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Original Research Article

The chemopreventive effects of onion and garlic oils against valproic acid-induced toxicity

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Abstract

Valproic acid (VPA) is a first-line antiepileptic drug that is used clinically in the treatment of various seizure disorders, in the management of bipolar disorder and migraine. In this study we wish to use natural oils, depending on their antioxidant effect, to reduce the side effects of the widely used VPA. We studied this effect on the highly affected organs (liver, kidney and heart). The animals were divided into five groups: the 1st was the control one, the 2nd was VPA treated group, the 3rd, the 4th and the 5th were the groups treated with onion, garlic or their mixture respectively. The study showed a significant increase in liver and kidney functions, myocardial toxicity and oxidation shift in VPA treated rats and all were ameliorated after treatments especially with the oils mixture.

Keywords: Valproic acid- toxicity- onion oil- garlic oil.

INTRODUCTION

VPA has been used clinically for epilepsy for years, as the second major option treatment discovered after lithium (Hu*etal*., 2011; Catarino *et al*., 2011). Due to its wide spectrum of antiepileptic activity, VPA is the most prescribed antiepileptic drugs (Perucca, 2002). However, its hepatotoxicity (Neuman *et al*., 2001), teratogenicity (Kultima *et al*., 2004) and common general side effects such as convulsions, facial edema, lassitude, hypoglycemia, vomiting, and heartburn (Williams *et al*., 2007) are disadvantages that limit its chronic use. Also, the serious toxicity and even deaths have been reported (Baselt, 2008). Valproic acid was found to reduce serum carnitine which is essential cofactor in transport of long chain fatty acids across the inner mitochondrial membrane (Fung *et al*., 2003).

Medicinal plants continue to provide valuable therapeutic agents, in both modern medicines and traditional system (Ahmed *et al*., 2007). Antioxidants delay or prevent oxidation of the substrate (Halliwell and Gutteridge, 2007*)*. Interest in finding naturally occurring antioxidants in foods or medicine to replace synthetic antioxidants has increased considerably, given that synthetic antioxidants are being restricted due to their side effects (Zneng and Wang, 2001). Therefore, attention has been directed toward the development and and isolation of natural antioxidants from plant sources.

Helen *et al*. (1999) indicated that garlic and onion oils are effective antioxidants against the oxidative damage.Also, Pedraza-Chaverri *et al*. (2000) reported that onion and garlic were effective in preventing or ameliorating oxidative stress. It was reported that *Allium sativa* and *Alliumcepa*are organic sulphides, capable of enhancing glutathione-S-transferase activity in the liver (Guyonnet *et al*., 2002), and isothiocyanate is a very potent inducer of phase II metabolising enzymes such as quinonereductase and glutathione-S-transferase (Andorfer*etal*., 2004).

MATERIALS AND METHODS

Experimental Animals

Male albino rats (*Rattusnorvegicus*) weighing about 130-170g were used as experimental animals in the present investigation. They were obtained from the animal house in the National Research Center, El-Giza, Egypt. They were kept under observation for 10 days before the onset of the experiment to exclude any intercurrent in-fection. The animals were housed at normal atmospheric

temperature (25 ± 5°C) as well as 12 hrs daily normal light periods. Rats were given access of water and supplied daily enough balanced standard diet *ad libitum*. All animal procedures are in accordance with the recommendations of the Candian committee for care and use of animals (Candian Council on Animal Care [CCAC], 1993).

Chemicals

VPA is a branched chain carboxylic acid (2-propyl-pentanoic acid or di-n-propylacetic acid), with a chemical structure very similar to that of short chain fatty acid (Johannessen and Johannessen, 2003). Depakine (valproic acid / sodium valproate) was purchased from Sanofi-synthelabo, France. The tablets were crushed, dissolved in distilled water and orally given to animals by gastric intubation at dose level of 500 mg/kg b. wt six days per week for two weeks.

Onion and garlic oils were obtained from Unit of Maceration and Extraction of Oils in the National Research Center, El-Giza, Egypt. The onion oil or garlic oil or both were dissolved in 10% tween 20 and orally given to animals by gastric intubation at dose level of 100 mg/kg b. wt (Ogunmodede *et al*., 2012) six days per week for four weeks.

Animals Grouping

This study included 50 adult male albino rats which were divided into five groups (ten animals for each) designed as follows:

1-The first group was regarded as control and each animal was orally given 10% tween 20 (as a vehicle) six days per week by gastric intubation for four weeks.

2-The second group was treated *via* the same route as normal group for 2 weeks, and then it was treated with valproic acid at a dose level of 500 mg/kg b. wt dissolved in distilled water and orally administered by gastric intubation six days per week during the last two weeks of the experimental period.

3-The third group was treated with onion oil at a dose level of 100 mg/kg b. wt (dissolved in 10% tween 20) six days per week for four weeks and valproic acid at a dose level of 500 mg/kg b. wt (dissolved in distilled water) six days per week during the last two weeks of the experiment.

4-The fourth group was treated with garlic oil at a dose level of 100mg/kg b. wt (dissolved in 10% tween 20) six days per week for four weeks and valproic acid at a dose level of 500 mg/kg b. wt (dissolved in distilled water) six days per week during the last two weeks of the experiment.

5-The fifth group was treated with a mixture of garlic oil and onion oil at a dose level of 100 mg/kg b. wt (dissolved in 10% tween 20) six days per week for four weeks and valproic acid at a dose level of 500 mg/kg b. wt (dissolved in distilled water) six days per week during the last two weeks of the experiment.

Sampling

At the end of the experiment, blood samples were collected from jugular vein of each animal in a centrifuge tube and left to clot at room temperature for 45 minutes and then centrifuged at 3000 r.p.m. for 15 minutes. The clear, non-heamolysed supernatant sera were quickly removed, divided into four portions for each individual animal and kept at -30°C for various biochemical analyses.. After decapitation and dissection, organs (liver, kidney and heart) were excised and weighed. 0.5g from each tissue were homogenized in 5ml 0.9% NaCl (10% w/v) using teflon homogenizer (**Glas-**Col, Terre Haute, USA). The obtained homogenate was kept in deep freezer at -30 °C to be used later for the measurement of oxidative stress markers. The homogenate supernatant for each liver, kidney and heart samples was obtained by centrifuging the homogenate at 3000 r.p.m. for 10 minutes.

