

by interacting with unsaturated fatty acid chain (10-13).

The essential amino acid methionine shows antioxidant properties in various models of oxidative stress<sup>(14)</sup>. The mechanisms responsible for the observed methionine-induced cytoprotection are not yet fully understood. The free radical scavenging activities of methionine can only partially be explained by the chelating function of its sulfur moiety<sup>(15)</sup>. There has been increased interest among researchers to use antioxidant nutrients and medicinal plants with antioxidant activity for protection against lead toxicity<sup>(16)</sup>.

In an effort to decrease the severity of lead exposure side effects on hematological parameters, the present study was designed to explore the protective role of vitamin E alone or in combination with the amino acid methionine against lead acetate side effects on hematological parameters of adult male rabbit for 90 days, as sub chronic exposure.

## **Methods**

### **Experimental design:**

Thirty adult male rabbits of local breed were divided randomly into equal five groups, each group of six animals treated as follow: **Group 1:** Control group that were orally administered with tap water daily. **Group 2:** Orally administered with 2.5 mg/Kg B.W. lead acetate (250 mg/100 ml) dissolved in tap water daily. **Group 3:** Orally administered with 2.5 mg/Kg B.W. lead acetate (250 mg/100 ml) dissolved in tap water + 100 mg/Kg B.W. methionine dissolved in 2 ml of tap water daily. **Group 4:** Orally administered with 2.5 mg/Kg B.W. lead acetate (250 mg/100 ml) dissolved in tap water + 100 IU/Kg B.W. vitamin E daily. **Group 5:** Orally administered with 2.5 mg/Kg B.W. lead acetate (250 mg/100 ml) dissolved in tap water + 100 IU/Kg B.W. vitamin E+ 100 mg/Kg B.W. methionine dissolved in 2 ml of tap water daily. The experiment lasts for 90 days, meanwhile, animals were observed daily for their behavior and health performance. At the end of the experiment all the experimental animals were

sacrificed and 6-8 ml of blood samples was collected into EDTA tube for immediate hematological measurements and reticulocytes count.

### **Hematological and biochemical changes:**

Total red blood cells (RBC) count, hemoglobin (Hb) concentration, packed cell volume % (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC), Platelet count, total and differential WBC as well as lymphocyte and neutrophil count were accomplished by using hematological analyzer (Hycel Hematology analyzer, version B, ver2.5x.) at Damawand general laboratory for processing in both hematology and biochemistry/ Sulaimaniya city. Reticulocytes were counted in 1000 cells of the total RBCs and expressed as percentage.

**Serum iron:** Serum iron concentration was enzymatically measured using enzymatic assay kit (Biolabo SA, Maizy-France).

**Serum Ferritin:** was measured by Ferritin enzyme immunoassay test Kit (Linear chemicals, Barcelona-Spain) using DANA 3200 ELISA Reader.

### **Histopathological changes:**

Liver tissues preserved in 10% neutral formalin buffer solution. After fixation, the tissue was trimmed and the specimens were washed with saline for (1-2 hrs) and transferred to following steps: **1. Dehydration:** specimens were passed through ascending grades of ethanol alcohol (70%, 80%, 90%, 100%) for 1 hour in each concentration. **2. Clearing:** two solutions of xylol commonly used for clearing. The specimens rested 1 hour in each step. **3. Impregnation with Paraffin wax, 4. Blocking, 5. Sectioning and 6. Staining with Prussian blue stain** for hemosidrine.

### **Statistical analysis**

Data are shown as the mean  $\pm$  stander error (SE). When a significant interaction between major factors was identified by ANOVA. SPSS version 11.5, Duncan's new multiple range test was used post-ANOVA to identify significant differences between mean values at probability level of ( $P < 0.05$ ) taken as significant.