Determination of blood indices of albino rats treated with aluminum chloride and investigation of antioxidant effects of vitamin E and C

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Abstract
The current study aims to investigate hematological and biochemical blood indices of albino rats administrated aluminum chloride (AlCl₃) for eight weeks, and to study the therapeutic effects of vitamin E and C. AlCl₃ decreased the total red blood cell count (by 18%), hemoglobin (7%) and hematocrit (20%), and increased white blood cell count (67%), lymphocytes (29%), mean corpuscular volume (14%), mean corpuscular hemoglobin (6%) and platelets (33%). Administration of vitamin E with or without vitamin C failed to restore levels of red blood cell counts, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin or platelets, but vitamin E on its own restored levels of white blood cells, hemoglobin and lymphocytes.

AlCl₃ decreased serum glucose levels by 30%, and increased triglyceride (28%) and cholesterol (20%) levels; neither vitamin treatments restored the levels of these components. AlCl₃ increased levels of urea (12%), uric acid (77%) and creatinine (25%) compared to the controls, and vitamin E separately or together with vitamin C restored the levels of these nitrogen compounds.

The activities of alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase were also increased by the AlCl₃ treatment; the first two but not aspartate aminotransferase were restored by vitamin E separately or together with vitamin C.

We conclude that vitamin E separately or together with vitamin C suppressed cytogenetic injury and damage to some biochemical pathways of rat organs induced by AlCl₃.

Keywords: albino rats, aluminum chloride, blood indices, rat organs, vitamin E, vitamin C.

Introduction
Aluminum is a well-known toxic agent and represents a severe problem in a variety of medical (Nicolini et al. 1992) and environmental situations (Meranger 1989). The evidence implicating aluminium as a neurotoxin has been continuously mounting. Research on both animals and humans has linked it with neurocognitive dysfunction and in some cases death (Rifat et al. 1990). The major sources of aluminum include air, food and water (Michel 1990), and the gastrointestinal tract constitutes the main route of entry into the body. However, the absorption rate is low in normal human subjects (Brown et al. 1986).

Aluminum hydroxide, administered therapeutically in large quantities as an antacid and phosphate binder has been suggested to contribute to aluminum accumulation and toxicity (Lione 1985). Chronic exposition can cause alterations in skeletal, nervous, hematopoietic and respiratory systems (Chen et al. 2002; Cambell 2002). Blood urea is the principal end product of protein catabolism and a good indicator of kidney function. Uric acid is the end product of catabolism of purine bases; increased concentrations in the blood over the normal range might be due to extra degradation of purines in the liver, or an inability to excrete uric acid by the kidneys (Varely 1987). Creatinine appears in the serum in amounts proportional to the body's muscle mass and is more readily excreted by the kidneys than urea or uric acid (Pevicharova et al. 1997).

Blood enzymes are normally found in small amounts in circulation because of normal tissue turnover. Alanine aminotransferase as a liver enzyme significantly elevates in hepatobiliary disease, but also in connection with damage to the heart or skeletal muscle as well as liver parenchyma. Alkaline phosphatase is present on the cell surfaces in most human
tissues, and belongs to a group of enzymes that catalyze the hydrolysis of phosphomonoesters at alkaline pH. High activity is found in the intestine, liver, bone, spleen and kidneys (Stryer 1995). Aluminum ions alter the properties and structure of cellular membranes, inhibiting many enzymes (Platt et al. 2001; Abreo & Glass 1993), and can act as antagonists for other elements such as calcium, magnesium, iron, silicon, phosphorus, copper and zinc (Ward et al. 2001).

Vitamin C is essential for the formation of collagen and intracellular material, bone, teeth and for the healing of wounds. It helps maintain elasticity of the skin aids the absorption of iron and improves resistance to infection. Vitamin E is the primary liposoluble antioxidant, perhaps important in scavenging free oxygen radicals and in stabilizing cell membranes, maintaining permeability (Packer 1993). Antioxidants such as vitamins E and C, coenzyme Q, glutathione and selenium ions can act synergistically, preventing lipid peroxidation and cell destruction (Escott-Stump & Mahan 2000).

