Effect of vitamin C and E administration on haemoglobin and packed cell volume in chicken

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ABSTRACT

There is an increasing interest on the effect of antioxidant in the treatment of certain pathological conditions.

A low haemoglobin (Hb) and packed cell volume (PCV) level in chicken were induced, by feeding them *Hypericum perforatum* (Aran) at a dose of (2.5) gm /kg orally in gelatin capsules and exposing them to direct sunlight at noon for (20) minutes.

Experimental animals were divided into seven groups; each group consists of six animals. Vitamins C or E was administered either before Aran administration or after cessation of Aran administration.

Aran toxicity associated with several clinical signs including: photosensitivity, itching, redness of skin, hyperkeratosis of comb and shaking of head and keratoconjunctivitis. In addition a significant lowering of Hb and PCV level. Vitamin C and E significantly effective in elevating the reduced Hb and PCV level when these vitamins were administered one hour before the administration of Aran or after cessation the administration of Aran, besides all the clinical signs were abolished except the persistence of mild keratinization of comb and redness of cornea, when these animals were examined in the last day of experiment.

This study suggests the use of either vitamin C or E either as prophylactic against toxicity with this plant, or as medication in case of toxicity.

Key Words: Chicken, packed cell volume, vitamins, Aran.

الخلاصة

لقد زاد الاهتمام بتأثير مضادات الأكسدة في علاج حالات مرضية خاصة. قلة خضاب الدم وحجم الخلايا المرصوصة قد استُحدِثَت في الدجاج بتغذيتهم بنبات العرن بجرعة فموية مقدارها (٢,٥) غرام/كغم على شكل كبسولات هلامية وتعريضهم إلى أشعة الشمس المباشرة ظهراً ولمدة عشرين دقيقة.

قُسِّمَت الحيوانات المختبرية إلى سبعة مجاميع، كل مجموعة مؤلفة من ستة. أُعطي فيتامين (ﺃ) أو (ج) إما قبل إعطاء العرن أو بعد التوقف عن إعطائه. التسمم بالعرن مصحوب بعدة أعراض سريرية تتضمن الحساسية الشديدة للضوء، حكة مع احمرار في الجلد، التقرن في عرف الديك، التهاب تقرني في الجفن بالإضافة إلى الانخفاض المعنوي في مستوى خضاب الدم وحجم الخلايا المرصوصة.

إعطاء كلا الفيتامينين بساعة قبل إعطاء جرعة العرن أو بعد التوقف عن إعطاء العرن يؤدي إلى زوال جميع الأعراض السريرية باستثناء تقرن خفيف الشدة واحمرار القرنية عند فحص هذه الحيوانات في اليوم الأخير من التجربة. تقترح هذه الدراسة استخدام فيتامين (ﺃ) أو (ج) إما كوقاية ضد التسمم نتيجة استخدام نبات العرن أو للعلاج في حالات التسمم.

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INTRODUCTION

Aran *(Hypericum perforatum)* is a toxic plant found in northern parts of Iraq[1]. It contains a red fluorescent pigment called hypericin [2, 3], which is responsible for the development of toxic signs [4].

The clinical signs include photosensitivity, redness of comb, beak and face, shaking the head, itching, hyperkeratosis of comb and keratoconjunctivitis with lowering of Hb and PCV level [5].

These toxic signs were due to the presence of hypericin that absorbs sunlight energy and has the ability to transfer this energy to molecular oxygen results in high reactive oxygen intermediate, such as hydrogen peroxide, and hydroxy radical [6,7]. Exposure of cell membrane to oxygen free radicals stimulate lipid peroxidation [8], this reaction may cause cellular damage [9]. This direct effect on all membrane of intact erythrocyte lead to haemolysis [10, 11], which is responsible for low level of Hb and PCV.

Vitamin E considered to provide protection against oxygen free radical by acting as antidote and by stabilizing cellular membrane with the formation of a complex with membrane fatty acid, thus stopping the destructive chains reaction associated with lipid peroxidation [12] and thus prevent erythrocyte haemolysis.

The pretreatment with vitamin E and selinum one hour before Aran administration found to be effective in restoring low Hb and PCV level to normal in addition minimizing all the clinical signs of toxicity [5]. Vitamin C is the most rapidly oxidised vitamin [13]. It is required for the synthesis of collagen and is a powerful reducing agent (antioxidant) which play a part in intracellular oxidation - reduction system and in mapping up oxidants (free radicals) produced endogenously or in environment [14]. The present study was planned to compare the effectiveness of vitamin C and E without selinum in elevating low Hb and PCV in chickens exposed to *H. perforatum*. This may draw the attention of using vitamin C or E clinically for the treatment of anaemia induced by toxic plants.

