The present study was conducted to elucidate the possible protective effect of vitamin E and β-carotene in case of toxic effect of the ethyl alcohol extract of faba beans. 42 male albino rats divided into 7 equal groups of 6 rats each. Group 1: taken paraffin oil daily for 15 days. Groups 2,3 y 4: injected intra-peritoneal with 1/3LD₅₀ of faba beans ethyl alcohol extract for 15 days but groups 3 y 4: received either vitamin E or β-carotene orally at the protective dose 1 hour prior to extract injection. Groups 5,6,7: rats injected intra-peritoneal with diethyl maleate (5µl/rat) and after 1 hour injected with 1/3 LD₅₀ of faba beans ethyl alcohol extract for 15 days to induce favism, but groups 6 y 7: received vitamin E and β-carotene orally at the protective dose 1 hour prior to favism induction. The results in favism-induced group revealed significant decrease in blood parameters, serum glucose, serum and liver proteins, serum and liver AST, serum ALT, blood and liver GSH, serum and liver G6-PD, liver ALT, serum and liver TBARs, while treatment with the protective dose of vitamin E or β-carotene shift all the above parameters to be near the control value. In conclusion, the treatment with both antioxidants may provide a partial protection against the toxic effects induced by favism. This indicates that vitamin E or β-carotene may be capable of greatly modifying rodents’ susceptibility to blood toxicity and oxidative stress induced by favism.

Keywords: Favism, vitamin E, β-carotene, faba beans.
LIST OF ABBREVIATIONS


INTRODUCTION

Favism is an acute hemolytic anemia known to occur in glucose 6-phosphate dehydrogenase (G6-PD) deficient individuals after intake of faba beans (McMillan and Jollow, 1999). After eating large amounts of fresh faba beans there was a blood in the urine, headache, dizziness, fatigue, loss of appetite, and jaundice in the eyes and hemolytic anemia, hyper-bilirubinemia, and G6-PD deficiency in 8-year-old male patient (Bicakci, 2009). The favic crisis begins several hours after ingestion of raw or cooked beans and can persist for several days. The clinical condition is characterized by a variety of symptoms including fever, free hemoglobin in the blood and urine, icterus, enlarged spleen, elevated reticulocyte counts and renal failure (Arese et al., 1989). Faba beans when extracted by ethyl alcohol contain high amounts (up to 6.7 g/100 g dry weight) of favism inducing factors (vicine and convicine). Their active glycogens (divicine and isouramil) are suspected to play a role in hemolysis (McMillan and Jollow, 1999). Upon hydrolysis of the inactive glycosides vicine and convicine, reduced divicine and isouramil are formed by the action of micro-flora in the gastrointestinal tract and are absorbed by the blood (Frohlich and Marquardt, 1983; Hegazy and Marquardt, 1984). The causative agents’ divicine and isouramil are highly reactive compounds generating free radicals (Arbid and Marquardt, 1986) which interact with the abundant supply of oxygen in the blood to produce super-oxide radicals which cause cell damage and lead to favism-like signs (Albano et al., 1984 and Marquardt, 1989). So, it is important to balance the ratio of antioxidants to oxidants by supplementation or cell induction of antioxidants (Gate et al., 1999). The use of the cleavage amplified polymorphism (CAP) markers can be efficiently used in breeding selection to track the introgression of the vicine and convicine- (vc-) allele to develop cultivars with low vicine and convicine (v-c) content and improved nutritional value of faba beans (Gutierrez et al., 2006). Certain antioxidant can protect against the toxic effects of favism-inducing factors. (Corash et al., 1980; Corash et al., 1982; Arbid et al., 2000) reported that vitamin E supplementation of glucose-6-phosphate dehydrogenase (G6-PD)-deficient individuals’ decreased chronic hemolysis. Vitamin E deficiency, which occurs in certain pathological conditions, is accompanied by increased concentration of malonaldehyde (in vitro study) and increased per-oxidative damage (in vivo study) which is the two factors responsible for short life span of red cells.

The aim of the present work is an attempt to test the protective effect of vitamin E or β-carotene free-radical scavengers which would quench the free radicals produced by induction of favism by intraperitoneal injection of the alcoholic extract of faba beans thereby neutralized its per oxidative damage in glutathione (GSH) depleted G6-PD normal albino rats.