The Gaza strip was exposed to Israeli bombing from Dec 27 2008 to Jan 18 2009, resulting in high concentrations of heavy metals. Such environmental contaminants can be transmitted to humans, causing many health complications (Manduca et al. 2009). Although many studies have been carried out on the toxic effect of aluminium ions and the antioxidant effects of vitamin E and C (Hayes et al. 2001; Eastmond et al. 2001; Manduca et al. 2009; Al-Faisal 2010), their effects on the body at a molecular level are still controversial. The present study investigates the different effects of AlCl₃ on blood indices of albino rats, and the subsequent response of rat tissues to therapeutic actions of vitamins E and C.

Materials & Methods

The study design involved one control and three treatment groups. It used 24 adult male albino rats, each weighing 100-120 gm, purchased from the breeding unit of the Biology Department, IUG. They were kept in plastic cages with wire mesh covers for one week before experimentation, and then divided in groups of six into one control and three treatment groups. Group one was administered 40 mg/l AlCl₃ dissolved in the drinking water (Fyiad 2007); group two was given 40 mg/l AlCl₃ plus vitamin E (150 mg/kg) (El-Nahas 1993); and group three had 40 mg/l AlCl₃ plus vitamins E and C (150 mg/kg) (El-Nahas 1993). Commercial balanced diet and water were continuously and regularly supplied ad libitum to the animals throughout the experimental period. The duration of the experiment was 8 weeks, when blood samples were collected from the jugular vein for hematological and biochemical examination.

Routine hematological parameters and a complete blood count was carried out using an automated 18-parameter hematology analyzer (ABX Micros 60, Horiba ABX, France). Clear serum samples were separated by centrifugation at 3000 rpm. for 20 min, collected and stored in a deep freeze at (-20°C) for biochemical analysis. Glucose, triglyceride and cholesterol were determined using classical methods described by Trinder (1979), Fossati & Prencipe (1982) and Allain et al. (1974), respectively. Serum urea measurement was based on cleavage of urea with urease (Fawcett & Scott 1960). Serum uric acid was determined according to Fossati et al. (1980). Serum creatinine was measured without protein precipitation according to Bartels et al. (1972). Activities of serum aspartate aminotransferase and alanine aminotransferase were determined according to the classic method of Reitman & Frankel (1957); measurement of serum alkaline phosphatase activity was also based on the method of Bessey et al. (1946).

Data were analyzed using SPSS version 13 for Windows. ANOVA was used to test for differences among groups; differences were considered significant if p < 0.05.
Results

Table 1 summarizes the effect of AlCl3 and vitamins C and E on hematological parameters. After eight week of AlCl3 administration, there was significant decrease in the total red blood cells, red blood cells, hemoglobin and hematocrit compared to the control. In contrast, white blood cells, lymphocytes, corpuscular volume, corpuscular hemoglobin and platelets showed a significant increase compared to the control. There was a non-significant increase in corpuscular hemoglobin concentration. Administration of vitamin E alone, or with vitamin C, failed to counteract the effect of AlCl3 on red blood cells, hematocrit, corpuscular volume, corpuscular hemoglobin, corpuscular hemoglobin concentration and platelets. Vitamin E alone counteracted the effect of the ion on white blood cells, lymphocytes and hemoglobin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>control</th>
<th>AlCl3 + vitamin E</th>
<th>AlCl3 + vitamins E &amp; C</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells (x 10^3 cell/μl)</td>
<td>3.90 ± 0.19</td>
<td>6.5 ± 0.24</td>
<td>4.10 ± 0.36</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>60.6 ± 2.5</td>
<td>78.2 ± 3.0</td>
<td>62.6 ± 3.2</td>
</tr>
<tr>
<td>Red blood cells (x 10^6 cell/μl)</td>
<td>11.19 ± 0.20</td>
<td>9.14 ± 0.35</td>
<td>9.25 ± 0.30</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>16.26 ± 0.70</td>
<td>15.18 ± 0.18</td>
<td>16.18 ± 0.19</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>65.9 ± 1.1</td>
<td>53.0 ± 1.2</td>
<td>52.5 ± 1.3</td>
</tr>
<tr>
<td>Corpuscular volume (fL)</td>
<td>14.5 ± 0.2</td>
<td>16.6 ± 0.2</td>
<td>17.4 ± 0.3</td>
</tr>
<tr>
<td>Corpuscular hemoglobin (pg)</td>
<td>27.14 ± 0.20</td>
<td>28.65 ± 0.16</td>
<td>30.84 ± 0.19</td>
</tr>
<tr>
<td>Corpuscular hemoglobin concentration (g/dl)</td>
<td>53.52 ± 0.25</td>
<td>57.95 ± 0.31</td>
<td>56.70 ± 0.36</td>
</tr>
<tr>
<td>Platelets (x 10^3/μl)</td>
<td>595.1 ± 23.3</td>
<td>790.0 ± 25.2</td>
<td>731.8 ± 31.3</td>
</tr>
</tbody>
</table>

