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, 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 (Huetal., 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 (Zheng and Wang, 2001). Therefore, attention has been directed toward the development 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 Allium cepa 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 et al., 2004).

MATERIALS AND METHODS

Experimental Animals

Male albino rats (Rattus norvegicus) 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 infection. 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 Canadian committee for care and use of animals (Canadian 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 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.

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-heatamolysed 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 Diagnostics 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. Totalantioxidant levels in liver, kidney and heart homogenate were determined using the 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>ALT (U/L)</th>
<th>% change</th>
<th>AST (U/L)</th>
<th>% change</th>
<th>ALP (U/L)</th>
<th>% change</th>
<th>LDH (U/L)</th>
<th>% change</th>
<th>Bilirubin (mg/dl)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (G1)</td>
<td></td>
<td>7.83 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>13.00 ± 1.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>25.98 ± 2.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>482.91 ± 11.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>0.81 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>VPA(G2)</td>
<td></td>
<td>21.33 ± 2.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>172.41</td>
<td>43.75 ± 6.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>236.53</td>
<td>45.91 ± 3.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.71</td>
<td>1273.90 ± 56.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>164.00</td>
<td>1.67 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.17</td>
</tr>
<tr>
<td>Onion oil + VPA (G3)</td>
<td></td>
<td>9.16 ± 0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-57.70</td>
<td>10.50 ± 1.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-76.00</td>
<td>25.48 ± 2.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-44.50</td>
<td>676.28 ± 23.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-46.87</td>
<td>0.59 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-66.46</td>
</tr>
<tr>
<td>Garlic oil + VPA(G4)</td>
<td></td>
<td>9.50 ± 0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-55.46</td>
<td>13.01 ± 1.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-70.28</td>
<td>28.06 ± 1.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-38.88</td>
<td>466.50 ± 9.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-63.35</td>
<td>0.74 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-55.68</td>
</tr>
<tr>
<td>Onion oil + Garlic oil + VPA(G5)</td>
<td>5.66 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-73.46</td>
<td>8.50 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-80.57</td>
<td>23.28 ± 1.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-49.29</td>
<td>725.86 ± 8.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-42.98</td>
<td>0.44 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-73.65</td>
<td></td>
</tr>
<tr>
<td>F-Probability</td>
<td>P&lt;0.001</td>
<td>-</td>
<td></td>
<td>P&lt;0.001</td>
<td>-</td>
<td>P&lt;0.001</td>
<td>-</td>
<td>P&lt;0.001</td>
<td>-</td>
<td>P&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>LSD at the 5% level</td>
<td>4.445</td>
<td>-</td>
<td></td>
<td>8.501</td>
<td>-</td>
<td>3.562</td>
<td>-</td>
<td>82.460</td>
<td>-</td>
<td>0.229</td>
<td>-</td>
</tr>
<tr>
<td>LSD at the 1% level</td>
<td>6.014</td>
<td>-</td>
<td></td>
<td>11.501</td>
<td>-</td>
<td>3.637</td>
<td>-</td>
<td>111.592</td>
<td>-</td>
<td>0.310</td>
<td>-</td>
</tr>
</tbody>
</table>

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.