MATERIALS AND METHODS

Forty two experimental chickens, (8) weeks old, white male with body weight (750-850 gm). Chickens were obtained from IPA Agricultural Research Center. They were housed at a temperature of (20-25)°C. Food and water were available *ad librium*. Chickens were randomly divided to seven groups, each consist of six animals.

Aran was dried in air, ground fine enough to be stored at room temperature in sealed plastic bags until used. The dried powder was placed in gelatin capsules. A dose of (2.5) gm/kg body weight was used daily in all groups [1]. Animals were divided into seven groups as follows: -

Group 1: control where normal feeding.

Group 2: chickens were forced fed with Aran daily at a dose of (2.5) gm/kg body weight for (12) days.

Group 3: (300) mg/kg of vitamin C in gelatin capsules were administered (60) minutes before Aran administration for (12) days.

Group 4: (300) mg/kg of vitamin E in gelatin capsules administered (60) minutes before Aran administration for (12) day.

Group 5: chickens were forced with Aran daily for (6) days. On day (7) Aran administration was stopped and (300) mg/kg of vitamin C in gelatin capsules was administered daily for the following days up to day (13).

Group 6: vitamin E was used at dose of (300) mg/kg, as in group (5).

Group 7: chicken forced fed with Aran for six days. On day seven chickens receive normal feeding.

Chickens in all group were exposed to direct sun light daily at noon for (30) minutes after the administration of Aran. This study was conducted during October.

In day (13), all chickens were slaughtered, blood samples were collected in test tubes containing EDTA (ethylene diamine tetraacetic acid) for haematological examination (Hb, PCV) [13].

The data were subjected to one way analysis of variance followed by Duncan test. The level of significance was at ($p < 0.05$).

RESULTS

Chickens fed with Aran at dose of (2.5) gm/kg produced severe signs of toxicity that was significant in day (2) of administration. The clinical signs of toxicity included photosensitivity, itching, redness of comb and face, keratoconjunctivitis, shaking of head as represented in table (1). Besides low Hb and PCV level (tables 2 and 3) and lowering in body weight (figure 1).

The preadministration of vitamin C and E (G3, G4) before Aran administration minimizes greatly the clinical signs of toxicity as represented in tables (2) and (3). Besides, vitamin C and E in groups (3) and (4), respectively tend to elevate the low Hb and PCV level (figures 2 and 3). Body weight restored to that of normal. In Groups (5) and (6), the administration of vitamin C and E in day (7) of experiment greatly and significantly elevate Hb and PCV than group (2) and even the control level ($p< 0.05$). Besides, the clinical signs of toxicity gradually decline and completely disappeared in day (13), except slight keratinization of comb and slight redness of eye. Body weight significantly increased.

In Group (7) cessation the administration of Aran produced gradual recovery from Aran toxicity except mild keratinization of comb and redness of eye. The drop in body weight was gradual and minimal compared to group (2) (figure 1).

DISCUSSION

Aran administration at (2.5) gm/kg in chickens produced severe signs of toxicity (table 1), when these animals were exposed to sunlight directly for (30) minutes at noon.

The clinical signs of toxicity started in the second day of Aran administration by photosensitivity, redness of comb, beak and face, shaking of head, itching, exaggeration of clinical signs of toxicity reaches its maximum after (1) week by hyperkeratosis of comb and keratoconjunctivitis. These clinical signs were in agreement with other workers [5,15], and could be attributed to the phototoxic compound contained in Aran called hypericin. Sun light energy absorbed by the phototoxic compound in the skin is thought to be transferred to molecular oxygen, resulting in a highly reactive oxygen intermediate such as superoxide radical, hydrogen peroxide and hydroxyl radical [6, 7].

Table (1): Clinical signs of Aran toxicity

The following table represents different clinical signs of toxicity in different groups (+) represent the presence of the signs, while (-) represent the absence of that clinical sign. (G) sign represent group number