**Table 1:** Hematological indices of the rats administrated AlCl3, vitamin E and vitamin C (all values expressed as mean ± SE). Means with different subscripts in the same row differ significantly (p<0.05).

Table 2 shows changes in glucose, triglycerides and cholesterol concentrations in the experimental groups. Aluminium treatment significantly decreased serum glucose levels (by 30%) and increased significantly triglycerides (28%) and cholesterol (20%). Treatments with vitamin E alone or with vitamin C did not restore these compounds to control levels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>control</th>
<th>AlCl3 + vitamin E</th>
<th>AlCl3 + vitamins E &amp; C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>95.2 ± 3.1</td>
<td>66.8 ± 2.2</td>
<td>68.8 ± 2.2</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>88.5 ± 2.5</td>
<td>113.3 ± 2.3</td>
<td>105.7 ± 3.4</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>129.1 ± 2.3</td>
<td>155.0 ± 3.2</td>
<td>152.2 ± 3.3</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>25.3 ± 0.1</td>
<td>28.3 ± 1.1</td>
<td>26.5 ± 1.2</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>3.51 ± 0.20</td>
<td>6.21 ± 0.26</td>
<td>3.77 ± 0.24</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.60 ± 0.01</td>
<td>0.75 ± 0.02</td>
<td>0.65 ± 0.01</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/ml)</td>
<td>25.5 ± 0.3</td>
<td>30.8 ± 0.4</td>
<td>28.1 ± 0.3</td>
</tr>
<tr>
<td>Aspartate amino transferase (IU/ml)</td>
<td>21.9 ± 0.4</td>
<td>31.4 ± 0.4</td>
<td>28.7 ± 0.5</td>
</tr>
<tr>
<td>alkaline phosphatase (IU/ml)</td>
<td>81.5 ± 0.6</td>
<td>100.1 ± 4.3</td>
<td>90.1 ± 2.2</td>
</tr>
</tbody>
</table>

**Table 2:** Chemical concentrations in albino rats administrated AlCl3, vitamin E and vitamin C (all values expressed as mean ± SE). Means with different subscripts in the same row differ significantly (p<0.05).

Levels of non-protein nitrogenous constituents for treatment groups are also given in Table 2. The aluminium treatment significantly increased urea (by 12%), uric acid (77%) and creatinine
levels (25%) compared to the control. Vitamin E separately or together with vitamin C significantly counteracted the effects of aluminium.

Activities of serum aspartate amino transferase, alanine aminotransferase and alkaline phosphatase (Table 2) increased significantly following aluminium treatment. As for the nitrogenous compounds, these increases were counteracted by treatment with vitamin E alone or together with vitamin C. In contrast, aspartate amino transferase activity was not counteracted at all.