MATERIALS AND METHODS

Tested Compound

a. Alcoholic extract of Vicia faba was prepared using Vicia faba Rebaya 40, which was obtained from the legume section, department of Agricultural, Ministry of Agricultural of Egypt.

b. Depletive material: Diethyl maleate
(C₈H₁₂O₄) was used as a depletive agent for Blood glutathione (GSH) in rats under experiment. It was obtained from E-Merk Company, Egypt (1L=1.07 kg, molecular weight (M)=173.18 g/mol (Jahn, 1982 and Uthus, 1992).

The antioxidants: 1-Vitamin E” α-tocopherol acetate” in the oily form, supplied by pharco Pharmaceutical Co., Egypt (each capsule contained 100 mg vitamin E). 2- β-carotene: in oily form as soft gels, supplied by Arab Co. for Pharm.& Medicinal Plants, Egypt (each gel contained 15 mg natural β-carotene).

**Determination of LD₅₀ of the extracts**
The LD₅₀ of the extract was determined in mg/100 g body weight for adult albino rats according to the equation of Behren and Karber (1953).

**Preparation of alcoholic extract**
Extraction was carried-out (El-Shabrawy, 1971) using 70% ethyl alcohol, as it is the most satisfactory solvent for extraction of both alcohol and water constituents of the sample.

**Technique for Hematological Parameters.**
Red and White blood cells (RBCs and WBCs), as well as, Hematocrit value (Hct.) were carried-out (Rodak, 1959) using hemocytometer.

**Diagnostic kits**
The entire reagent kits used was for rat serum. Hemoglobin (Hb) concentration was determined (Van Kampen and Zijlstra, 1961) using hemoglobin test kits (bio-merieux). Erythrocyte and Liver glutathione concentrations were determined (Beutler et al., 1963). Determination of Protein Content in serum and liver were estimated (Gornall et al., 1949) by using protein kits (Egyptian American Company for laboratory services, Egypt). Serum Albumin was estimated (Henery, 1968) by using kits of bio-analytcs laboratory reagents and products, Egypt. Serum globulin was calculated by subtracting the individual data of serum albumin from the individual data of serum total protein. Aspartate and alanine amino-transferase (AST y ALT) activities in serum and liver tissue were estimated (Reitman and Frankel, 1957) colorimetrical using Quimica clinical kits, Spain. Serum glucose was measured (Trinder, 1969) using the kits of bio-Analytics laboratory reagents and products, Egypt. Lipid per-oxidation [thiobarbituric acid reactive substances (TBARs)] is estimated in serum and liver tissue (Yoshioka et al., 1979). Glucose 6-phosphate dehydrogenase (G6-PD) activity in serum and liver tissue was estimated (Lohr and Waller, 1974) using kits from Sigma.

**Experimental design**
A total number of 42 male albino rats (Sprague Dawley) from Animal house laboratory, National Research Center, Dokki, Egypt. All animals were maintained on a balanced diet and were offered drinking water ad.libitum. The experiments were carried-out in accordance with the national regulations on animal welfare and Institutional Animal Ethical Committee (IAEC), “National Research Centre (NRC), Cairo – Egypt” (Permission no.186). Rats were allocated randomly into 7 groups of 6 rats each. **Group 1:** taken paraffin oil (0.25 ml/rat) daily by using stomach tube for 15 days. **Group 2:** injected intra-peritoneal with 1/3LD₅₀ of alcoholic extract of faba beans daily for 15 days. **Group 3:** received vitamin E orally at the protective dose 100 mg/kg body weight, (Mustafa et al., 1999) then injected intra-peritoneal after 1 hour with 1/3LD₅₀ of the alcoholic extract of faba beans daily for 15 days. **Group 4:** Rats received β-carotene orally at the protective dose 70 mg/kg body weight, (Ibrahim et al., 2008) then injected intra-peritoneal after 1 hour with 1/3LD₅₀ of the alcoholic extract of faba beans daily for 15 days. **Group 5:** rats injected intra-peritoneal with diethyl ma-
leate at a dose of 5μl/rat and after 1 hour injected with 1/3 LD$_{50}$ of faba beans alcoholic extract for 15 days. This was to induce favic-like symptoms when administrated to G6-PD-normal rats (favism-induced group). **Group 6**: rats received vitamin E orally, then injected intra-peritoneal after 1 hour with diethyl maleate at a dose of 5μl/rat and after 1 hour of maleate treatment, injected with 1/3 LD$_{50}$ of faba beans alcoholic extract for 15 days. **Group 7**: rats received β-carotene orally, then injected intra-peritoneal after 1 hour with diethyl maleate at a dose of 5μl/rat and after 1 hour of maleate treatment, injected with 1/3 LD$_{50}$ of faba beans alcoholic extract for 15 days.