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were measured by colorimetric method using kits developed by Spectrum Diagnostics according to the method of Sherwin (1984). Serum alkaline phosphatase (ALP) activity was measured by colorimetric method using kits obtained from Spectrum Diagnostics according to the method of Marsh *et al*. (1959). Serum lactate dehydrogenase (LDH) activity was determined according to method of Young (1990) using reagent kits purchased from Spectrum, Egypt. Serum urea concentration was determined according to the method of Shephard and Mezzachi (1983) using reagent kits purchased from Spectrum, Egypt. Uric acid in serum was measured by enzymatic method using kits developed by Spectrum Diagnostic according to the method of Fossati *et al.* (1980). Serum creatinine level was determined by kinetic method using kits obtained from Diamond Diagnostics according to Young (2001). Serum creatine kinase MB (CK-MB) activity in serum was determined according to method of Young (1997) using reagent kits purchased from Spectrum, Egypt. Totalthiols levels in liver, kidney and heart homogenate were determ**in**ed us**in**gthe method of Koster *et al*. (1986). Liver, kidney and heart glutathione content was determined according to the procedure of Beutler *et al*. (1963)with some modifications. Glutathione peroxidase activity in liver and kidney was assayed according to the method suggested by Matkovics *et al*. (1998). Glutathione-S-transferase was assayed according to the method of Mannervik and Gutenberg (1981) with little modifications. Lipid peroxidation in liver, kidney and heart homogenates was

Table 1. Effect of onion oil and/or garlic oil on serum parameters related to liver function of valproic acid –administered rats.

Groups \ Parameters	ALT (U/L)	% change	AST (U/L)	% change	ALP (U/L)	% change	LDH (U/L)	% change	Bilirubin (mg/dl)	% change
Normal (G1)	7.83 ± 0.79 ^{b}	-	13.00 ± 1.09 ^{b}	-	25.98 ± 2.93 ^{b}	-	482.91 ± 11.06 ^{c}	-	0.81 ± 0.09 ^{b}	-
VPA(G2)	21.33 ± 2.98 ^{a}	172.41	43.75 ± 6.07 ^{a}	236.53	45.91 ± 3.72 ^{a}	76.71	1273.90 ± 56.14 ^{a}	164.00	1.67 ± 0.08 ^{a}	106.17
Onion oil + VPA (G3)	9.16 ± 0.75 ^{b}	-57.70	10.50 ± 1.80 ^{b}	-76.00	25.48 ± 2.49 ^{b}	-44.50	676.28 ± 23.36 ^{b}	-46.87	0.59 ± 0.07 ^{bc}	-66.46
Garlic oil + VPA(G4)	9.50 ± 0.88 ^{b}	-55.46	13.01 ± 1.09 ^{b}	-70.28	28.06 ± 1.65 ^{b}	-38.88	466.50 ± 9.83 ^{c}	-63.35	0.74 ± 0.05 ^{b}	-55.68
Onion oil + Garlic oil + VPA(G5)	5.66 ± 0.91 ^{b}	-73.46	8.50 ± 0.55 ^{b}	-80.57	23.28 ± 1.59 ^{b}	-49.29	725.86 ± 8.19 ^{b}	-42.98	0.44 ± 0.06 ^{c}	-73.65
F-Probability	P<0.001	-	P<0.001	-	P<0.001	-	P<0.001	-	P<0.001	-
LSD at the 5% level	4.445	-	8.501	-	7.662	-	82.480	-	0.229	-
LSD at the 1% level	6.014	-	11.501	-	10.367	-	111.592	-	0.310	-

Data are expressed as mean ± standard error. Number of animals/group = six.
Means, which have the same superscript symbol(s), are not significantly different.
Percentage (%) changes were calculated by comparing valproic acid – administered group with the normal group and pre-treated valproic acid groups with the valproic acid – administered group.

determined according to the chemical method of Preuss *et al.* (1998). Superoxide dismutase activity in liver, kidney and heart homogenates was determined according to the method of Marklund and Marklin (1974). Catalase activity in liver, kidney and heart homogenates was determined according to the method of Cohen *et al.* (1970). Peroxidase activity in liver, kidney and heart homogenates was determined according to the chemical method of Kar and Mishra (1976).

The data were analyzed using the one-way analysis of variance (ANOVA) (Roa *et al.*, 1985) followed by LSD to compare various groups with each other. Results were expressed as mean ± standard error (SE). F-probability, obtained from one-way ANOVA, expresses the effect between groups.

RESULTS

The administration of valproic acid at dose level of 500 mg/kg b.w. to albino rats for 2 weeks produced a highly significant elevation (P<0.01) of serum ALT, AST, ALP, LDH and bilirubin levels. The treatment of valproic acid-administered animals with onion oil and/or garlic oil succeeded to produce potential amelioration (P<0.01) of the elevated serum parameters related to liver function (table 1).

Liver total thiol and glutathione contents were remarkably decreased in valproic acid-administered rats. The treatment of these animals with onion oil and/or garlic oil prospered to produce potential improvement(P<0.01) of the total thiol and glutathione levels. The activities of liver GPO, GST, SOD, catalase and peroxidase (table 2 and 3) were detectably decreased in valproic acid-administered animals. The effect of valproic acid on SOD activity (-59.08%) seemed to be the most potent as compared with GPO (-29.80%), GST (-44.59%), catalase (-52.66%) and peroxidase (-48.95%).

The treatment of valproic acid-administered rats with onion oil and/or garlic oil succeeded to cause ahighly significant alleviation (P<0.01) of the GST, SOD and catalase activities. The liver peroxidase activity, on the other hand, was significantly (P<0.05) decreased as a result of treatment of animals with garlic oil, and it was highly significant (P<0.01) affected as a result of treatment with onion oil alone (94.63%) and treatment with onion oil and garlic oil in combination (53.62%). Furthermore, the administration of onion oil and garlic oil on combination

Table 2. Effect of onion oil and/or garlic oil on liver total thioland glutathione contents and the activities of glutathione peroxidase and glutathione-S-transferase of valproic acid – administered rats.

Parameters / Groups	Total thiol (nmol/100mg tissue)	% change	Glutathione (nmol/100mg tissue)	% change	Glutathione Peroxidase (U/100mg tissue)	% change	Glutathione-S-transferase (U/100mg tissue)	% change
Normal (G1)	189.10 ± 9.90^{a}	-	93.50 ± 6.76^{a}	-	74.60 ± 2.80^{c}	-	145.30 ± 7.37^{b}	-
VPA (G2)	118.50 ± 2.13^{c}	-37.57	68.00 ± 2.70^{b}	-27.27	52.80 ± 2.00^{d}	-29.72	80.50 ± 1.95^{c}	-44.59
Onion oil + VPA (G3)	160.00 ± 10.60^{b}	35.59	106.00 ± 9.42^{a}	55.88	116.60 ±1.80^{a}	120.83	144.10 ± 7.58^{b}	79.00
Garlic oil + VPA (G4)	200.50 ± 3.60^{a}	69.49	93.00 ± 2.41^{a}	36.76	103.10 ± 2.60^{b}	94.69	154.90 ± 5.68^{b}	92.42
Onion oil + Garlic oil + VPA (G5)	166.60 ± 6.14^{b}	40.67	106.50 ± 2.78^{a}	56.61	100.10 ± 2.20^{b}	89.39	178.00 ± 2.45^{a}	121.11
F-Probability	P<0.001	-	P<0.001	-	P<0.001	-	P<0.001	-
LSD at the 5%level	21.566	-	16.407	-	6.066	-	16.260	-
LSD at the 1%level	29.177	-	22.197	-	9.307	-	21.998	-

Data are expressed as mean ± standard error. Number of animals/group = six.
Means, which have the same superscript symbol(s), are not significantly different.
Percentage (%) changes were calculated by comparing valproic acid – administered group with the normal group and pre-treated valproic acid groups with the valproic acid – administered group.