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 prospered to produce potential improvement (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 animals. The treatment of these animals with onion oil and/or garlic oil succeeded to produce potential amelioration (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 highly 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 thiol and glutathione contents and the activities of glutathione peroxidase and glutathione-S-transferase of valproic acid – administered rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Total thiol (nmol/100mg tissue)</th>
<th>% change</th>
<th>Glutathione (nmol/100mg tissue)</th>
<th>% change</th>
<th>Glutathione Peroxidase (U/100mg tissue)</th>
<th>% change</th>
<th>Glutathione-S-transferase (U/100mg tissue)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (G1)</td>
<td>189.10 ± 9.90</td>
<td>-</td>
<td>93.50 ± 6.76^</td>
<td>-</td>
<td>74.60 ± 2.80^</td>
<td>-</td>
<td>145.30 ± 7.37^</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>VPA (G2)</td>
<td>118.50 ± 2.13^</td>
<td>-37.57</td>
<td>68.00 ± 2.70^</td>
<td>-27.27</td>
<td>52.80 ± 2.00^</td>
<td>-29.72</td>
<td>8.50 ± 1.95^</td>
<td>-44.59</td>
</tr>
<tr>
<td></td>
<td>Onion oil + VPA (G3)</td>
<td>160.00 ± 10.60^</td>
<td>35.59</td>
<td>106.00 ± 9.42^</td>
<td>55.88</td>
<td>116.60 ± 1.80^</td>
<td>120.83</td>
<td>144.10 ± 7.58^</td>
<td>79.00</td>
</tr>
<tr>
<td></td>
<td>Garlic oil + VPA (G4)</td>
<td>200.50 ± 3.60^</td>
<td>69.49</td>
<td>93.00 ± 2.41^</td>
<td>36.76</td>
<td>103.10 ± 2.60^</td>
<td>94.69</td>
<td>154.90 ± 5.68^</td>
<td>92.42</td>
</tr>
<tr>
<td></td>
<td>Onion oil + Garlic oil + VPA (G5)</td>
<td>166.60 ± 6.14^</td>
<td>40.67</td>
<td>106.50 ± 2.78^</td>
<td>56.61</td>
<td>100.10 ± 2.20^</td>
<td>89.39</td>
<td>178.00 ± 2.45^</td>
<td>121.11</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Lipid peroxidation (nmol MDA/100 mg tissue /hr)</th>
<th>% change</th>
<th>SOD (U/g tissue)</th>
<th>% change</th>
<th>catalase (k.10^2)</th>
<th>% change</th>
<th>Peroxidase (U/g tissue)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (G1)</td>
<td>122.83 ± 11.06^</td>
<td>-</td>
<td>14.20 ± 0.72^</td>
<td>-</td>
<td>36.08 ± 1.0^</td>
<td>-</td>
<td>135.50 ± 7.78^</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>VPA (G2)</td>
<td>223.00 ± 18.41^</td>
<td>81.59</td>
<td>5.81 ± 0.69^</td>
<td>-59.08</td>
<td>17.08 ± 1.84^</td>
<td>-52.66</td>
<td>69.16 ± 5.02^</td>
<td>-48.95</td>
</tr>
<tr>
<td></td>
<td>Onion oil + VPA (G3)</td>
<td>130.66 ± 15.16^</td>
<td>-41.43</td>
<td>15.46 ± 1.84^</td>
<td>166.09</td>
<td>38.66 ± 4.95^</td>
<td>126.34</td>
<td>134.16 ± 8.71^</td>
<td>94.63</td>
</tr>
<tr>
<td></td>
<td>Garlic oil + VPA (G4)</td>
<td>134.16 ± 10.65^</td>
<td>-39.86</td>
<td>12.66 ± 1.11^</td>
<td>117.90</td>
<td>39.66 ± 3.11^</td>
<td>132.20</td>
<td>87.41 ± 2.54^</td>
<td>26.38</td>
</tr>
<tr>
<td></td>
<td>Onion oil + Garlic oil + VPA (G5)</td>
<td>107.50 ± 5.32^</td>
<td>-51.79</td>
<td>13.91 ± 1.59^</td>
<td>139.41</td>
<td>40.00 ± 2.25^</td>
<td>134.19</td>
<td>106.00 ± 6.4^</td>
<td>53.62</td>
</tr>
</tbody>
</table>

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 (94.83%) 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Creatinine (mg/dl)</th>
<th>% change</th>
<th>Urea (mg/dl)</th>
<th>% change</th>
<th>Uric acid (mg/dl)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (G1)</td>
<td></td>
<td>4.04 ± 0.34b</td>
<td>-</td>
<td>37.16 ± 2.86a</td>
<td>-</td>
<td>5.20 ± 0.82b</td>
<td>-</td>
</tr>
<tr>
<td>VPA G2</td>
<td></td>
<td>7.04 ± 0.35a</td>
<td>75.00</td>
<td>20.50 ± 2.72c</td>
<td>-44.83</td>
<td>7.26 ± 0.12a</td>
<td>39.60</td>
</tr>
<tr>
<td>Onion oil + VPA (G3)</td>
<td></td>
<td>3.83 ± 0.36b</td>
<td>-45.71</td>
<td>31.60 ± 1.50b</td>
<td>54.14</td>
<td>4.58 ± 0.28b</td>
<td>-36.88</td>
</tr>
<tr>
<td>Garlic oil + VPA (G4)</td>
<td></td>
<td>4.48 ± 0.2</td>
<td>-37.14</td>
<td>28.05 ± 2.36b</td>
<td>36.82</td>
<td>4.61 ± 0.21b</td>
<td>-36.50</td>
</tr>
<tr>
<td>Onion oil + Garlic oil + VPA (G5)</td>
<td></td>
<td>4.21 ± 0.16b</td>
<td>-40.00</td>
<td>28.71 ± 0.64b</td>
<td>40.04</td>
<td>4.48 ± 0.10b</td>
<td>-38.29</td>
</tr>
</tbody>
</table>