**Blood Sampling and Handling**

Blood samples were collected from retro-orbital plexus of rats using capillary tubes (Schermer, 1967) into clean centrifuge tubes. Part of blood sample was collected on EDTA as anticoagulant for blood parameters [Hemoglobin content, hematocrit (Hct.) value, red blood cells (RBCs), white blood cells (WBCs) counts and glutathione peroxidase (GPx)]. The other part of the blood sample was allowed to coagulate and centrifuged at 4000 r.p.m. for 15 minutes to separate blood serum. Separated serum was stored at -20°C for the determination of liver function aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin and globulin were measured also in serum.

**Prepared of homogenate liver tissue**

After collection of the blood, the animals were decapitated and then dissected whereby the liver was obtained, washed in cold saline and dried between filter papers. The liver was weighed, homogenized and kept at -20°C for further investigation. 0.5 gm of liver tissue was dissolved in 2.5 ml of tris buffer solution, then homogenate in the homogenizer for exactly 30 minutes. Then centrifuge for exactly 20 minutes at 7,000 r.p.m then separate the supernatant, which proceed in the same manner of blood serum.

**STATISTICAL ANALYSIS**

Data were evaluated with SPSS/10 software. Comparisons of multiple groups were done with ANOVA with corrections for multiple comparisons. In vivo changes were analyzed between-group repeated-measures ANOVA and Data were tabulated. Results were expressed as mean ± SD and significant difference according to negative positive control groups at P<0.05.

**RESULTS**

The procedure of the study was gone in two parallel directions:-

**First**: Determination of the antioxidant protective effect of both vitamin E and β-carotene against 1/3 LD$_{50}$ of ethyl alcoholic extract of faba beans.

**Second**: Evaluation of antioxidant protective effect of both vitamin E and β-carotene against favism-induced group [diethyl maleate (5μl/rat) then taken 1/3 LD$_{50}$ of ethyl alcoholic extract of faba beans].

Faba beans alcoholic extract has been observed to be toxic when injected (i.p) at high doses which may lead to death after 5 minutes of injection, so the LD$_{50}$ of the extract must be determined to get the appropriate dose that can induce favic like-symptoms, in G6-PD normal rats. The results of the experiment concerning the determination of the intra-peritoneal LD$_{50}$ of the alcoholic extract of faba beans for male albino rats are presented in table 1.

The investigation of the data found in table 2 showed that 1/3 LD$_{50}$ of the ethyl alcohol extract of faba beans are responsible for significant decrease (P<0.05) in hematocrit (Hct.) value, serum ALT, albumin, serum and liver glucose 6-phosphate dehydrogenase (G6-PD) while, liver
The Protective Effect of Some Antioxidants against the Toxic Effect

**Table 1.** Data used for the determination of LD$_{50}$ of the alcoholic extract of faba beans on male albino rats by intra-peritoneal injection (Data were collected 96 hours after injection).

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Dose (mg/100g B.wt.)</th>
<th>No. of rats</th>
<th>No. of dead</th>
<th>d.</th>
<th>Z.</th>
<th>Z.d</th>
<th>Mortality rate</th>
<th>LD$_{50}$ (mg/100g B.wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>287.5</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>200</td>
<td>6</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>IV</td>
<td>250</td>
<td>6</td>
<td>3</td>
<td>50</td>
<td>2</td>
<td>150</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>V</td>
<td>400</td>
<td>6</td>
<td>4</td>
<td>150</td>
<td>3.5</td>
<td>525</td>
<td>0</td>
<td>66.6</td>
</tr>
<tr>
<td>VI</td>
<td>500</td>
<td>6</td>
<td>6</td>
<td>100</td>
<td>5</td>
<td>500</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

ALT showed significant increase (P<0.05) but highly significant decrease (P<0.01) in serum AST and highly significant increase (P<0.01) in serum total protein, on the other hand very highly significant increase (P<0.001) in serum globulin, serum and liver thiobarbituric acid reactive substance (TBARs).