Table 3. Effect of onion oil and/or garlic oil on liver lipid peroxidation and the activities of SOD, catalase and peroxidase in valproic acid – administered rats.

Parameters / Groups	Lipid peroxidation (nmol MDA/100 mg tissue /hr)	% change	SOD (U/g tissue)	% change	catalase (k.10^{2})	% change	Peroxidase (U/g tissue)	% change
Normal (G1)	122.83 ± 11.06^{b}	-	14.20 ± 0.72^{a}	-	36.08 ± 1.0 ^{a}	-	135.50 ± 7.78^{a}	-
VPA (G2)	223.00 ± 18.41^{a}	81.59	5.81± 0.69^{b}	-59.08	17.08 ± 1.84^{b}	-52.66	69.16 ± 5.02^{c}	-48.95
Onion oil + VPA (G3)	130.66 ± 15.16^{b}	-41.43	15.46 ± 1.84^{a}	166.09	38.66 ± 4.95^{a}	126.34	134.16 ± 8.71^{a}	94.63
Garlic oil + VPA (G4)	134.16 ± 10.65^{b}	-39.86	12.66 ± 1.11^{a}	117.90	39.66 ± 3.11^{a}	132.20	87.41± 2.54^{b}	26.38
Onion oil + Garlic oil + VPA (G5)	107.50 ± 5.32^{b}	-51.79	13.91± 1.59^{a}	139.41	40.00 ± 2.25^{a}	134.19	106.00 ± 6.47^{b}	53.62
F-Probability	P<0.001	-	P<0.001	-	P<0.001	-	P<0.001	-
LSD at the 5% level	37.890	-	3.764	-	8.596	-	18.974	-
LSD at the 1% level	51.262	-	5.093	-	11.630	-	25.671	-

Data are expressed as mean ± standard error. Number of animals/group is six.
Means, which have the same superscript symbol(s), are not significantly different.
Percentage (%) changes were calculated by comparing valproic acid – administered group with the normal group and pre-treated valproic acid groups with the valproic acid – administered group.

seemed to induce the most potent effect on GST (121.11%) and catalase (134.19%) activities, while the administration of onion oil alone appeared to have the most potent effect on GPO (120.83%), SOD (166.09%) and peroxidase (94.63%) activities. Liver lipid peroxidation content was highly significantly increased (P<0.01) in valproic acid-administered animals. The treatment of these animals with onion oil and/or garlic oil succeeded to produce a potential amendment (P<0.01).

Table 4 showed the prophylactic effect of

Table 4. Effect of onion oil and/or garlic oil on creatinine, urea and uric acid levels in serum of valproic acid – administered rats.

Groups / Parameters	Creatinine (mg/dl)	% change	Urea (mg/dl)	% change	Uric acid (mg/dl)	% change
Normal (G1)	4.04 ± 0.34^{b}	-	37.16 ± 2.86^{a}	-	5.20 ± 0.82^{b}	-
VPA G2)	7.04 ± 0.35^{a}	75.00	20.50 ± 2.72^{c}	-44.83	7.26 ± 0.12^{a}	39.60
Onion oil + VPA (G3)	3.83 ± 0.36^{b}	-45.71	31.60 ± 1.50^{ab}	54.14	4.58 ± 0.28^{b}	-36.88
Garlic oil + VPA (G4)	4.48 ± 0.2^{b}	-37.14	28.05 ± 2.36^{b}	36.82	4.61 ± 0.21^{b}	-36.50
Onion oil + Garlic oil + VPA (G5)	4.21 ± 0.18^{b}	-40.00	28.71 ± 0.64^{b}	40.04	4.48 ± 0.10^{b}	-38.29
F-Probability	P < 0.001	-	P < 0.001	-	P < 0.001	-
LSD at the 5% level	0.905	-	6.396	-	1.188	-
LSD at the 1% level	1.224	-	8.654	-	1.607	-

Data are expressed as mean ± standard error. Number of animals/group = six.
Means, which have the same superscript symbol(s), are not significantly different.
Percentage (%) changes were calculated by comparing valproic acid – administered group with the normal group and pre-treated valproic acid groups with the valproic acid – administered group.

Table 5. Effect of onion oil and/or garlic oil on kidneytotal thiol and glutathione content and the activities of glutathione peroxidase and glutathione-S-transferaseofvalproic acid – administered rats.

Groups / Parameters	Total thiol (nmol/100mg tissue)	% change	Glutathione (nmol/100mg tissue)	% change	Glutathione Peroxidase (U/100mg tissue)	% change	Glutathione-S-transferase (U/100mg tissue)	% change
Normal (G1)	201.80 ± 6.55^{b}	-	101.10± 9.42^{a}	-	160.10 ± 4.70^{ab}	-	174.83 ± 7.38^{a}	-
VPA (G2)	129.80 ± 5.32^{c}	-35.82	51.83± 4.91^{c}	-48.71	121.00 ± 3.60^{c}	-24.37	92.66 ± 4.92^{b}	-46.99
Onion oil + VPA (G3)	210.30 ± 10.24^{ab}	62.79	73.81± 5.73^{b}	42.47	172.60 ± 2.90^{a}	42.14	164.33 ± 9.89^{a}	77.34
Garlic oil + VPA (G4)	227.10± 9.83^{a}	75.96	99.30 ± 9.42^{a}	91.69	157.10 ± 6.10^{b}	29.75	168.50 ± 13.66^{a}	81.84
Onion oil + Garlic oil + VPA (G5)	227.50 ± 8.60^{a}	75.97	99.11± 5.73^{a}	91.31	153.30 ± 6.90^{b}	26.44	163.00 ± 5.63^{a}	75.91
F-Probability	P < 0.001	-	P < 0.001	-	P < 0.001	-	P < 0.001	-
LSD at the 5% level	24.840	-	21.624	-	15.060	-	25.809	-
LSD at the 1% level	33.606	-	29.256	-	20.370	-	34.918	-

Data are expressed as mean ± standard error. Number of animals in each group is six.
Means, which have the same superscript symbol(s), are not significantly different.
Percentage (%) changes were calculated by comparing valproic acid – administered group with the normal group and pre-treated valproic acid groups with the valproic acid – administered group.

Table 6. Effect of onion oil and/or garlic oil on kidney lipid peroxidation and the activities of SOD, catalase and peroxidase in valproic acid administered rats.

