating effect of pomegranate juice consumption on carbon tetrachloride-induced sperm damages, lipid peroxidation, and testicular apoptosis. Toxicol. Indust. Health, 32(1): 126-137.

Ustundag, U.V.; Tunali, S.; Alev, B.; Ipekci, H.; Emekli-alturfan, E.; Tunali-akbay, T.; Yanardag, R. and Yarat, A. (2016): Effects of chard (Beta Vulgaris L. Var. Cicla) on cardiac damage in valproic acid–induced toxicity. J. Food Biochem., 40(2): 132-139.

Valko, M.; Izakovic, M.; Mazur, M.; Rhodes, C. J. and Telser, J. (2004): Role of oxygen radicals in DNA damage and cancer incidence. Mol. Cell. Biochem., 266(1-2): 37-56.

Verrotti, A.; Basciani, F.; Trotta, D.; Pomilio, M.; Morgese, G. and Chiarelli, F. (2002): Serum copper, zinc, selenium, glutathione peroxidase and superoxide dismutase levels in epileptic children before and after 1 year of sodium valproate and carbamazepine therapy. Epilepsy Res., 48(1-2):71-75.

Wiseman, H. and Halliwell, B. (1996): Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem. J., 313(1): 17-29.

Yegin, A.; Akbas, S.H.; Ozben, T. and Korgun, D.K. (2002): Secretory phospholipase A2 and phospholipids in neural membranes in an experimental epilepsy model. Acta. Neurol. Scand, 106:258–262.

Young, D.S. (1995): Effects of drugs on clinical lab Tests (4th ed). AACC Press, Washington D.C.