The receiving of vitamin E prior to alcoholic extract administration (table 2) induced significant increase (P<0.05) in hemoglobin (Hb.) concentration, serum and liver AST, serum total protein while significant decrease (P<0.05) in serum and liver glucose 6-phosphate dehydrogenase (G6-PD). Moreover, highly significant de-

**Table 2.** Blood parameters, serum glucose, liver function and Antioxidants of rats treated with ethyl alcohol extract, Vitamin E+ ethyl alcohol extract and β-Carotene+ ethyl alcohol extract.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (oil) (-ve Control)</th>
<th>Extract (+ve Control)</th>
<th>Vitamin E + Extract</th>
<th>β-carotene + Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb. (mg/dl)</td>
<td>14.6±0.3</td>
<td>12.9±1.6</td>
<td>15.4±0.9*</td>
<td>14.0±0.7</td>
</tr>
<tr>
<td>Hct. (%)</td>
<td>43.3±1.2</td>
<td>38.9±3.2</td>
<td>42.5±1.3</td>
<td>41.5±1.9</td>
</tr>
<tr>
<td>RBCs (106/mm3)</td>
<td>5.3±0.3</td>
<td>5.2±0.8</td>
<td>5.7±0.2</td>
<td>5.0±0.6</td>
</tr>
<tr>
<td>WBCs (103/mm3)</td>
<td>9.4±0.6</td>
<td>8.4±2.5</td>
<td>6.6±0.8**</td>
<td>8.7±1.5</td>
</tr>
<tr>
<td>Serum glucose (mg/dl)</td>
<td>102±14.5</td>
<td>103±11.7</td>
<td>133±15.4*</td>
<td>124±19.0**</td>
</tr>
<tr>
<td>Serum AST (u/l)</td>
<td>18.0±4.6</td>
<td>6.0±0.9</td>
<td>15.7±1.2</td>
<td>28.7±4.1***</td>
</tr>
<tr>
<td>Serum ALT (u/l)</td>
<td>8.8±3.6</td>
<td>3.5±1.6</td>
<td>14.0±0.9*</td>
<td>8.7±2.3</td>
</tr>
<tr>
<td>Liver AST (u/g of wet tissue)</td>
<td>44.2±12.3</td>
<td>41.7±9.8</td>
<td>71.3±9.5*</td>
<td>61.5±3.6*</td>
</tr>
<tr>
<td>Liver ALT (u/g of wet tissue)</td>
<td>50.5±3.3</td>
<td>73.3±15.1</td>
<td>80.0±8.9**</td>
<td>53.7±3.9*</td>
</tr>
<tr>
<td>Serum Total Protein (g/dl)</td>
<td>8.4±0.4</td>
<td>10.4±0.4</td>
<td>6.6±1.0*</td>
<td>6.4±0.7**</td>
</tr>
<tr>
<td>Serum Albumin (g/dl)</td>
<td>4.9±0.5</td>
<td>3.9±0.6</td>
<td>4.4±0.4</td>
<td>3.7±1.0</td>
</tr>
<tr>
<td>Serum Globulin (g/dl)</td>
<td>3.5±0.3</td>
<td>6.5±0.5</td>
<td>2.2±0.7**</td>
<td>2.7±1.3</td>
</tr>
<tr>
<td>Liver Protein (u/g of wet tissue)</td>
<td>2.9±0.78</td>
<td>2.4±0.5</td>
<td>1.4±0.1**</td>
<td>3.5±0.3**</td>
</tr>
<tr>
<td>Blood GSH (mg/dl)</td>
<td>34.8±1.6</td>
<td>33.4±3.4</td>
<td>33.9±4.6</td>
<td>28.7±2.0**</td>
</tr>
<tr>
<td>Liver GSH (mg/g of wet tissue)</td>
<td>14.2±1.4</td>
<td>15.3±2.7</td>
<td>14.6±4.5</td>
<td>13.2±4.1**</td>
</tr>
<tr>
<td>Serum TBARs (nmol/l)</td>
<td>15.7±2.4</td>
<td>54.1±5.2</td>
<td>35.9±2.6**</td>
<td>31.0±1.8**</td>
</tr>
<tr>
<td>Liver TBARs (nmol/g of wet tissue)</td>
<td>166±19.8</td>
<td>281±20.5</td>
<td>254±33.7**</td>
<td>238±10.2***</td>
</tr>
<tr>
<td>Serum G6-PD (u/l)</td>
<td>31.4±4.9</td>
<td>21.8±2.2</td>
<td>22.9±2.4**</td>
<td>30.3±4.3*</td>
</tr>
<tr>
<td>Liver G6-PD (u/g of wet tissue)</td>
<td>116±29.4</td>
<td>66±10.4</td>
<td>79±6.8*</td>
<td>68±6.5*</td>
</tr>
</tbody>
</table>

