The prophylactic effect of vitamins C and E against induced dietary iron overload in rats

Emhemmed M. Belaid * **Almahdi M. Aljadi ****

Abstract

The aim of the present study was to examine the effect of exogenous antioxidant vitamins on dietary iron overload. Eighty(80) adult female albino rats were divided into 4 main groups, made up of 20 rats each, and were fed the following dietary regimen: control group(Gp1) fed a basal diet and water ad libitum. Groups 2,3 and 4 were fed a basal diet and intraperitoneally injected with iron dextran(200mg/kg b.wt.) once/ week up to 13 weeks**.** Group 2(Gp2) fed a basal diet and treated with iron only. Group 3(Gp3) received vitamin C(0.5 g/L) in drinking water**.** Group 4 was divided into 2 subgroups (10 rats each); group 4A(Gp4A) and group 4B(Gp4B); they were received vitamin E and selenium (0.25 g/L and0.50 g/L respectively) in drinking water**.** All of the treated groups showed a significant increase($p<0.05$) in the total serum iron and transferrin saturation compared with the control group.Gp2 and Gp4A showed a significant increase ($p<0.05$) in serum AST and ALT levels compared with the control group; however, Gp3 and Gp4B showed no significant change compared with the control group. It can be concluded that treatment with high doses of vitamin C and vitamin E could prevent or delay iron overload-induced toxicity in rats.

Keywords: *antioxidants, iron overload, vitamin C, vitamin E.*

Introduction

Iron(Fe) is an essential metal for the growth, development, and long-term survival of most organisms. The accumulation of iron in some tissues have been associated with the development and progression of several pathological

* Staff member Department of Medical Laboratory, Faculty of Engineering and Technology Sciences, Brack;

**Staff member Department of Public Health ,Faculty of Medical Technology, Murzuk; Sebha University, Libya

conditions, including certain cancers, liver and heart diseases, diabetes, and immune system dysfunction(1). Iron overload disorders are characterized by the accumulation of Fe in mononuclear phagocytes and parenchymal cells of the liver and other organs(2,3), and is identified to be the direct cause of liver damage(4).

As a transition element, the ionic form of iron participates in one-electron transfer reactions, and this is an important attribute in its role as a prosthetic group in enzymes that catalyze redox reactions. However, this capacity also enables iron to generate free radicals. For example, iron participating in the Fenton reaction will react with less potent reactive oxygen species (ROS) and free radicals to produce more potent secondary ROS (2). These reactive radicals cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular diseases, stroke, cancer, neurodegenerative diseases and cataractogenesis(5).

Free radicals and ROS are normally removed or inactivated *in vivo* by an antioxidant defense system. This include some enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and coenzyme Q10, which protect cells from the toxic effects of ROS and free radicals (5). SOD converts $O_2^{\bullet-}$ into H_2O_2, while CAT and GPx convert H_2O_2 to oxygen and water. Other antioxidants of biological importance are vitamins C, E, beta carotene and minerals such as selenium(2,6).

Ascorbic acid (vitamin C) is a potent water soluble antioxidant species against ROS and reactive nitrogen radical, at very low concentrations (7). Interestingly, the high intake of vitamin C have not been found to increase oxidative damage in humans and in fact, lessen the risk of chronic heart disease (CHD) or cancer (8). A factor that may affect the pro-oxidant and antioxidant property of ascorbic acid is its concentration. The *in vitro* data suggests that at low concentrations and in the presence of transition metals such as iron or copper, ascorbic acid act as a pro-oxidant, but at higher levels of concentration in a living organism, its major function is as an antioxidant(9). In addition, ascorbic acid can regenerate other antioxidants such as α-tocopheroxyl, urate and β-carotene radical cation from their radical species (10).

Vitamin E is the primary lipid soluble antioxidant, which may has an

important role in scavenging of free oxygen radicals and stabilizes the cell membrane maintaining its permeability (11). Supplementation with vitamin E and/ or selenium has been known to protect against oxidative damage and reduced iron concentrations in iron-overloaded mice (12).

Though some *in vitro* and *in vivo* studies showed promising antioxidant effects of vitamins C and E, some other studies on the other hand, showed no benefit and even possible harm (2,6,13,14); the aim of the present study was therefore to examine the probable prophylactic effect of vitamins C and E as antioxidants against induced dietary iron overload in rats.