Groups / Parameters	Lipid peroxidation (nmol MDA/100 mg tissue /hr)	% change	SOD (U/g tissue)	% change	catalase (k.10^{2})	% change	Peroxidase (U/g tissue)	% change
Normal (G1)	138.33 ± 8.19^{b}	-	14.25 ± 1.80^{a}	-	21.20 ± 1.46^{b}	-	142.16 ± 5.65^{ab}	-
VPA (G2)	175.33 ± 5.32^{a}	26.81	3.45 ± 0.59^{c}	-75.78	10.53 ± 0.36^{c}	-50.33	79.33 ± 7.11^{d}	-44.36
Onion oil + VPA (G3)	121.50 ± 3.58^{c}	-30.57	7.91 ± 1.40^{bc}	192.27	27.91 ± 2.14^{a}	165.05	114.83± 6.69^{c}	45.31

Table 6. Continue

Garlic oil + VPA (G4)	106.16± 3.01^{d}	-39.42	10.60 ± 0.80^{ab}	207.24	26.66 ± 2.91^{a}	153.18	125.00 ± 4.99^{bc}	57.60
Onion oil + Garlic oil + VPA (G5)	142.33 ± 3.79^{b}	-18.85	12.71 ± 2.53^{a}	268.11	24.73 ± 0.47^{ab}	134.85	148.50 ± 5.76^{a}	87.20
F-Probability	P < 0.001	-	P < 0.001	-	P < 0.001	-	P < 0.001	-
LSD at the 5% level	14.647	-	4.628	-	5.131	-	17.686	-
LSD at the 1% level	19.816	-	6.262	-	6.942	-	23.928	-

Data are expressed as mean ± standard error. Number of animals/group = six.
Means, which have the same superscript symbol (s), are not significantly different.
Percentage (%) changes were calculated by comparing valproic acid – administered group with the normal group and pre-treatedvalproic acid groups with the valproic acid – administered group.

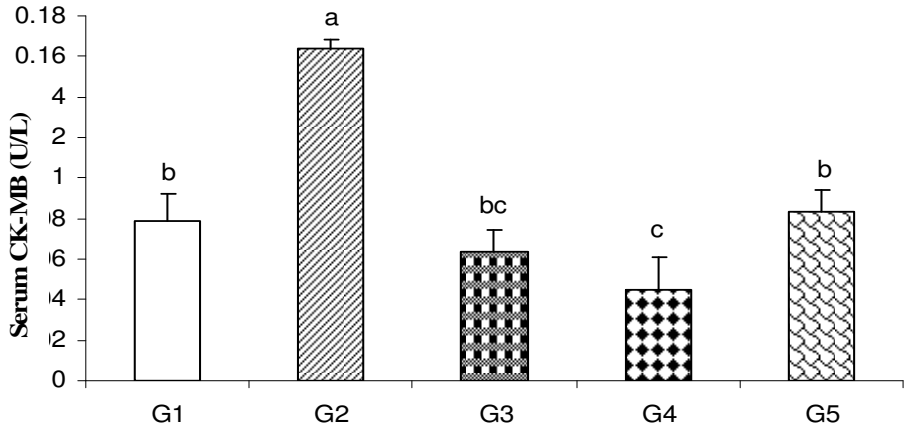

Figure 1. Effect of onion oil and/or garlic oil on serum CK-MB activity of valproic acid-administered albino

onion oil and/or garlic oil on serumcreatinine, urea and uric acid levels of valproic acid-administered animals**.**The administration of valproic acid induced a highly significant elevation (P<0.01) of serum creatinine and uric acid levels, recording percentage changes +75.00% and +39.60% respectively. In contrast there is a highly significant decrease (P<0.01) in the kidney urea level. The treatment of valproic acid-administered animals with onion oil and/or garlic oil prospered to produce potential amendment (P<0.01) of the elevated creatinine and uric acid levels. On the other hand urea level was remarkably increased as a result of onion oil administration.

Table 5 and 6 indicated thatvalproic acid-administered rats exhibited a highly significant decrease (P<0.01) of the kidney total thiols, glutathione, GPO, GST, SOD, catalase and peroxidase levels. In contrast there is a highly significant increase (P<0.01) of the kidney lipid peroxidation content. The treatment of valproic acid-administered rats with onion oil and/or garlic oils prospered to significantly alleviate the kidney total thiols, glutathione, GPO, GST, SOD, catalase and peroxidase levels as well as lipid peroxidation.

The present study showed that CK-MB activity (Figure 1) was highly significant increased (P<0.01) as a result of the administration of valproic acid (107.59%). The serum CK-MB

Table 7. Effect of onion oil and/or garlic oil on heart total thiol and glutathione content and the activities of glutathione, peroxidase and glutathione-S-transferase of valproic acid – administered rats.

Groups \ Parameters	Total thiol (nmol/100 mg tissue)	% change	Glutathione (nmol/100mg tissue)	% change	Glutathione Peroxidase (U/100mg tissue)	% change	Glutathione-S-transferase (U/100mg tissue)	% change
Normal (G1)	141.50± 8.19^{b}	-	90.16 ± 5.06^{b}	-	137.10 ± 3.60^{a}	-	123.50 ± 7.11^{ab}	-
VPA (G2)	120.00 ± 4.50^{c}	-14.89	58.25 ± 2.83^{c}	-35.39	118.60 ± 3.90^{b}	-13.86	64.60 ± 2.86^{c}	-47.69
Onion oil + VPA (G3)	173.00 ± 3.69^{a}	44.17	95.16 ± 10.27^{b}	63.36	133.00± 2.20^{a}	12.71	123.30 ± 7.37^{b}	90.86
Garlic oil + VPA (G4)	141.00 ± 2.09^{b}	17.50	93.00 ± 7.86^{b}	59.65	132.30 ± 5.70^{a}	11.86	139.00 ± 5.05^{a}	115.00
Onion oil + Garlic oil + VPA (G5)	225.00 ± 0.18^{a}	87.50	117.16 ± 0.90^{a}	100.85	129.50 ± 2.20^{a}	9.32	127.50 ± 0.18^{ab}	97.51
F-Probability	P<0.001	-	P<0.001	-	P<0.05	-	P<0.001	-
LSD at the 5% level	13.475	-	18.454	-	10.690	-	15.406	-
LSD at the 1% level	18.231\	-	24.967	-	14.470	-	20.843	-

Data are expressed as mean ± standard error. Number of animals/group = six.
Means, which have the same superscript symbol(s), are not significantly different.
Percentage (%) changes were calculated by comparing valproic acid – administered group with the normal group and pre-treated valproic acid groups with the valproic acid – administered group.

Table 8. Effect of onion oiland/or garlic oil on heart lipid peroxidation and activities of SOD, catalase and peroxidase in valproic acid- administered rats.