Results were expressed as mean ± SD and significant difference according to control group at P<0.05, ANOVA showed a highly significant difference between all groups at P<0.0001.*P<0.05 Significant difference compared to control, **P<0.01 Moderate significant differences compared to control, ***P<0.001 High significant Differences compared to control.
crease (P<0.01) in white blood cells (WBCs), serum globulin and liver total protein but significant increase (P<0.01) in liver ALT and TBARs, in addition, very highly significant increase (P<0.001) in serum (TBARs) compared to alcoholic extract group.

The receiving of β-carotene prior to alcoholic extract administration (table 2) caused significant increase (P<0.05) in serum ALT, liver glutathione (GSH) but highly significant decrease (P<0.01) in serum AST and highly significant increase (P<0.01) in serum and liver TBARs compared to alcoholic extract group.

The analysis of the data found in table 3 revealed that in favism group a significant decrease (P<0.05) in Hb. concentration, Hct. value, serum glucose, ALT, liver protein, while a highly significant decrease (P<0.01) in serum AST, protein, globulin and serum G6-PD and a highly significant increase (P<0.01) in liver AST and ALT. But very highly significant decrease (P<0.001) in red blood cells count, liver GSH and G6-PD and highly significant decrease (P<0.01) in liver GSH. On the other hand, highly significant increase (P<0.01) in blood GSH and very highly significant decrease (P<0.001) in serum and liver TBARs compared to control groups.

The administration with vitamin E prior to favism-induced group (table 3) exhibited significant increase (P<0.05) in Hb. concentration, Hct. value, serum AST, protein, liver GSH and G6-PD, but, revealed significant decrease (P<0.05) in serum ALT and albumin. A highly significant decrease (P<0.01) was observed in blood GSH but, very highly significant decrease (P<0.001) in serum and liver TBARs compared to favism-induced group.