Materials and Methods

Animals and Treatment: A total number of eighty(80) adult female albino rats(110± 10 gm) were obtained from the animal house, and fed commercial basal diet and water ad libitum. They were kept for 2 weeks before the beginning of experiments for adaptation. They were then divided into 4 main groups made up of 20 rats each and were fed the following dietary regimen: the first group, the control group(Gp1) was fed a basal diet and water ad libitum. Groups 2,3 and 4 were fed a basal diet, water ad libitum and intraperitoneally injected with iron dextran(Laboratories STEROP s.a., Belgium; 200mg/kg b.wt.) once/ week up to 13 weeks(15).Group 2(Gp2) was fed a basal diet and treated with iron but totally devoid of vitamins. Differently, group 3(Gp3) received vitamin C(El-ESRAA Company, Egypt; 0.5 g/L) in drinking water daily(16).Group 4 was divided into 2 subgroups (10 rats each); group 4A(Gp4A) received vitamin E and selenium(El-ESRAA company, Egypt; 0.25 g/L) in drinking water day by day(17).And group 4B (Gp4B) received vitamin E and selenium (0.50 g/L) in drinking water day by day. All of the animals were observed for clinical signs and employed at the end of the 13th week for blood samples.

Methods: Blood hemoglobin level was measured according to the method described by *Barbera*(18). Commercial kits for the measurement of serum iron, total serum iron-binding capacity(TIBC), total protein, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) were obtained from Biomeurex Company (France), and were utilized according to the manufacturer's instructions.

Statistical analysis: All data were expressed as mean± standard error (S.E.), and statistically analyzed using statistical analysis system(SAS) package(2002). For all analysis, P value < 0.05 was considered statistically significant.

Results

Clinical signs:

The clinical signs including focal loss of body hair were observed on day 71 in group 2 (Gp2) and nervous manifestations in the form of hyperactivity in response to stimulation in all treated groups. No mortalities were recorded in any of the experimental groups.

Blood analysis:

As shown in table (1) Hemoglobin (Hb) level of the control group (Gp1), group 2 (Gp2), group 3 (Gp3), group 4A (Gp4A) and group 4B (Gp4B) were 14.26 ± 0.19, 11.09 ± 0.19, 12.56 ± 0.28, 11.14 ± 0.34 and 14.53 ± 0.19(g/dl) respectively. Gp2, Gp3 and Gp4A showed a significant decrease ($p< 0.05$) in Hb level in comparison with control group (Gp1), however there were no significant difference ($p>0.05$) between Gp4B and control group (Gp1). Serum iron (SI) level of the control group (Gp1), Gp2, Gp3, Gp4A and Gp4B were 191.00 ± 3.24, 324 ± 6.85, 395.11 ± 4.03, 380.50 ± 11.32 and 357.75 ± 16.5 (μg/dl) respectively. All of the treated groups showed a significant increase ($p<0.05$) in the total serum iron compared with the control group (Gp1). Moreover, Vitamin treated groups(Gp3, Gp4A and Gp4B) showed the highest levels.

Table (1): Hb, Serum iron, Total iron binding capacity and Transferrin saturation of rats treated with iron with or without concurrent administration of vitamin C or vitamin Eat the end of the experiment (13 weeks).

Parameter	Group				
	1	2	3	4A	4B
Hb (g/dl)	14.26 ± 0.19 a	11.09 ± 0.19 c	12.56 ± 0.28 b	11.14 ± 0.34 c	14.53 ± 0.19 a
Iron (μg/dl)	191.00 ± 3.42 d	324.00 ± 6.85 c	395.11 ± 4.03 a	380.50 ± 11.32 ab	357.75 ± 16.50 b
Total iron binding capacity (μg/dl)	372.00 ± 20.75 a	252.50 ± 6.91 c	233.78 ± 7.61 c	288.25 ± 4.64 b	238.00 ± 15.23 c
Transferrin saturation (%)	51.77 ± 2.64 d	123.43 ± 4.06 c	170.63 ± 6.63 a	131.93 ± 2.72 bc	152.35 ± 12.39 ab

Groups: 1. Control; 2. Iron; 3. Iron + Vitamin C; 4A. Iron + Vitamin E (1/4 g); 4B. Iron + Vitamin E (1/2 g).

Values are means ± standard errors.

Means in a row without a common letter differ significantly (P<0.05).

