The role of Vitamins A and E and their combination on some Physiological Parameters of Cobalt Treated Laboratory Mice

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الخلاصة:

قسمت الدراسة الحالية إلى جزئين. تناول الجزء الأول تقيم دور فيتامين C وفيتامين E والتداخل بينهم (C و E) على المعايير الدمية والكيماحيوية في الفئران المعرضة إلى كبريتات الكوبالت. بينت نتائج الدراسة وجود انخفاض معنوي عالي في العدد الكلي لكريات الدم الحمر وقيمة خضاب الدم وقيمة حجم خلايا الدم المرصوص،فضلا عن ارتفاع معنوي عالي في تعداد خلايا الدم البيض .كما وأظهرت النتائج الدور الايجابي الذي يلعبه فيتامين C وفيتامين E والتداخل بينهما (Cو E) في استرجاع كافة القيم الدموية والكيماحيوية المتدهورة و لتصل اقرب إلى القيم الطبيعية قبل التعرض إلى الكوبالت.

Abstract

The present study is investigate the effect of vitamins A and vitamin E and their combination on the relief of the toxic effect of cobalt exposure on some hematological, and biochemical parameters. The study was showed that the treatment with cobalt chloride is caused significant increased in the RBC, PCV and decreased in Hb. The total number of WBC count was elevated. Biochemical tests show significant increase in the level of total cholesterol, triglyceride, LDL-c and significant decrease of HDL-c compared with control group..The present study shows the positive role of vitamin A and E in the improvement of the physiological and biochemical alterations which resulted from the exposure to cobalt.

Introduction:

It has been shown that cobalt and cobalt compounds may produce adverse health effects (Lison, 2007) such as cardiovascular toxicity, and reprotoxicity, with possible DNA alterations (Colognato *et al*., 2008). Cobalt toxicity may also be caused through oxidant based and free radical-based processes (Hoet *et al*., 2002). Cobalt may act by inducing haemoxygenase and causing haem oxidation in organs. Haem-containing proteins that would be affected include monooxygenase enzymes (cytochrome P450) and catalase. Cobalt may also increase erythropoietin, which results in the increased production of red blood cells. An erythropoietic effect was observed by study of Paternain *et al*., (1988). When Cobalt chloride (25, 50 or 100 mg cobalt/kg bw/day) was administered to mice on days 6 to 15 of gestation by oral gavages.

Glucose metabolism has been also demonstrated to be affected by cobalt. Animals treated with cobalt exhibit depressed serum and tissue glucose levels (Lison, 2007). Previous study indicated that the effect of cobalt administration on the blood is an increase in triglycerides, cholesterol, and free fatty (Taylor and Marks, 1978). In another study, the result revealed that administration of 0.5% H_2O_2 in drinking water to rats for six weeks caused significant increase in platelet count and in serum TC, TAG, LDL-C, and VLDL-C concentration with significant decrease in prothrombin time and HDL-C concentration as compared to other groups. On the other hand, oral intubation of

vitamin E in addition to H_2O_2 decreased the serum concentration of TC, TAG, LDL-C, VLDL-C, and platelet count comparing to H_2O_2 treated group (Abdul-Katum and Khudair, 2008). This study is an attempt to evaluate role of Vitamin A and Vitamin E and their combination on hematological and biochemical parameters of cobalt exposure mice.

MARERIALS AND METHODS:

The experiment was conducted at the animal house of the Veterinary Medicine College–University of Basrah, where 40 females and 68 males mice (*Mus musculus*) sexually mature, 12 weeks old, and of 20–25 grams weights were use.