Groups \ Parameters	Lipid peroxidation (nmol/g tissue)	% change	SOD (U/g tissue)	% change	catalase (k.10^{2})	% change	Peroxidase (U/g tissue)	% change
Normal (G1)	84.00 ± 9.80^{b}	-	19.73 ± 0.94^{a}	-	28.24 ± 0.39^{a}	-	78.75 ± 3.89^{bc}	-
VPA (G2)	107.16 ± 7.78^{a}	27.57	7.73 ± 0.75^{b}	-61.58	17.80 ± 2.00^{c}	-36.96	67.00 ± 5.39^{c}	-14.86
Onion oil + VPA (G3)	71.00 ± 5.17^{b}	-33.64	12.41 ± 0.65^{b}	63.58	28.40 ± 1.28^{a}	59.55	89.33 ± 4.20^{b}	33.32
Garlic oil + VPA (G4)	77.00 ± 2.88^{b}	-28.03	23.16 ± 3.52^{a}	205.50	24.60 ± 0.29^{b}	34.83	118.33 ± 7.40^{a}	76.11
Onion oil + Garlic oil + VPA (G5)	74.00 ± 0.77^{b}	-30.84	11.80 ± 0.20^{b}	55.67	29.00 ± 0.51^{a}	62.92	68.00 ± 0.68^{c}	1.49
F-Probability	P<0.01	-	P<0.001	-	P<0.001	-	P<0.001	-
LSD at the 5% level	8.275	-	4.937	-	3.256	-	14.069	-
LSD at the 1% level	24.725	-	6.679	-	4.406	-	19.034	-

Data are expressed as mean ± standard error. Number of animals/group = six.
Means, which have the same superscript symbol(s), are not significantly different.
Percentage (%) changes were calculated by comparing valproic acid – administered group with the normal group and pre-treated valproic acid groups with the valproic acid – administered group.

activity was remarkably decreased (P<0.01) as a result of treatment with onion oil, garlic oil and onion oil and garlic oil in combination, recording percentage changes of -60.97% , -72.59% and -49.39% respectively.

Heart total thiol and glutathione content, GPO, GST, SOD and catalase activities were remarkably decreased in valproic acid-administered rats (table 7). Moreover the heart lipid peroxidation and peroxidase levels was significantly increased (P<0.05) and non-significantlydecreased respectively, as a result of valproic acid administration. Moreover, valproic acid produced more potent effect on SOD (-61.58%) than total thiol (-14.89%), glutathione (-35.39%), GPO (-13.86%), GST (-47.69%) and catalase (-36.96%).

The treatment of these animals with onion oil and garlic oil in combination prospered to produce a highly significant increase (P<0.01) of the total thiol (87.50%), glutathione (100.85%) and

catalase (62.92%) levels while it induced only a significant increase in GPO (9.32%) activity. In contrast, the heart SOD and peroxidase activities were not significantly affected as a result of valproic acid – administered rats. It was also noticed that the administration of onion oil alone caused a significant increase in heart total thiol (44.17%), glutathione (63.36%), GPO (12.71%), GST (90.86%), catalase (59.55%) and peroxidase (33.32%) levels while it treatment with onion oil and garlic oil in combination toinduced a significant decrease in lipid peroxidation (-33.64%). The administration of garlic oil significantly improved all tested parameters of oxidative stress and antioxidant defense markers.

DISCUSSION

Our study showed that valproic acid administration profoundly increased serum enzyme (ALT, AST, ALP and LDH) activities and bilirubin level. On the other hand, the serum albumin concentration was significantly decreased in the valproic acid-administered animals.The present study is in agreement with several authors (Banerjea *et al*., 2002; Felker *et al*., 2003; Sonmez *et al*., 2006; Lheureux and Hantson, 2009; Erdoğan, 2012).

Aminotransferases are elevated in some diseases such as myocardial infraction, infectious hepatitis or other damage to either the heart or liver (Murray *et al*., 1993). The elevation in the levels of serum AST, ALT and ALP is an indicative of hepatocellular damage (Bansal *et al*., 2005). This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane. Serum AST, ALT and ALP are biomarkers in the diagnosis of hepatic damage because they are released into the circulation after cellular damage (Naik and Panda, 2007).Elevation of AST and ALT indicates the utilization of amino acids for the oxidation or, for glucogenesis and is used to determine liver damage (Philip *et al*., 1995). It was demonstrated that VPA caused toxicity to rat hepatocytes and induced apoptosis in the rat hepatoma cell line (Phillips *et al*., 2003). The activities of ALT, AST and LDH are often used for evaluating the hepatotoxicity induced by chemicals or toxicants (Wang *et al*., 2007;Zhang *et al*., 2008). It was reported that the administration of VPA produced hepatotoxicity characterized by elevated serum ALT, AST, gamma-glutamyltransferase (GGT) and LDH activities (Erdoğan, 2012).

Theactivities of serum enzymes related to liver function obviously increased with the repeated administration of the drug, which suggested that valproic acid led to the damage of liver cell membrane and the leakage of intracellular enzymes.

The present study shows that administration of onion oil or garlic oil, alone or in combination to valproic acid-administered rats potentially decreased the elevated ALT, AST, LDH and ALP activities as well as bilirubin level. The total proteins and albumin levels were also remarkably increase as result of treatment with onion and garlic oils.

These results are concomitant with the improvement of histological integrity of liver as a result of treatment with onion and garlic oils and are also in accordance with many publications. Garlic extract decrease liver enzymes in serum and prevent liver damage of rats with liver fibrosis due to its ability to reduce free radical-induced oxidative damage in the liver (Gedik*etal*., 2005). Organosulfur components (as diallyl sulfide) present in garlic exhibit protective effects against toxicants (Kwak *et al*., 1993). El-Shatter *et al.* (1997) and Augusti *et al.* (2001) found that, the enzyme activities AST, ALT and ALP in serum of rats decreased significantly when they fed on a diet containing 5% garlic.Also, they explained that *Allium sativum*, may cause stabilized cell membrane and protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells. This is reflected by the reduction of liver enzymes. Garlic helps the liver to maintain its normal function by accelerating the regenerative capacity of its cells. Hoffman (1997) reported that the sulfur compound called allicin is formed from garlic by the action between the compound alliin and the enzyme allinase, both naturally present in garlic, and allicin has been listed as an inhibitor for lactate dehydrogenase, the main distinguishing enzyme for cancer cell metabolism.

The present study shows that administration of valproic acid remarkably decreased liver total thiols and glutathione level as well as GPO, GST, SOD, catalase (CAT) and peroxidase activity. The liver lipid peroxidation level, on the other hand, was detectably increased in these animals.

It was reported that glutathione homeostasis may be altered as a consequence of reactive metabolites and/or reactive oxygen species produced during VPA treatment (Cengiz *et al*., 2000). Reduced glutathione (GSH) is an important cell-protecting biomolecule against chemical-induced cytotoxicity by direct or enzymatic (glutathione-S-transferase and glutathione peroxidase) conjugation with electrophilic compounds and ROS (reactive oxygen species) (Reed, 1990).

There are a number of studies suggesting that excessive generation of free-radical intermediates are associated with VPA, possibly as a consequence of VPA biotransformation, alterations in glutathione homeostasis (Tang *et al*., 1995; Seckin *et al*., 1999), and/or depletion of cofactors required for antioxidant defense (Graf *et al*., 1998).

The present study shows that the treatment of valproic acid-administered animals with onion oil and/or garlic oil prospered to produce potential increase of the total thiols and glutathione levels. The treatment of valproic acid-administered rats with onion oil and/or garlic oil

successfully caused potential alleviation of the GST, SOD and CAT activities. Helen *et al.* (1999) indicated that garlic and onion oils are effective antioxidants against the oxidative damage. Pedraza-Chaverri *et al*. (2000) reported that onion and garlic were effective in preventing or ameliorating oxidative stress. Prackash *et al.* (2006) revealed that onion is a rich source of polyphenols with promising antioxidant and free radical scavenging activities and has the ability to provide protection against DNA damage caused by reactive oxygen species.