Serum total iron binding capacity (TIBC) of Gp1. Gp2. Gp3. Gp4A and Gp4B were 372.00 ± 20.75. 252 ± 6.91. 233.78 ± 7.61. 288.25 ± 4.64 and 238.00 ± 15.23(μg/dl) respectively (Table 2). All of the treated groups showed a significant decrease (p<0.05) compared with the control. Transferrin saturation (TS) in the Gp1. Gp2. Gp3. Gp4A and Gp4B were 51.77 ± 2.64. 123.43 ± 4.06. 170.63 ± 6.63. 131.93 ± 2.72 and 152.35 ± 12.39(%) respectively. All of the treated groups. particularly the vitamin treated groups. showed a significant increase (p<0.05) in transferrin saturation compared to the control group.

Table (2) shows serum total protein. aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in control and treated groups. Serum total protein in Gp1. Gp2. Gp3. Gp4A and Gp4B were 2.53 ± 0.27. 2.58 ± 0.06. 2.61 ± 0.04. 2.73 ± 0.05 and 2.58 ± 0.05 (g/dl) respectively. Results showed no significant differences between all groups. Serum AST levels of Gp1. Gp2. Gp3. Gp4A and Gp4B were 153.75 ± 12.36. 275.13 ± 31.47. 152.78 ± 0.78. 315.00 ± 6.45 and 149.75 ± 13.31 (U/L) respectively. Serum ALT levels of Gp1. Gp2. Gp3. Gp4A and Gp4B were 71.25 ± 5.30. 214.88 ± 12.43. 78.78 ± 0.57. 236.75 ± 8.50 and 73.75 ± 4.99 (U/L) respectively. Gp2 and Gp4A showed a significant increase (p<0.05) in serum AST and ALT levels compared with the control group; however. Gp3 and Gp4B showed no significant change compared with the control group.

Table (2): Serum total protein. AST and ALT of rats treated with iron with or without concurrent administration of vitamin C or vitamin Eat the end of the experiment (13 weeks).

Parameter	Group				
	1	2	3	4A	4B
Total protein. (g/dl)	2.53 ± 0.27 a	2.58 ± 0.06 a	2.61 ± 0.04 a	2.73 ± 0.05 a	2.58 ± 0.05 a
Aspartate aminotransferase (U/L)	153.75 ± 12.36 b	275.13 ± 31.47 a	152.78 ± 0.78 b	315.00 ± 6.45 a	149.75 ± 13.31 b
Alanine aminotransferase (U/L)	71.25 ± 5.30 b	214.88 ± 12.43 a	78.78 ± 0.57 b	236.75 ± 8.50 a	73.75 ± 4.99 b

Groups: 1. Control; 2. Iron; 3. Iron + Vitamin C; 4A. Iron + Vitamin E (1/4 g); 4B. Iron + Vitamin E (1/2 g).

Values are means ± standard errors.

Means in a row without a common letter differ significantly ($P<0.05$).

Discussion

Iron is an essential element for all cells functioning and survival however, excess iron in tissues is potentially toxic, mutagenic, mitogenic and liver is primarily vulnerable (3). Iron overload disorders are characterized by the accumulation of Fe in mononuclear phagocytes and parenchymal cells of the liver and other organs (2). Considering the benefits as well as the toxic role of iron, the question is whether antioxidant vitamins (vitamin C) and (vitamin E) supplementation, have prophylactic effects in supporting the antioxidant defense system in dietary iron overload against liver damage.

The present study showed that all of the treated groups(Gp2, Gp3, Gp4A& Gp4B) had almost double serum iron levels, and 3-folds transferrin saturation (%) compared to that of the control group (Gp1). Serum iron reflects the degree of current hepatic inflammation and necrosis where transferrin saturation is the best predictor for the status of hepatic iron deposition (19). The participation of iron is essential to induce hydroxyl radical formation via the Fenton reaction ($Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^{\bullet} + OH^{-}$) and subsequently, to initiate lipid oxidation or oxidize almost every molecule present in biological systems (20).