**Discussion**

This study aimed to determine the toxic effects of the aluminium ion, and the therapeutic effects of vitamin E and vitamin C, on rats. We found highly significant decreases in hemoglobin, red blood cells and hematocrit among aluminium-treated rats, as have others (Karmaker et al. 2000). The reduction in hemoglobin content might be due to increased rate of destruction or reduction in the rate of formation of red blood cells. This interpretation was supported by the low levels of red blood cells in the treated groups. Reductions in hematocrit, red blood cells and hemoglobin might be attributed to hyperactivity of bone marrow, leading to production of red blood cells with impaired integrity that are easily destroyed in the circulation (Karmaker et al. 2000). On the other hand, these decreases could alternatively reflect a lower oxygen supply to different tissues, resulting in low energy production in the rats. The decrease in hemoglobin could be not only due to decrease in red blood cells count but also to impaired biosynthesis of heme in the bone marrow.

The significant increase in white blood cell levels of aluminium-treated rats might indicate activation of the immune system, a normal cell-mediated immune response (El-Demerdash 2004). The increase in lymphocytes could be due to the toxic action of the aluminium ion that stimulates the hemopoietic system to release more of these cells, causing an increase in their number in the blood stream.

The increase in corpuscular volume, corpuscular hemoglobin, corpuscular hemoglobin concentration and platelets were consistent with changes in red blood cell counts and hemoglobin levels. These changes may be correlated with some pathological changes developed in blood-forming organs, or with the destruction of red blood cells, or with both factors. In this regard, from similar results Naylor (1971) concluded that anemia resulted from hemodilution, extra vascular hemolysis and toxic dyshemopiosis.

Our findings show that vitamin E on its own counteracted the effects of the aluminium ion on white blood cells, lymphocytes and hemoglobin. Vitamin E and vitamin C separately increase the activities of antioxidant enzymes in various tissues of rats, especially liver tissues and also vitamin E on bone marrow, where the different blood cells are formed (Shireen et al. 2008).

Our findings also revealed a decrease in serum glucose levels in response to aluminium. Indirectly, aluminium is known to play a specific role in carbohydrate metabolism (Thirunavukkarasu & Sakthiasekaran 2003). Concerning lipid metabolism, our results demonstrated that triglycerides and total cholesterol levels increased in response to aluminium, consistent with increasing lipogenesis in the liver (Thirunavukkarasu & Sakthiasekaran, 2003). Vitamin E separately or together with vitamin C could not counteract the effects of the aluminium ion on glucose, cholesterol and triglycerides. This indicates that vitamin E is only indirectly involved in metabolism of these compounds via defending the integrity of cells against oxidating agents. Vitamin E mediates mitochondrial superoxide generation, suggesting a possible mode of action at tissue level, and it also modulates the expression and/or activation of redox-sensitive biological response modifiers that regulate important cellular events (Chow 2004).
Enhanced protein catabolism and accelerated amino acid deamination in response to low glucose levels caused by aluminium ion administration is the best interpretation for the elevated levels of urea. The presence of toxic compounds can increase blood urea and decrease plasma protein (Berne & Levy 1998). The observed increase in uric acid concentration might be due to extra degradation of purines in the liver, or an inability to excrete uric acid by the kidneys (Varely 1987). An increase in creatinine has been seen, interpreted as caused by a decrease in muscle mass (Pevicharova et al. 1997) or abnormal glomerular function of the kidneys induced by AlCl₃ administration (Berne & Levy 1998). The observation that vitamin E separately or together with vitamin C counteracted the toxic effects of the aluminium ion in forming these nitrogenous compounds indicates that the vitamins reverse and thus inhibit interactions with metabolic enzymes involved in their synthesis in the liver and muscles.

Serum transaminases (aspartate amino transferase and alanine aminotransferase) and alkaline phosphatase exhibited a significant increase in treated rats, perhaps indicating persistent cellular injury (Bansal et al. 2005). Elevated activities of serum transaminases could be a sign of impaired liver function. Alkaline phosphatase has a specific location within both sinusoidal and bile canalicular membranes, accounting for its more predominant elevation in certain disorders (Bansal et al. 2005): acute cell necrosis liberates alkaline phosphatase into the blood circulation and its level is elevated. As with biosynthetic enzymes of nitrogen compounds, vitamin E separately or together with vitamin C reversed the toxic effects of aluminium ions on the activities of alkaline phosphatase and alanine aminotransferase, but not aspartate amino transferase. Their effect on aspartate amino transferase requires further investigation.