The trial divided in to two parts. The first part was dealt with role of Vitamin A and Vitamin E and their combination on hematological and biochemical parameters of cobalt exposure mice. The hematological parameters measures were: Red Blood Cell (RBC), Hemoglobin (Hb), Packed Cell Volume (PCV). The biochemical parameters were Asparate Aminio Transferase (AST), Alanine Amino Transferase (ALT), total Cholesterol, HDL-c and LDL-c. The second part was dealt with role of Vitamin A and Vitamin E and their combination on reproductive efficiency of cobalt exposure mice. Animals were divided into five groups, consists of 8 male mice each. The first group consisted with 8 male mice .They were provided with normal water (*ad libitum*) for 45 days, then administered 0.1 olive oil for 15 days then terminated for the necessary tests. All other groups were treated with cobalt chloride (400 ppm) in drinking water provided as (*ad libitum*) for 45 days. Then separated into:

a-The first treated group: This group consists of 8 male mice which were treated with cobalt chloride (400 ppm) and terminated for the necessary tests.

b-The second treated group: This group consists of 8 male mice which were treated with cobalt chloride 400 ppm and then administered 0.1 ml of vitamin A dissolved in olive oil and admnistered orally for additional 15 days.

c- The third treated group: This group consists of 8 male mice which were treated with cobalt chloride 400 ppm and then administered 0.1 ml vitamin E 8 IU dissolved in olive oil and admnistered orally for additional 15 days.

d- The fourth treated group: This group consists of 8 male mice which were treated with cobalt chloride 400 ppm and then administered 0.1 ml vitamin A and 0.1 vitamin E 8 IU both dissolved in olive oil and admnistered orally for additional 15 days.

In this study, ANOVA Analysis and LSD tests are used according to (SPSS version) program at the ($P \leq 0.05$) to find the means for all treatments (SPSS, 1998).

RESULTS:

It seems from table (1) that the cobalt exposure caused significant elevation in RBC, and PCV, compared with control group. The Hb showed reduction in it s value compared with the control. When the cobalt treated animals group was administrated to Vitamin A and Vitamin E individually, the RBCs and PCV decrease significantly and PCV was raised compared with cobalt treated animals groups. It seems also that when the both vitamin A plus E offered together to the cobalt treated animals, the blood parameters almost returned to their normal values compared with the control group. It seems that both vitamins acted together in a way significantly better than when each vitamin works on its own.

Table (1): Effect of vitamin A, E and their combination on some blood parameters of cobalt chloride-treated male mice. (Mean ± SD)

Parameters / Groups	RBC Count ×106	PCV %	Hb gm/100ml
Control	c 8.00 ± 0.43	b29.67 ± 1.36	a 13.75 ± 0.61
Cobalt chloride	a 11.05 ± 1.75	a 46.33 ± 3.445	b 10.66 ± 0.75
Vitamin A	ab10.03 ± 0.64	ab41.14 ± 4.634	a 12.64 ± 1.67
Vitamin E	b 9.48 ± 1.10	b 40.71 ± 8.01	a 12.5 ± 1.37
Vitamin A/E	b 9.78 ± 0.62	ab41.57 ± 2.37	a 13.48± 0.41
LSD	1.27	5.61	1.33

The different letters refer to significant differences among groups ($P \leq 0.05$)

The W.B.C. and its differential numbers also have affected by cobalt exposure (table 2). The W.B.C. value increased significantly compared with control value. It seems from the results that the increasing of WBC comes from the increment of lymphocytes and monocytes percentage in which both increased significantly compared with the control group. The Neutrophils percentage was showed a reduction in its value compared with the control group. A significant reduction in WBC account, lymphocytes and monocytes percentages as well reductions in the neutrophils were observed when either vitamin A or E had given individually or as a combination compared with the control groups.

According to the hepatic enzymes (AST, ALT) and urea, the cobalt administration effect on them was noticed (table 3). The values were rising up significantly compared with the control group. When either vitamins A or E has given, a significant reduction in all values of AST, ALT enzymes and urea of the cobalt treated animals were observed, compared with the control value. However, the both vitamins (A Plus E) seemed to be acting in a perfect way in reducing liver enzymes and urea values compared with the vitamin A and Vitamin E treated groups, and both were able to return the liver enzymes and urea to their normal values compared with control group.