Serum AST and ALT level are the most sensitive markers employed in the diagnosis of hepatic damage (21). Elevation in serum activity of these enzymes (ALT & AST) reflects alterations in cell membrane and hepatocellular damage secondary to hemosderosis (22). In the present study, group 2 (iron/ non-vitamin) and 4A (iron/vitamin E(0.25g)) showed the highest serum ALT & AST levels. This elevation in liver function tests reflected the extent of liver damage by iron, as evidenced by degeneration and necrosis of hepatocytes. Furthermore, these findings suggest that a low dose of vitamin E couldn't attenuate the oxidative damage on the liver of iron-overloaded rats. Similar observations were made by some other researchers (2,23,24).On the other hand, group 3(iron/vit. C) and group 4B (iron/vit.E (0.5g)) showed normal

AST & ALT levels, suggesting that vitamin C and high dose of vitamin E could provide antioxidant protection against iron induced liver damage. The protective effect of vitamin C and E observed in this study did not involve enhanced elimination of excess iron, since treatment with vitamin C and E did not reduced the hepatic concentration of iron. The protective effect of both vitamins is associated with their antioxidant action at the site of iron storage in the liver. Vitamin C &E treatment prevented iron-induced liver damage and restored serum activity of ALT & AST to normal levels indicating a restoration of liver function (25).

Hemoglobin(Hb), were found to have a significant decrease in groups 2, 3 and 4A. The observed anemia in these groups could be attributed to the degeneration and necrosis of the liver and spleen, as they are the main organs of extramedullary hemopoiesis in rats. These hematological changes were in agreement with *Narama, et al*. (26) who**se** study revealed that rats fed 5% of iron lactate for 3 months, exhibited a slight decrease in Hb values**.** Conflicting research results however was recorded by *Santos, et al*. (27) and *Brandsch, et al*. (28)who found no change in Hb in iron-overload mice and rats. Serum iron(SI) and transferrin saturation (TS)% increase in all the treated groups compared to the control group, suggesting a high hepatic iron deposition due to iron supplementation; that was in agreement with some other studies (23,24). Decreased total iron binding capacity (TIBC) in groups 2, 3 and 4B is supported by Herbert, (29) who reported that TS% and SI levels tend to increase as iron storage increase over the normal range, whereas TIBC levels tend to decrease as iron stores increase (30).

Conclusion

From the above mentioned results, it can be concluded that prophylactic treatment with high doses of vitamin C and vitamin E could prevent or delay iron overload-induced toxicity in rats. With these positive findings further studies should be made utilizing humans as subjects for this would open wide arrays of benefits for human health.

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التأثير الوقائي لكل من فيتامين ج وفتيامين هضد الأحمال الزائدة للحديد المحدثه غدائيا في الفئران

■ امحمد بلعيد * ■ المهدي الجدي **

الملخص

هدفت هده الدراسة لاختبار تأثير الفيتامينات كمضادات للأكسدة ضد الأحمال الزائدة للحديد، حيث تم استخدام ثمانون(80) أنثى من الفئران البيضاء، قسمت إلى أربع مجموعات (20فأر لكل مجموعة). المجموعة الأول الضابطة أعطيت عليقة متوازية ومياه شرب، المجموعة الثانية والثالثة والرابعة أعطيت عليقة متوازية وحقنت بالحديد في الغشاء البروتوني (20مجم/كجم وزن الجسم) أسبوعيا لمدة 13 أسبوعا. المجموعة الثالثة أعطيت فيتامين ج (50 جرام/لبتر) بمياه الشرب. المجموعة الرابعة قسمت لمجموعتين فرعيتين (10 لكل مجموعة). حيث أعطيت المجموعة الرابعة (أ) و (ب) فيتامين هـ والسيلينيوم (25. ،50. جرام/ ليتر على التوالي) بمياه الشرب . أظهرت المجموعات المعالجة الثلاثة زيادة معنوية لحديد المصل وتشبع الترانسفيرين بالمقارنة بالمجموعة الضابطة. أظهرت المجموعة الثانية والرابعة (أ) زيادة معنوية في إنزيمات الكبد (ALT،AST) بالمقارنة بالمجموعة الضابطة، بينما لم يحدث أي تغيير معنوي لإنزيمات الكبد لدى المجموعة الثالثة والرابعة (ب) بالمقارنة بالمجموعة الضابطة. يمكن استنتاج ان المعالجة بفيتامين ج وفيتامين هـ تمنع أو تؤخر التسمم بالأحمال الزائدة للحديد.

عضو هيئة التدريس بكلية العلوم الهندسية والتقنية جامعة سبها *

عضو هيئة التدريس بكلية التقنية الطبية جامعة سبها **