We conclude that aluminium ions significantly decrease red blood cell counts, hemoglobin, hematocrit and glucose, and significantly increase white blood cell counts, lymphocytes, corpuscular volume, corpuscular hemoglobin, corpuscular hemoglobin concentration, platelets, triglycerides, cholesterol, urea, uric acid, creatinine and the activity levels of alanine aminotransferase, aspartate amino transferase and alkaline phosphatase. Vitamin E on its own counteracts the effect of AlCl₃ on white blood cell counts, hemoglobin and lymphocytes. Vitamin E alone or together with vitamin C counteracts the effects of aluminium ions on urea, uric acid, creatinine and on the activities of alanine aminotransferase and alkaline phosphatase. Consequently, vitamin E separately or together with vitamin C suppresses cytogenetic injuries and damage to some biochemical organ pathways (e.g. liver, kidney, bone marrow) induced by AlCl₃ administration.

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Abdel Aziz & Zabut: Effect on rat blood of aluminium chloride and vitamins E and C
تحديد المؤشرات الخاصة بدم الفئران البيضاء المعالجة بكلوريد الألومينيوم للتحقق من الآثار المضادة للأكسدة لفيتامينى (ج) و (هـ)

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الملخص العربي
تهدف الدراسة الحالية إلى تحديد الأعراض الفسيولوجية والكيميائية لدم الفئران البيضاء المعالجة بكلوريد الألومينيوم (AlCl3) لمدة ثمانية أسابيع لدراسة الآثار العلاجية لفيتامينى (ج) و (هـ). يؤدي كلوريد الألومينيوم إلى انخفاض عدد خلايا الدم الحمراء (نسبة 18 %) والهيموجلوبين (نسبة 7 %)، كما يؤدي لزيادة عدد خلايا الدم البيضاء (نسبة 67 %) والخلايا الليمفاوية (نسبة 29 %) ومتوسط حجم خلايا الدم (نسبة 14 %) ومتوسط الخلايا الليموجلوبيني لثلاثي الدم (نسبة 6 %) أو الصفاك الدموية (نسبة 33 %). فشل فيتامينى (هـ) عند إعطائه للقرابينات وحده أو مع فيتامين (ج) في استعادة المستويات الطبيعية لأعداد خلايا الدم الحمراء والهيموتوكريت وностخر حجم الخلايا وكذا متوسط الخلايا الليموجلوبيني لثلاتي الدم أو الصفاك الدموية، بينما نجح في استعادة المستويات الطبيعية لخلايا الدم البيضاء والهيموجلوبين والخلايا الليمفاوية.

عمل كلوريد الألومينيوم على خفض مستويات السكر في الدم بنسبة 30 % وزيادة مستويات الدهون الثلاثية (نسبة 28 %) والكولسترول (نسبة 20 %) ولم يسبب العلاج بانتي علاج فيتامينى (ج) في استعادة مستويات تلك المكونات، كما أسهم في زيادة مستويات بوريا (نسبة 12 %) وحسوب البوليك (نسبة 77 %) والكيراتينين (نسبة 25 %) للقرابينات غير المعالجة، إلا أن فيتامينى (هـ) على حد ذاته أو بالاشتراك مع فيتامين (ج) قام باستعادة المستويات الطبيعية لهذه المركبات النتريجوية.

من الملاحظ أن نشاط إنزيمات الألودين الأفيتاميني والأكيتان فيتامينى (ج) و (هـ) قد زاد نتيجة للاستعمال بكلوريد الألومينيوم، إلا أن أول إنزيمين (ما عدا الأسيتامين فيتاميني (ج) قد تأثر بالعلاج بفيتامينى (ج)) وضعده.

وقد سبق لنا تحليل أن فيتامينى (هـ) يمكنه بشكل متقطع أو مع فيتامين (ج) وقف الإصابات الخلوية والأضرار التي تلحق بعض المسارات الحيوية لاعضاء الفئران والتي تسبب فيها مادة كلوريد الألومينيوم.