The receiving of β-carotene prior to favism-induced group (table 3) caused

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (oil) (-ve Control)</th>
<th>Favism group (+ve Control)</th>
<th>Vitamin E + Favism</th>
<th>β-carotene + Favism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb. (mg/dl)</td>
<td>14.6±0.3</td>
<td>11.0±2.5</td>
<td>13.5±0.5*</td>
<td>14.0±0.7</td>
</tr>
<tr>
<td>Hct. (%)</td>
<td>43.3±1.2</td>
<td>32.0±6.7</td>
<td>39.9±2.7*</td>
<td>41.5±1.9</td>
</tr>
<tr>
<td>RBCs (106 cell/mm3)</td>
<td>5.3±0.3</td>
<td>4.0±0.1</td>
<td>4.6±0.8</td>
<td>5.0±0.6</td>
</tr>
<tr>
<td>WBCs (103 cell/mm3)</td>
<td>9.4±0.6</td>
<td>8.7±1.3</td>
<td>10.8±2.8</td>
<td>8.7±1.5</td>
</tr>
<tr>
<td>Serum glucose (mg/dl)</td>
<td>102±14.6</td>
<td>82±5.8</td>
<td>102±15.4</td>
<td>124±19.0</td>
</tr>
<tr>
<td>Serum AST (u/l)</td>
<td>18.0±4.6</td>
<td>3.5±1.3</td>
<td>26.0±3.0*</td>
<td>28.7±4.1*</td>
</tr>
<tr>
<td>Serum ALT (u/l)</td>
<td>8.8±3.6</td>
<td>3.0±1.3</td>
<td>7.7±1.5</td>
<td>8.7±2.3</td>
</tr>
<tr>
<td>Liver AST (u/g of wet tissue)</td>
<td>44.2±12.3</td>
<td>87.8±2.9</td>
<td>50.0±8.9</td>
<td>61.5±3.6*</td>
</tr>
<tr>
<td>Liver ALT (u/g of wet tissue)</td>
<td>50.5±3.3</td>
<td>74.0±17.7</td>
<td>62.5±6.7*</td>
<td>53.7±3.9*</td>
</tr>
<tr>
<td>Serum protein (mg/dl)</td>
<td>8.4±0.4</td>
<td>6.2±0.5</td>
<td>6.5±1.0*</td>
<td>6.4±0.7**</td>
</tr>
<tr>
<td>Serum albumin (mg/dl)</td>
<td>4.9±0.5</td>
<td>5.0±0.6</td>
<td>3.5±0.7*</td>
<td>3.7±1.0</td>
</tr>
<tr>
<td>Serum globulin (mg/dl)</td>
<td>3.5±0.3</td>
<td>1.2±0.9</td>
<td>3.0±0.8</td>
<td>2.7±1.3</td>
</tr>
<tr>
<td>Liver protein (mg/g of wet tissue)</td>
<td>2.9±0.8</td>
<td>1.4±0.5</td>
<td>2.2±0.3</td>
<td>3.5±0.3</td>
</tr>
<tr>
<td>Blood GSH (mg/dl)</td>
<td>34.8±1.6</td>
<td>20.8±3.8</td>
<td>24.2±2.7**</td>
<td>28.7±2.0**</td>
</tr>
<tr>
<td>Liver GSH (mg/g of wet tissue)</td>
<td>14.2±1.4</td>
<td>4.5±0.6</td>
<td>9.7±3.2*</td>
<td>13.2±4.1</td>
</tr>
<tr>
<td>Serum TBARs (nmol/dl)</td>
<td>15.7±2.4</td>
<td>65.2±3.6</td>
<td>33.6±3.2***</td>
<td>31.0±1.8***</td>
</tr>
<tr>
<td>Liver TBARs (nmol/g of wet tissue)</td>
<td>166±19.8</td>
<td>351±19.8</td>
<td>248±10.7***</td>
<td>238±10.2***</td>
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<tr>
<td>Serum G6-PD (u/l)</td>
<td>31.4±4.9</td>
<td>16.7±2.4</td>
<td>27.1±6.0</td>
<td>30.3±4.3</td>
</tr>
<tr>
<td>Liver G6-PD (u/g of wet tissue)</td>
<td>116±29.4</td>
<td>56±4.6</td>
<td>66±15.3*</td>
<td>68±6.5*</td>
</tr>
</tbody>
</table>

Results were expressed as mean ± SD and significant difference according to control group at P<0.05, ANOVA showed a highly significant difference between all groups at P<0.0001.*P<0.05 Significant difference compared to control, **P<0.01 Moderate significant differences compared to control, ***P<0.001 High significant Differences compared to control.
significant increase (P<0.05) in serum AST, liver G6-PD, serum protein but highly significant increase (P<0.01) in blood GSH and highly significant decrease (P<0.01) in liver TBARs and finally, a very highly significant decrease (P<0.001) in serum TBARs compared to favism-induced group.

**DISCUSSION**

The metabolic disease favism is an acute hemolytic anemia. It has been recognized sine antiquity. This disease may be fatal, usually occurs in children and is related to a genetic deficiency of glucose-6-phosphate dehydrogenase (G6-PD) in erythrocytes. It has been related to the consumption of faba beans. Results of the present investigation revealed that hemoglobin (Hb.) content, hematocrit (Hct.) Value and red blood cells (RBCs) count were significantly decreased in favism-induced group. The reduction in the three blood parameters attributed to the hemo-toxicity of β-glycosides found in the alcoholic extract of faba beans which resulted from removal of damaged red cells by the spleen (McMillan and Jollow, 1999). This reduction was observed in divicine and vicine injected rats and in G6-PD deficient children (Arbid et al., 2000; El-Sayed, 2000; Mohamed, 2004). The decrease in RBCs count was owing to conversion of hemoglobin into hemoglobin-peroxide complex (ferryl species) and methemoglobin as a result of a fall in the concentration of erythrocyte GSH below a certain critical level (Arbid and Marquardt, 1986; Marquardt, 1989). Regarding the protective effect of vitamin E or β-carotene against induced favism, results showed an increase in the hematological parameters above the normal level and the dietary vitamin E decreased tissue peroxidize ability, prevented incidence of myo-degeneration, and reduced erythrocyte hemolytic stress (Arbid et al., 2000; El-Sayed, 2000; Mohamed, 2004).