Days	1	2	3	4	5	6	7	8	9	10	11	12	13
Photosensitivity													
G1	-	-	-	-	-	-	-	-	-	-	-	-	-
G2	-	+	+	+	+	+	+	+	+	+	+	+	+
G3	-	+	-	-	-	-	-	-	-	-	-	-	-
G4	-	+	+	-	-	-	-	-	-	-	-	-	-
G5	-	+	+	+	+	+	+	-	-	-	-	-	-
G6	-	+	+	+	+	+	+	-	-	-	-	-	-
G7	-	+	+	+	+	+	+	+	+	-	-	-	-
Itching													
G1	-	-	-	-	-	-	-	-	-	-	-	-	-
G2	-	+	+	+	+	+	+	+	+	+	+	+	+
G3	-	+	-	-	-	-	-	-	-	-	-	-	-
G4	-	+	+	-	-	-	-	-	-	-	-	-	-
G5	-	+	+	+	+	+	+	+	-	-	-	-	-
G6	-	+	+	+	+	+	+	+	-	-	-	-	-
G7	-	+	+	+	+	+	+	+	+	+	-	-	-
Redness of Face, Comb, Beak													
G1	-	-	-	-	-	-	-	-	-	-	-	-	-
G2	-	+	+	+	+	+	+	+	+	+	+	+	+
G3	-	+	+	mild	mild	-	-	-	-	-	-	-	-
G4	-	+	+	-	-	-	-	-	-	-	-	-	-
G5	-	+	+	+	+	+	+	-	-	-	-	-	-
G6	-	+	+	+	+	+	+	+	-	-	-	-	-
G7	-	+	+	+	+	+	+	+	+	+	-	-	-
Hyperkeratosis of Comb													
G1	-	-	-	-	-	-	-	-	-	-	-	-	-
G2	-	-	mild	+	+	+	+	+	+	+	+	+	+
G3	-	-	-	-	-	-	-	-	-	-	-	-	-
G4	-	-	-	-	-	-	-	-	-	-	-	-	-
G5	-	-	mild	+	+	+	+	+	-	-	-	-	-
G6	-	-	-	mild	+	+	+	+	+	-	-	-	-
G7	-	-	-	+	+	+	+	+	+	+	+	+	+
Keratoconjunctivitis													
G1	-	-	-	-	-	-	-	-	-	-	-	-	-
G2	-	-	mild	+	+	+	+	+	+	+	+	+	+
G3	-	-	-	-	-	-	-	-	-	-	-	-	-
G4	-	-	mild	-	-	-	-	-	-	-	-	-	-
G5	-	mild	+	+	+	+	+	-	-	-	mild	mild	mild
G6	-	-	mild	+	+	+	+				mild	=	=
G7	-	-	=	+	+	+	+	+	+	mild	=	=	=
Shaking of Head													
G1	-	-	-	-	-	-	-	-	-	-	-	-	-
G2	-	-	+	+	+	+	+	+	+	+	+	+	+
G3	-	-	-	-	-	-	-	-	-	-	-	-	-
G4	-	-	-	-	-	-	-	-	-	-	-	-	-
G5	-	+	+	+	+	+	+	+	+	-	-	-	-
G6	-	+	+	+	+	+	+	+	+	-	-	-	-
G7	-	+	+	+	+	+	+	+	+	mild	-	-	-

G1 = control.
G2 = Aran alone.
G3 = vitamin C + Aran
G4 = vitamin E + Aran
G5 = vitamin C postoperative Aran stoppage.
G6 = vitamin E postoperative Aran stoppage.
G7 = Aran alone with no medication after day (7).