Garlic oil is as effective as onion oil. Garlic has anti-oxidant effects, which means it can reduce toxicity associated free-radical damage and it contains the trace elements germanium and selenium, which have been thought to play a role in improving host immunity and 'normalizing' the oxygen utilization in cells. In addition, garlic compounds have been found to inhibit lipid peroxidation, which is considered one of the main features of aging in liver cells (Hebel *et al.,* 2001; Park *et al.,* 2005). MacDonald *et al*., (2004) reported that there are varieties of antioxidants in garlic, which protect against disease-causing oxidative damage. Garlic extracts increase antioxidant action by scavenging reactive oxygen species, enhancing the cellular antioxidant enzymes, glutathione peroxidase and increasing glutathione in the cells (Carmia, 2001).

In the current study, the administration of valproic acid induced a highly significant elevation of serum creatinine and uric acid levels. In contrast to serum creatinine and uric acid levels, serum urea concentration was decrease as a result of valproic acid administration. This decrease in serum urea level due to valproic acid administration is explained in different ways by many authors.

A metabolite (4-en-VPA) of valproic acid indirectly inhibits fatty acid oxidation resulting in the reduction of free coenzyme A (CoA) and acetyl CoA. Low acetyl CoA levels inhibit mitochondrial production of N-acetyl-glutamate synthetase, which is a required activator of CPS I, the first enzyme in the urea cycle, and thereby leads to an accumulation of ammonia in the blood (McCall and Bourgeois, 2004) and supperssion of the incorporation of ammonia into the urea cycle (Raby, 1997; Hamer *et al*., 2000; Feil *et al*., 2002). These drugs may inhibit N-acetylglutamate, an activator of CPSI that is, needed for the normal function of this enzyme (Champe and Harvey, 1994). They can also increase the concentration of pyruvate, a potent inhibitor of CPS I which prevents the incorporation of ammonia into the urea cycle (Lennkh and Simhandl, 2000).

Valproic acid inhibits intramitochondrial β-oxidation of long chain fatty acids leading to activation of the cytosolic ω-oxidation pathway and increased utilization of free CoA, acetyl-CoA synthase, and acetyl CoA by this pathway that makes these substances less available for use in the urea cycle, which becomes less efficient in converting ammonia to urea (Latour *et al*., 2004). All of these different pathways lead to a decrease in urea production and, as a result, an increase in ammonia concentrations.

The present study showed that valproic acid-administered rats exhibited a significant decrease in the kidney total thiols, glutathione, GPO, GST, SOD, CAT and peroxidase levels while there is a significant increase of the kidney lipid peroxidation content.

The metabolism of valproate may trigger oxygen dependent tissue injury and elevate the free radicals in the body (Cengiz *et al*., 2000). The free radicals generated cause a cascade of neurochemical events leading to cell degeneration and cell death (Gilgun-Sherki *et al*., 2002). Valproate, a widely used drug for the treatment of epilepsy enhances the clearance of selenium, copper and zinc, subsequently leading to decreased synthesis of free radical scavenging enzymes. It has been reported by some investigators that glutathione peroxidase decreases in epileptic children receiving valproate (Yüksel *et al*., 2001). Glutathione activity is also reported to be inhibited by valproate (Cotariu *et al*., 1992).

The administration of onion and/or garlic oils to valproic acid-treated rats produced a potential improvement of the deteriorated kidney antioxidant defense system indicated by increase of kidney total thiols and glutathione levels as well as glutathione peroxidase, glutathione-s-transferase, SOD, catalase and peroxidase activities. The kidney lipid peroxidation was decrease as a result of treatment with onion and/or garlic oils. These result are in concurrence with many publications (Mamdouh and Abdel-Raheim, 2003; Saravanan and Prakash, 2004; Popova and Popve, 2005; Abd Al-Ameer, 2011).

The present study showed that serum CK-MB, AST and LDH activities which are markers of heart function, was significantly increased as a result of the administration of valproic acid.

These results are in accordance with Custalow *et al*. (2001) and Daniels *et al*. (2004) who demonstrated that elevated CK-MB and myocardial damage which reflect VPA cardiotoxicity. Those authors proposed that the mechanism of cardiotoxicity was impairment of myocardial fuel oxidation.

In conclusion, valproic acid administration increases the formation of oxygen free radicals, reduces antioxidants and increases lipid peroxidation which leads to organ damage. Treatment with onion oil and/or garlic oil before and during VPA administration reduced the changes in oxidative stress markers. A possible protective effect of the three tested treatments on VPA-induced hepatotoxicity, nephrotoxicity and cardiotoxicity may be explained on the basis of oxidant-antioxidant system management. Thus, onion oil and/or garlic oil may act as a useful therapeutic agent in preventing toxicity which may occur during valproic acid treatment. However, further clinical studies are required to assess

the safety and the efficacy of these agents in human beings.

REFERENCES

Abd Al-Ameer HA (2011). The protective role of onion oil (*Allium cepa* L.) extract on some physiological parameters on streptozotocin induced diabetes in male mice. Kufa Journal for Veterenary Medical Sciences 2(1), 59-76.

Ahmed OM, Abdel-Reheim ES, Ashour MB, Abdel-Tawab SM (2007). Evaluation of anti-hyperglycemic effect of some medicinal plant ingredients and their mechanism on streptozotocin-induced diabetic rats. J. Egypt. Ger. Zool. 53A, 53-100

Andorfer JH, Tchaikovskaya T, Listowsky I (2004). Selective expression of glutathione S-transferase genes in the murine gastrointestinal tract in response to dietary organosulfur compounds. Carcinogenesis 25(3), 359-367.

Augusti KT, Narayanan A, Pillai LS, Ebrahim RS, Sivadasan R, Sindhu KR, Subha I, Abdeen S, Nair SS (2001). Beneficial effects of garlic (*Allium sativum* L.) on rats fed with diets containing cholesterol and either of the oil seeds, coconuts or groundnuts. Indian J. Exp. Bio. 39, 660-667.

Banerjea MC, Diener W, Kutschke G (2002). Pro-and anticoagulatory factors under sodium valproate therapy in children. Neuropediatrics 33, 215-220.

Bansal AK, Bansal M, Soni G, Bhatnagar D (2005). Protective role of Vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. Chem. Biol. Interact. 156, 101-111.

Baselt R (2008) Disposition of Toxic Drugs and Chemicals in Man, 8th edition, Biomedical Publications, Foster City, CA. 1622-1626.

Beutler E, Duren O, Kelly BM (1963). Improved method for thedetermination of blood glutathione. J. Lab. Clin. Med. 61, 882-888.

Candian Council on Animal Care (CCAC) (1993). Guide to the Care and Use of Experimental Animals, CCAC, Ottawa, Ontario, Canada, 1-298

Carmia B (2001). Antioxidant Health Effects of Aged Garlic Extract. J. Nutr. 131, 1010-1015.

Catarino CB, Liu JY, Liagkouras I (2011).Dravet syndrome as epileptic encephalopathy:Evidence from long-term course and neuropathology. Brain 134(10), 2982-3010.