Table (2 The Effect of vitamin A, vitamin E and their combination on WBC count and deferential WBC number of cobalt chloride treated male mice. (Mean ± SD

Groups	WBC count ($X10^3$)	Neutrophil %	Eosinophil %	Basophil %	Lymphocyte %	Monocyte %
Control	b 3.70 ± 0.44	a 55.17 ± 1.72	b 1.33 ± 0.51	٠	b 42.00 ± 1.09	b 1.67± 0.81
Cobalt chloride	a 7.30 ± 0.70	d 35.38 ± 3.24	a 2.38 ± 0.51	٠	a 52.50 ± 2.33	a 9.75 ± 2.86
Vitamin A	b 5.16 ± 0.86	c 41.50 ± 3.78	b 1.63 ± 0.51	٠	a 50.63 ± 2.61	b 5.88 ± 1.642
Vitamin E	b 4.69 ± 0.76	b 47.57 ± 3.15	b 1.71 ± 0.48	٠	b 46.29 ± 2.13	b 4.57 ± 1.134
Vitamin A and E	c 4.08 ± 0.61	a 53.00 ± 1.30	b 1.38 ±0.518	٠	c 42.75 ± 1.66	c 2.75 ± 0.707
LSD	0.99	5.42	0.66	٠	3.53	1.82

The different letters refer to significant differences among groups ($P \leq 0.05$).

Table (3) the effect of vitamin A and vitamin E or their combination on some liver enzymes activity and urea levels of cobalt chloride-treated. (Mean± SD)

group	ALT (IU/L)	AST (IU/L)	Urea
Control	73.18 c±4.01	253.18 b±13.87	b 62.97 ±4.23
Cobalt chloride	95.81 a±8.74	353.43 a±49.78	a 103.53 ±4.22
Vitamin A	88.39 ab±3.06	285.73 b±8.01	78.79 b±7.52
Vitamin E	82.28 b±3.98	270.45 b±10.61	80.29 b±4.82
Vitamin A and E	87.08 b±10.76	255.86 b±19.90	69.45 b±6.75
LSD	8.72	32.55	9.33

The different letters refer to significant differences among groups (P≤0.05).

According to table (4) , it seems the cobalt exposure caused significant elevation in total cholesterol, triglyceride and LDL, whereas there was a reduction in the HDl value compared with control group .However, the combination of both vitamins (A Plus E) were able to reduce the abnormal elevation of plasma lipid to their normal values compared with the control group.

Table (4) The effect of vitamin A, vitamin E and their combination on some plasma lipid

Parameter / Groups	Total Cholesterol mg/dl	Triglyceride mg/dl	HDL-C mg/dl	LDL-C mg/dl
Control	b 132.97 ±9.93	b 128.19 ±4.06	a 51.67 ±0.74	b 59.0 ±3.42
Cobalt chloride	a 171.49 ±6.19	a 166.99 ±5.32	c 44.28 ±1.41	a 93.90±5.16
Vitamin A	a 164.02 ±7.31	b 154.45 ±8.53	b 49.24 ±1.85	b 83.89±9.04
Vitamin E	b 150.86 ±5.93	c 144.08 ±4.84	ab50.47±0.75	c 71.58±6.2
Vitamin A and E	b 132.96 ±7.68	b 131.22 ±4.52	a 51.09 ±0.81	b 55.62±7.84
LSD	13.15	10.37	1.85	10.0

The different letters refer to significant differences among groups (P≤0.05).

Discussion:

The results show that administration of cobalt chloride had significantly (p≤0.05) increased RBCs count and PCV compared with the control. It seems that Cobalt has an erythropoietic action, increasing blood volume and total erythrocyte number in several animal species. Doses necessary for eliciting an erythropoietic effect have been in the order of 1 mg/kg given daily. This effect is probably the result of the capacity of cobalt to mimic the pathophysiological response to hypoxia that involves various genes, including those coding for erythropoiesis and growth factors for angiogenesis (Lison, 2007).

The Hb concentration of cobalt chloride treated group was significantly ($p \leq 0.05$) decreased compared with the control, this may result from Hb destruction or to a decrease in the rate of Hb synthesis (Atamanalp *et al.*, 2010). Cobalt can also inhibit heam synthesis in the liver (De Matteis and Gibbs, 1977). They suggest that cobalt inhibits the induced synthesis of the enzyme activity rather than its formation. Administration of the cobalt chloride treated group with vitamin A had elevated RBCs count and PCV without reaching significant ($p \leq 0.05$) differences, but managed to increase Hb concentration significantly ($p \leq 0.05$) compared with cobalt chloride treated group. While administration of vitamin E or Vitamins A and E combination had increased Hb concentration significantly ($p \leq 0.05$) compared with cobalt chloride treated group. Similar results have been reported by Hassan (2006), AL-Mousawy (2009), Abdul Wahap (2009) and Jassim and Hassan (2010) suggested that administration of vitamin E or vitamin A to animals exposed to oxidative stress by heavy metals such as cadmium, lead, copper, and aluminum decreased the adverse effects produced by heavy metals on blood parameters.