The present study showed a decrease in white blood cells (WBCs) count in favism-induced group. This decrease in leukocytes counts attributed mainly to a decrease in lymphocytes. This may be due to direct action of the two compounds (vicine and divicine) or their glycogens (divicine and isouramil) on the lymphatic tissue (Kaneko, 1980). The reduction in WBCs count after injection of vicine, convicine and divicine in male albino rats was reported by many authors (Arbid and Marquardt, 1986; Yannai and Marquardt, 1985). With respect to the protective effect of vitamin E and β-carotene against favism, results showed that the decrease in WBCs count that was observed in favism group is persisted in group received the protective dose of β-carotene before incidence of favism, while those received the protective dose of vitamin E showed an increase in WBCs compared to control rats. Such finding is in agreement with that recorded a significant increase in WBCs count in rats received a protective dose of vitamin E against radiation and attributed that increase to the effect of vitamin E on hemopiotic system, where Vitamin E significantly increase platelets counts and total leucocytes count (TLC) while, Vitamin E pretreatment improved the morphology of the red blood cells (Garg et al., 2008; Cay and Naziroglu, 1999).

Serum glucose level showed a significant decrease in favism-induced group. These findings were observed in rats injected with convicine (Arbid and Marquardt, 1986). They attributed this reduction to the impairment of liver function which decreased production of glucose, altered production of glycogenic hormones including insulin and glucagon hormones or damage to kid-
nary tubules resulting in reduced recovery of glucose from the blood during glomeruli filtration (Cay and Naziroglu, 1999). Regarding the protective doses of vitamin E or β-carotene increased the level of serum glucose to reach the normal level in case of vitamin E, while β-carotene showed a slight increase compared to control group. These findings were in agreement with that recorded an increase in the level of serum glucose in rats received vitamin E as protector against radiation (Garg et al., 2008). In contrast, many authors (Naziroglu, 1999; Mikkelsen et al., 2009; Catal and Bolkent, 2008) observed that blood glucose levels were decreased in diabetic humans and rats by administration of vitamin E, where combined administration of Vitamin E and C lowered inflammation and improved insulin action through a rise in non-oxidative glucose metabolism.

Liver enzymes (AST and ALT) were highly significantly decreased in favism-induced group, while their activity in the hepatic tissue showed a significant increase but serum and liver protein in addition to globulin exhibited highly significant decrease, while serum albumin showed a trend almost around the control level. This decrease in the levels of AST and ALT may be correlated with the decrease in the level of serum and hepatic total protein, where biosynthesis of protein in favism group was decreased. This decrease in protein and albumin levels explained by liver injury is associated with decreased albumin level secondary to decreased protein synthesis and increased globulin level due to deteriorated hepatic activity while the decline in serum albumin attributed to enhanced degradation and loss of albumin through the gastrointestinal tract (Marquardt et al., 1997 and Rosengren et al., 1995). Supplementation with the protective dose of vitamin E or β-carotene was associated with a slight increase in the activity of serum AST and ALT. However, their activity in the hepatic tissue was retained around the normal control value especially in case of β-carotene treatment. But, serum protein showed a little improvement in the antioxidant pre-treated groups especially in vitamin E compared to control group. The increase in the AST and ALT activity in rats pretreated with the protective dose of vitamin E attributed to vitamin E toxicity that induce damage in hepatic cells and enzymatic release to the blood (Comporti, 1985). On the other hand, when these enzyme activities were decreased in the hepatic tissue and these explained by being protein in nature, so these enzymes were exposed to oxidative de-naturation, decreased protein synthesis by the hepatic cells and the release of the enzymes from the injured cells to the blood (Abbady, 1994). The protective dose of vitamin E against the toxicity of CCl₄ and γ-radiation, respectively resulted in a decrease in the serum levels of AST and ALT (Ibrahim et al., 2008 and Garg et al., 2008). In the case of carotinaemia (patients with excessive beta-carotene through food-intake), blood samples showed a significant increased in serum beta-carotene, but normal liver enzymes, where the supplementation with β-carotene in combination with selenium, ascorbic acid, and α-tocopherol help to prevent the development of liver injury and increased the level of serum protein to reach the normal range but albumin levels were decreased in male rats received vitamin E as protectors against radiation (Yanardag et al., 2001 and Marquardt et al., 1997).