Cengiz M, Yukcel A, Seven M (2000). The effects of carbamazepine and valproic acid on the erythrocyte glutathione, glutathione peroxidase, superoxide dismutase and serum lipid peroxidation in epileptic children. Pharmacol. Res. 41, 423–425.

Cengiz M, Yukcel A, Seven M (2000). The effects of carbamazepine and valproic acid on the erythrocyte glutathione, glutathione peroxidase, superoxide dismutase and serum lipid peroxidation in epileptic children. Pharmacol. Res. 41, 423–425.

Champe PC, Harvey RA (1994). Lippincott's Illustrated Reviews. Biochemistry, 2nd edition J. B. Lippincott, Philadelphia, Pa, USA.

Cohen G, Dembiec D, Marcus J (1970). Measurement of catalase activity in tissue. Anal. Biochem, 34, 30-38.

Cotariu D, Evan, S, Lahat E, Theitler J, Bistritzer T, Zaidman JL (1992). Inhibition of human red blood cell glutathione reductase by valproic acid. Biochem. Pharmacol. 43, 425–429.

Custalow BC, Watts JA, Thorrnton L, O'Malley PO, Barbee RW, Grattan RM (2001) Role of fatty acids in recovery of cardiac function during resuscitation from hemorrhagic shock. Shock 15, 231-238.

Daniels T, Gallanger M, Tremblay G, Rodgers (2004). Effects of valproic acid on cardiac metabolism. Can. J. Pharmacol.82, 927-933

EL-Shatter MA, Sobbhy MH, Shehab JG (1997). Some pharmacological and pathological studies on Allium sativum (garlic). AppliedVet. Med. J. 36, 264-283.

Erdoğan S.(2012).Massive creatine kinase and hepatic enzyme elevation due to quetiapine and valproic acid treatment: A case report. Archives of Neuropyschiatry 49, 238-240.

Feil D, Chuang K, Sultzer DL (2002).Valproateinduced hyperammonemia as a cause of altered mental status American Journal of Geriatric Psychiatry 10 (4), 476–478.

Felker BL, Sloan KL, Dominitz JA, Barnes RF (2003).The safety of valproic acid use for patients with hepatitis C infection. Am. J. Psychiatry. 160, 174–178.

Fossati P, Prencipe L, Berti G (1980). Use of 3,5-dichloro-2-hydroxy-benzenesulfonic acid/4-aminophenoazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin. Chem. 26, 227-231.

Fung EL, Tan VL, Ho CS (2003).Carnitine level in Chinese epileptic patients taking sodium valproate. Pediatr. Neurol. 28(1)1, 24-37.

Gedik N, Kabasakal L, Sehirli O, Ercan F, Sirvanci S, Keyer Uysal M, Sener G (2005). Long-term administration of aqueous garlic extract (AGE) alleviates liver fibrosis and oxidative damage induced by biliary obstruction in rats. Life Sci. 76, 593-606.

Gilgun-Sherki Y, Rosenbaum Z, Melamed E, Offen D (2002).Antioxidant therapy in acute central nervous system injury. Current state. Pharmacol. Rev. 54, 271–84.

Graf WD, Oleinik OE, Glauser TA, Maertens P, Eder DN, Pippenger CE (1998). Altered antioxidant enzyme activities in children with a serious adverse experience related to valproic acid therapy. Neuropediatrics, Aug. 29(4), 195-201.

Guyonnet D, Belloir C, Suschetet M, Siess MH, Le Bon AM (2002). Mechanisms of protection against aflatoxin B1 genotoxicity in rats treated by organosulfur compounds from garlic. Carcinogenesis. 23, 1335–1341.

Halliwell B, Gutteridge (2007). J. Free Radicals in Biology and Medicine. Oxford, UK. Oxford Univ.

Hamer HM, Knake S, Schomburg U, Rosenow F (2000). Valproateinduced hyperammonemic encephalopathy in the presence of topiramate. Neurology 54 (1), 230–232.

Hebel SK, Katstrup EK (2001). Drug Facts and Comparisons. St. Louis, Mo.

Helen A, Krishnakumar K, Vijayammal PL, Augusti KT (2000). Antioxidant effects of onion oil (*Allium cepa*. Linn) on the damages induced by nicotine in rats as compared to alpha-tocopherol. Toxicol.Lett. 116, 61-68.

Helen A, Rajasree CR, Krishakumar K, Augusti KT, Vijayammal PL (1999).Antioxidant role of oils isolated from garlic (*Allium sativum* Linn) and onion (*Allium cepa* Linn) on nicotine-induced Lipid peroxidation. Vet. Hum.Toxicol. 41(5), 316-319.

Henry TR (2003). The History of Valproate in Clinical Neuroscience. Psychopharmacol.Bulletin 37 (S2), 5–16.

Hoffman EJ (1997). Enzyme Inhibitors for Cancer Cell Metabolism: Townsend Letter for Doctors and Patients,114.

Hu X, Wang J, Dong W, Fang Q, Hu L, Liu C (2011). A meta-analysis of polycystic ovary syndrome in womentaking valproate for epilepsy. Epil. Res. 97 (1–2), 73–82

Johannessen CU, Johannessen SI (2003). Valproate. past, present and the future. CNS Drug Rev. 9, 199-216.

Kar M, Mishra D (1976).Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. Plant Physiol. 57, 315-319.

Koster JF, Biemond P, Swaak AJG (1986). Intracellular and extracellular sulpy-hydryl levels in rheumatoid arthritis. Ann. Rheum. Dis. 45, 44-46.

Kultima K, Nystrom AM, Scholz B, Gustafson AL, Dencker L, Stigson M (2004).Valproic acid teratogenicity: a toxicogenomics approach. Environmental Health Perspectives 112 (12), 1225–1235.

Kwak MK, Kim SG ,Kwak JY, Novak RF, Kim ND (1993). Inhibition of cytochrome P4502E1 expression by organosulfur compounds allylsulfide, allylmercaptan and allylmethyl-sulfide in rats. Biochem. Pharmacol., 47. 531–539.

Latour P, Biraben A, Polard E, Bentue-Ferrer D, Beauplet A, Tribut O, Allain H (2004). Drug induced encephalopathy in six epileptic patients.Topiramate? Valproate? or both? Hum Psychopharmacol. 19, 193-203.

Lennkh C, Simhandl C (2000). Current aspects of valproate in bipolar disorder. International Clinical Psychopharmacology 15 (1), 1–11.

Lheureux PE, Hantson P (2009).Carnitine in the treatment of val-proic acid-induced toxicity. Clin. Toxicol. (Phila). 47, 101-111.