The PCV results agreed with Corrier *et al*. (1985) in rats exposed to cobalt chloride. The increased Hb content may be attributed to increased erythropoiesis and hemoglobin synthesis. (Atamanalp *et al*., 2011). Administration of cobalt chloride had significantly ($p \leq 0.05$) increased WBCs count and Eosinophil, Lymphocyte and Monocyte compared with the control, while a significant decrease in Neutrophil was observed in cobalt chloride treated group compared with the control. Vitamin A or vitamin E treating decreased WBCs count, Esionophil and Monocyte number significantly ($p \leq 0.05$) but not lymphocyte, and significantly ($p \leq 0.05$) increased Neutrophil number compared with cobalt chloride treated group, but still has a significant ($p \leq 0.05$) difference with the control. This result agreed with Abdul Wahap, (2009) in mice treated with aluminum chloride then treated with vitamin A and with AL-Mousawy (2009) in mice treated with copper then treated with vitamin E. Both of them found that administration of vitamin A or vitamin E to mice treated with aluminum and Cupper had restored WBCs count and differential WBC number to normal value.

Vitamin E and vitamins A and E combination treating decreased WBCs count, Esionophil, lymphocyte and monocyte number significantly ($p \leq 0.05$), and increased Neutrophil number significantly ($p \leq 0.05$) compared with cobalt chloride treated group. The anti- oxidant effect of vitamins E and A, that scavenges ROS and may prevent WBC demargination to peripheral blood (Suermen-Guer *et al*., 1999).

After administration of vitamin A, ALT enzyme was decreased but hadn't reached significant difference with cobalt chloride treated group, while AST and urea levels were significantly decreased. This result agreed with Abdul Wahap, (2009).

Administration of vitamin E had significantly ($p \leq 0.05$) decreased ALP, AST, and urea compared with cobalt chloride treated groups. This result agreed with Mine *et al*. (2009). While vitamins A and E combination had significantly ($p \leq 0.05$) decreased ALP, AST, and urea levels. Numerous studies have demonstrated that antioxidant vitamin supplementations can be beneficial in lowering markers indicative of oxidant stress and lipid peroxidation (Brown *et al*., 1997). Serum cholesterol, triglyceride, and LDH of cobalt chloride treated group were increased significantly ($p \leq 0.05$), whereas HDL was decreased significantly ($p \leq 0.05$) compared with the control. The result agreed with Taylor and Marks (1978), who suggest that may be caused by the inhibition of tissue lipoprotein lipase, resulting in the failure to clear very low-density lipoprotein, and

perhaps by stimulation of lipoprotein synthesis by the liver (Eaton, 1972). In contrast Garoui *et al*. (2011) found that exposure of mice to cobalt had significantly decreased Serum cholesterol and triglyceride. Administration of vitamin A reduces cholesterol level but still had no significant ($p \leq 0.05$) difference with cobalt treated group. Whereas triglyceride and LDL were reduced significantly compared with cobalt chloride treated animals, as well as HDL level was increased significantly.

Treated with cobalt chloride exposure animals with vitamin E has reduced significantly ($p \leq 0.05$) cholesterol, triglyceride, and LDL levels. Whereas HDL levels were significantly ($p \leq 0.05$) decreased compared with the control group.

The conclusions of the presented study are that the administration of cobalt chloride has increased RBCs count, PCV,WBC count, Eosinophil, Lymphocyte , Monocyte, ALT, AST,urea, total cholesterol, Triglyceride and LDL- C .In conversely administration of cobalt chloride has reduced the value of Hb , Neutrophil, HDL-C. Administration of vitamin A, vitamin E and their combination has reduced toxic effect of cobalt chloride though the combination of vitamins A and E was acted in better way than when each vitamin acted by its own.

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