A highly significant decrease was observed in blood and hepatic tissue levels of glutathione (GSH) while highly significant increase in the level of lipid per-oxidation malondialdehyde (MDA) or thiobarbituric acid reactant substance (TBARs) in serum and liver tissue in favism-induced group. The decrease in GSH level by faba beans due anti-nutritional factors (vicine and convicine) that participate in the oxidation-reduction shuttle system. In early stages of oxidative stress the endogenous antiox-
dant (GSH) was stimulated to combat the increased free radicals production. By time when oxidative stress becomes chronic, the endogenous antioxidants are consumed and depleted. The oxidation of GSH to GSSG was accelerated in the presence of isouramil and divicine (Olaboro et al., 1981; Yu and Paetau Robinson 2006). On the other hand, a marked significant increase in the level of blood GSH of G6-PD deficient patients (Mohamed, 2004). The glycogens of vicine and convicine produce free radicals, which not only react with erythrocyte but also with other membranes and tissues to cause tissue damage and loss of functional properties and there is an increase in the plasma lipid peroxide concentration in hens fed faba beans β-glycosides. This increase was a degree of spontaneous hemolysis of the erythrocyte (Yu and Paetau-Robinson, 2006 and Subudhi et al., 2009). With respect to the protective doses of vitamin E or β-carotene the present study showed that an increase in the level of glutathione in blood and liver tissue especially in rats received the protective dose of β-carotene but reduced the level of lipid peroxidation in serum and hepatic tissue but not to the control value. These results were in agreement with many authors (Arbid et al., 2000; El-Sayed, 2000; El-Habibi., 2000; Ozden et al., 2009) where vitamin E provides a complete protection against the toxic effect of divicine by its great power to modify individual susceptibility to divicine or favism, where the dietary supplements of vitamins E and beta-carotene reduced oxidative stress in cats with renal insufficiency disease. On the contrary, the supplementation with vitamin E enhances oxidative stress parameters and increases the plasma level of TBARS and lipid hydro-peroxides associated with a decrease in vitamin E after administration with alcoholic leaf-extract of faba beans (El-Habibi, 2000; Ozden et al., 2009; Subudhi et al., 2009; Pushpavalli et al., 2009).

The present study indicated a highly significant decrease in the activity of serum and liver glucose-6-phosphatase dehydrogenase (G6-PD) in favism-induced group as compared to control group. This reduction attributed to a compensatory response to oxidative stress to maintain sufficient levels of NADPH in response to an oxidative stress (El-Sayed, 2003). These results were in agreement with that showed significant decrease in the enzyme activity of G6-PD among children suffering from G6-PD deficiency (Mohamed, 2004 and Pushpavallati et al., 2009). The treatment with vitamin E or β-carotene as protectors against the toxicity of faba beans extract, showed marked increase in the activity of serum G6-PD, while its activity in the hepatic tissue showed slight increase. Such observation was coincides with that found G6-PD activity significantly elevated as response to oxidative stress of iron-load rats, El-Sayed (2003) explained this increase in G6-PD activity as response to enhanced lipid per-oxidation. The treatment of CCl₄-rats with Nigella sativa as a protector produces an increase in the activity of G6-PD in serum and liver. This increase attributed to the fact that G6-PD activity maintain the thiol redox status in the liver (by increased the production of NADPH), protecting the tissue from the damage effect of CCl₄ toxicity. So, the increase in G6-PD level after vitamin E or β-carotene administration may be due to that α-tocopherol protect dehydrogenase-SH group from oxidation or reaction with other metal ions because of its locations close to the sulfhydryl groups in the cell (Ismaeil, 2001).

CONCLUSION

42 male albino rats divided into 7 equal groups of 6 rats each. Group 1: taken paraffin oil daily for 15 days. Groups 2,3 y 4: injected intra-peritoneal with 1/3LD₅₀ of ethyl alcohol extract of faba beans daily for 15 days but groups 3 y 4: received either vitamin E or β-carotene orally at the protective dose 1 hour prior to alcohol extract injection. Groups 5,6,7: rats injected intra-
peritoneal with diethyl maleate (5μl/rat) and after 1 hour injected with 1/3 LD_{50} of faba beans ethyl alcohol extract for 15 days to induce favism, but groups 6 and 7: received vitamin E and β-carotene orally at the protective doses 1 hour prior to favism induction. Favism-induced group showed significant decrease in blood parameters, serum and liver proteins, serum and liver AST, serum ALT, blood GSH, serum and liver G6-PD but significant increase the liver ALT, liver GSH, serum and liver TBARs compared to favism group. While the protective dose of vitamin E or β-carotene pushes the above parameters towards the normal values.

REFERENCES