MacDonald JA, Marchand ME, Langler RF (2004). Improving upon the in vitro biological activity of antithrombotic disulfides. Blood Coagul. Fibrinolysis 15, 447-450.
Mamdouh MA, Abdel-Raheim MA (2003). Oxidative stress in streptozotocin-induced diabetic rats: Effects of garlic oil and melatonin. Comparative Biochemistry and Physiology 135(A), 539-547.
Mannervik B, Gutenberg C (1981). Glutathione transferase (Human placenta). Meth. Enzymol. 77, 231-235.
Marklund S, Marklund G (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and convenient assay for superoxide dismutase. Eur. J. Biochem.. 47, 469-474.
Marsh WH, Fingerhut B, Kirsch E (1959). Adaptation of an alkaline phosphatase method for automatic colorimetric analysis. Clin. Chem. 5, 119-126.
Matkovics B, Kotorman M, Varga IS, Hai DQ, Varga C (1998). Oxidative stress in experimental diabetes induced by streptozotocin. Acta Physiol. Hung. 85, 29-38.
McCall M, Bourgeois JA (2004).Valproic acid-induced hyperammonemia. a case report, Journal of Clinical Psychopharmacology 24 (5), 521–526.
Murray RK, Granner DK, Mayes PA , Rodwell VW (1993).Catabolism of proteins and of amino acid nitrogen. In. Harper's Biochemistry, 23rd edn. A Lange Medical Book. Prentice-Hall International Limited, London, 293–302.
Naik SR, Panda VS (2007). Antioxidant and hepatoprotective effects of *Ginkgo biloba*phytosomes in carbon tetrachloride-induced liver injury in rodents. Liver Int. 27, 393-399.
Neuman MG, Shear NH, Jacobson-Brown PM, Katz GG, Neilson HK, Malkiewicz IM, Cameron RG, Abbot F (2001). CYP2E1 mediated modulation of valproic acid-induced hepatotoxicity. Clinical Biochemistry 34, 211–218.
Ogunmodede OS, Saalu LC, Ogunlade B, Akunna GG, Oyewopo AO (2012). An Evaluation of the Hypoglycemic, Antioxidant and Hepatoprotective Potentials of Onion (*Allium cepa* L.) on Alloxan-induced Diabetic Rabbits. International Journal of Pharmacology 8, 21-29.
Park EY, KI SH, Ko MS, Kim CW, Lee MH, Lee YS, Kim SG (2005). Garlic oil and DDB, comprised in a pharmaceutical composition for the treatment of patients with viral hepatitis, prevents acute liver injuries potentiated by glutathione deficiency in rats. ChemBiol Interact. 82-96.
PC-STAT (1985). One-way analysis of variance. Version 1A (C) copyright. The University of Georgia. Programs coded by Rao, M.,Blane, K. and Zonneberg, M. University of Georgia, USA.
Pedraza-Chaverri J, Maldonado PD, Medina-Campos ON, Olivares-Corichi IM, Granados-Silvestre MA, Hernandez- Pando R, Ibarra-Rubio ME (2000). Garlic ameliorates gentamicin nephrotoxicity. relation to antioxidant enzymes. Free Radi. Biol. and Med. 29, 602–611.
Pedraza-Chaverri J, Maldonado PD, Medina-Campos ON, Olivares-Corichi IM, Granados-Silvestre MA, Hernandez- Pando R, Ibarra-Rubio ME (2000). Garlic ameliorates gentamicin nephrotoxicity. relation to antioxidant enzymes. Free Radi. Biol. and Med. 29, 602–611.
Perucca E (2002). Pharmacological and therapeutic properties of valproate: A summary after 35 years of clinical experience. CNS Drugs 16, 695–714.
Philip GH, Reddy PM, Sridevi G (1995).Cypermethrin induced in vivo alterations in the carbohydrate of 34 freshwater fish Labeorohita. Ecotoxicol. Environ. Safety 31, 173-178.
Phillips A, Bullock T, Plant N (2003).Sodium Valproate induces apoptosis in the rat hepatoma cell line, FaO. Toxicol. 192, 219-227.
Popova MA, Popove CS (2005). Effect of chemical agents on some enzyme activities and on the stability of membrane structures. Bulg. J. Vet. Med. 8, 163-171.
Prackash D, Singh BN, Upadhyay G (2006). Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). 1-5.
Preuss HG, Jarrel ST, Scheckenbach R, Lieberman S, Anderson RA (1998). Comparative effects of *Chromium vanadium* and *Gymnemasylvestre* on sugar-induced blood pressure elevations in SHR. J. American Collage of Nutrition 17, 116-123.
Raby WN (1997).Carnitine for valproic acid-inducedhyperammonemia. Ame. J. Psych. 154 (8), 1168–1169.
Reed DJ (1990). Glutathione. Toxicological implications. Annu. Rev. Pharmacol. Toxicol. 30, 603–631.
Roa M, Blame M, Zonneberg M (1985).PC-STAT. One way analysis of variance: Version 1A (c) copyright. University of Georgia, USA.
Saravanan G, Prakash J (2004). Effect of garlic (allium sativum) on lipid peroxidation in experimental myocardial infarction in rats. J Ethanopharmacol. 94, 155-158.
Seckin S, Basaran-Kucukgergin C, Uysal M (1999). Effect of acute and chronic administration of sodium valproate on lipid peroxidation and antioxidant system in rat liver. Pharmacol. Toxicol. 85, 294–298.
Shephard MD, Mezzachi RD (1983). colorimetric method for the determination of serum urea concentration. Clin. Biochem. Revs. 4, 61-67.
Sherwin J (1984).Liver function. In. Kaplan LA, Pesce AJ, eds. Clin. Chem. Theo. Anal. Correl. St louis. Mosby, 420-438.
Sonmez FM, Demir E, Orem A, Yildirmis S, Orhan F, Aslan A, Toobas M (2006). Effect of antiepileptic drugs on plasma lipids, lipoprotein (a), and liver enzymes. J. Child. Neurol. 21, 70-74.
Tang W, Borel AG, Fujimiya T, Abbott FS (1995). Fluorinated analogues as mechanistic probes in valproic acid hepatotoxicity:Hepatic microvesicularsteatosis and glutathione status. Chem. Res. Toxicol. 8, 671–682.
Wang H, Xu DX, Lv JW, Ning H, Wei W (2007). Melatonin attenuates lipopolysaccharide (LPS)-induced apoptotic liver damage in D-galactosamine-sensitized mice. Toxicol. 237, 49-57.
Williams DC, Massey GV, Russell EC, Riley RS, Ben-Ezra J (Mar 2007). Translocation positive acute myeloid leukemia associated with valproic acid therapy. Pediatric Blood and Cancer 50 (3), 641–643.
Young DS (1990). Effects of drugs on clinical laboratory tests. AACC press, Washington D.C.
Young DS (1997). Effects of drugs on clinical laboratory tests. 3th ed.
Young DS (2001).Effects of disease on Clinical Lab. Tests, 4th edition, AACC.
Yüksel A, Cengiz M, Seven M, Ulutin T (Aug 2001).Changes in the antioxidant system in epileptic children receiving antiepileptic drugs: two-year prospective studies. J. Child. Neurol. 16(8), 603-606.
Zhang X, Yang F, Xu C, Liu W, Wen S, Xu Y (2008). Cytotoxicity evaluation of three pairs of hexabromocyclododecane (HBCD) enantiomers on Hep G2 cell. Toxicology *in Vitro* 22, 1520-1527.
Zneng W, Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. 49, 5165-5170.