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 kidney total thiol and glutathione content and the activities of glutathione peroxidase and glutathione-S-transferase of valproic acid – administered rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Total thiol (nmol/100mg tissue)</th>
<th>% change</th>
<th>Glutathione (nmol/100mg tissue)</th>
<th>% change</th>
<th>Glutathione Peroxidase (U/100mg tissue)</th>
<th>% change</th>
<th>Glutathione-S-transferase (U/100mg tissue)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (G1)</td>
<td></td>
<td>201.80 ± 6.55b</td>
<td>-</td>
<td>101.10 ± 9.42b</td>
<td>-</td>
<td>160.10 ± 4.70b</td>
<td>-</td>
<td>174.83 ± 7.38b</td>
<td>-</td>
</tr>
<tr>
<td>VPA (G2)</td>
<td></td>
<td>129.80 ± 5.32c</td>
<td>-35.82</td>
<td>51.83 ± 4.91c</td>
<td>-48.71</td>
<td>121.00 ± 3.60c</td>
<td>-24.37</td>
<td>92.66 ± 4.92c</td>
<td>-46.99</td>
</tr>
<tr>
<td>Onion oil + VPA (G3)</td>
<td></td>
<td>210.30 ± 10.24c</td>
<td>62.79</td>
<td>73.81 ± 5.73b</td>
<td>42.47</td>
<td>172.60 ± 2.90b</td>
<td>42.14</td>
<td>164.33 ± 9.89c</td>
<td>77.34</td>
</tr>
<tr>
<td>Garlic oil + VPA (G4)</td>
<td></td>
<td>227.10 ± 9.83c</td>
<td>75.96</td>
<td>99.30 ± 9.42b</td>
<td>91.69</td>
<td>157.10 ± 6.10b</td>
<td>29.75</td>
<td>168.50 ± 13.66c</td>
<td>81.84</td>
</tr>
<tr>
<td>Onion oil + Garlic oil + VPA (G5)</td>
<td></td>
<td>227.50 ± 8.60c</td>
<td>75.97</td>
<td>99.11 ± 5.73b</td>
<td>91.31</td>
<td>153.30 ± 6.90b</td>
<td>26.44</td>
<td>163.00 ± 5.83c</td>
<td>75.91</td>
</tr>
</tbody>
</table>

F-Probability 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Lipid peroxidation (nmol MDA/100 mg tissue /hr)</th>
<th>% change</th>
<th>SOD (U/g tissue)</th>
<th>% change</th>
<th>catalase (k.10^6)</th>
<th>% change</th>
<th>Peroxidase (U/g tissue)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (G1)</td>
<td></td>
<td>138.33 ± 8.19b</td>
<td>-</td>
<td>14.25 ± 1.80a</td>
<td>-</td>
<td>21.20 ± 1.46b</td>
<td>-</td>
<td>142.16 ± 5.65b</td>
<td>-</td>
</tr>
<tr>
<td>VPA (G2)</td>
<td></td>
<td>175.33 ± 5.32a</td>
<td>26.81</td>
<td>3.45 ± 0.59c</td>
<td>-75.78</td>
<td>10.53 ± 0.36c</td>
<td>-50.33</td>
<td>79.33 ± 7.11c</td>
<td>-44.36</td>
</tr>
<tr>
<td>Onion oil + VPA (G3)</td>
<td></td>
<td>121.50 ± 3.58c</td>
<td>-30.57</td>
<td>7.91 ± 1.40c</td>
<td>192.27</td>
<td>27.91 ± 2.14a</td>
<td>165.05</td>
<td>114.83 ± 6.69c</td>
<td>45.31</td>
</tr>
</tbody>
</table>

F-Probability P < 0.001 - LSD at the 5% level 1.607 - 8.654 - 1.224 - LSD at the 1% level - - - - -
Table 6. Continue

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum CK-MB (U/L)</th>
<th>Percentage (%)</th>
<th>F-Probability</th>
<th>LSD at the 5% level</th>
<th>LSD at the 1% level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic oil + VPA (G4)</td>
<td>106.16± 3.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-39.42</td>
<td>P &lt; 0.001</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Onion oil + Garlic oil + VPA (G5)</td>
<td>142.33 ± 3.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-18.85</td>
<td>P &lt; 0.001</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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.

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 that valproic 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.

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

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

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

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.

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%).
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 infarction, 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).

The activities 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 et al., 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 allin 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. Prakash 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 antioxidant 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-acetylglutamate 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 suppression 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 results 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 injected diabetes in male mice. Kufa Journal for Veterinary Medical Sciences 2(1), 59-76.