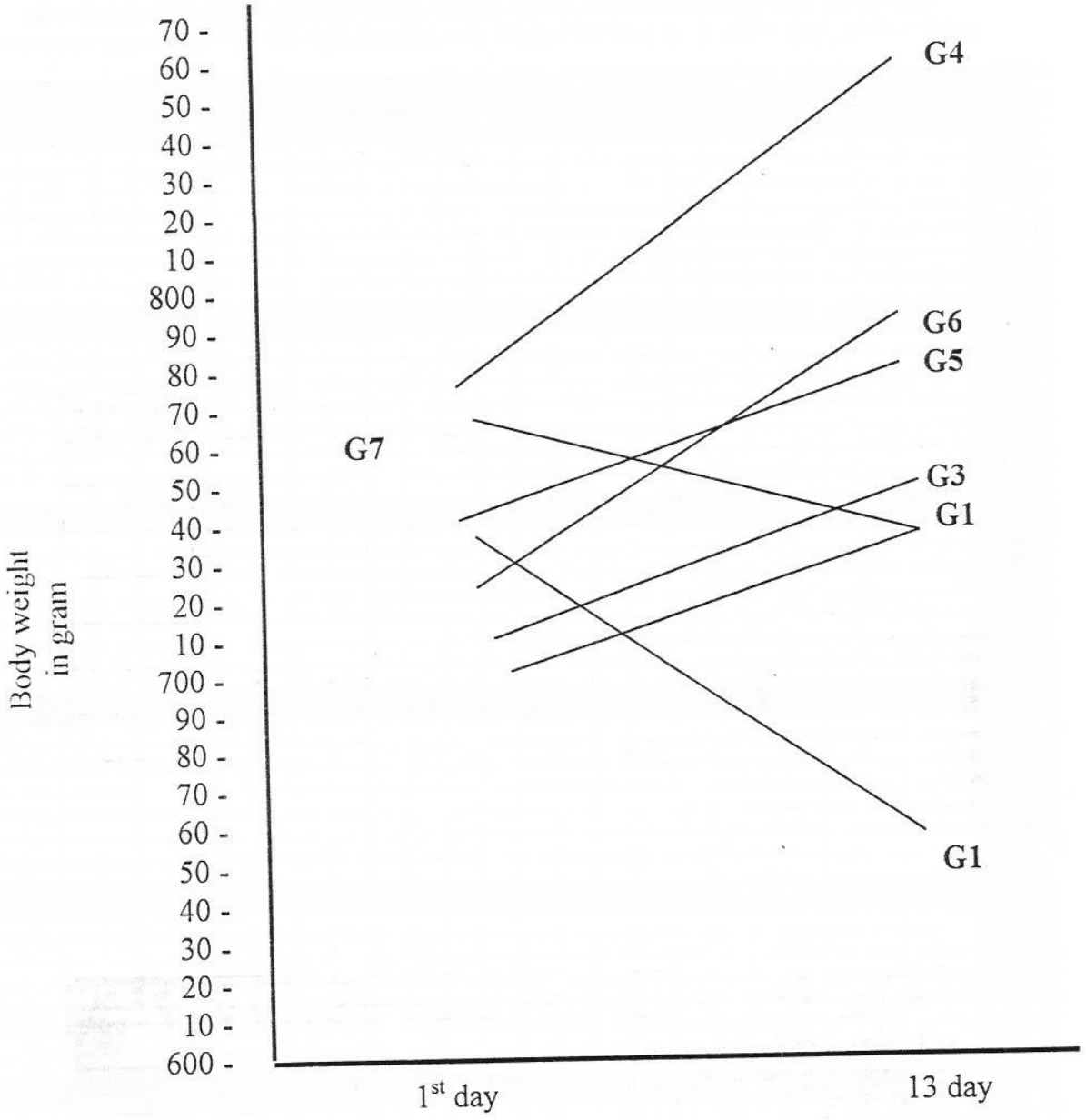

Figure (1): Weight of chickens in group with in the experimental days

The principal finding in this study is the significant reduction in Hb and PCV which agree the result obtained by other [5,10]. This effect can be explained due to the presence of hypericin that cause haemolysis of intact erythrocyte cell membrane lead to their haemolysis [10], or due to the effect of oxygen free radicals on erythrocyte membrane causing their haemolysis [11]. The effect of vitamin E could be explained by its protective antioxidant effect and by stabilizing the erythrocyte membrane through the formation of complex within membrane fatty acid [12,15], thus stopping the destructive chain reaction associated with lipid peroxidation [12] and thus prevents

haemolysis of erythrocyte. In addition, vitamin E considered as the most effective chain breaking membrane antioxidant, competes for peroxyl radicals at a faster rate than polyunsaturated fatty acid so protecting polyunsaturated fatty acid against oxidation [18].

Similarly, vitamin C elevated Hb and PCV level, its effect may be correlated by its antioxidant effect, by scavenges superoxide anion (O_2), nitric oxide radical (NO), and peroxyl radicals [16]. Furthermore, vitamin C have other important role as a co-antioxidant effect by regenerating - tocopherol (vitamin E) from the tocopheroxyl radical [17]. This effect draws our attention for the combination of vitamins C and E in the treatment of Aran toxicity and the clinical evaluation of vitamins C and E combination in the treatment of anaemia associated with plants toxicity.

Table (2): The effect of vitamin C and E on haemoglobin concentration in chickens

Group Number	Mean Hb Concentration	Duncan Test
G1	11.8 ± 0.1	(D)
G2	7.2 ± 0.1	(A)
G3	8.86 ± 0.153	(B)
G4	10.02 ± 0.13	(B)
G5	17.7 ± 0.12	(C)
G6	16.9 ± 0.11	(C)
G7	7.0 ± 0.1	(A)

Values with different letter are significantly different ($p<0.05$).
N= 6 in each group.
Group keys as in table (1).

Table (3): The effect of vitamin C and E on PCV in chicken

Group Number	Mean PCV (%)	Duncan Test
G1	34.5 ± 0.1	(D)
G2	21.0 ± 0.12	(B)
G3	30.2 ± 0.18	(A)
G4	32.1 ± 0.13	(A)
G5	56 ± 0.8	(C)
G6	54 ± 1.0	(C)
G7	19.9 ± 0.1	(B)

Values with different letter are significantly different ($p<0.05$).
N = 6 in each group.
Group keys as in table (1).

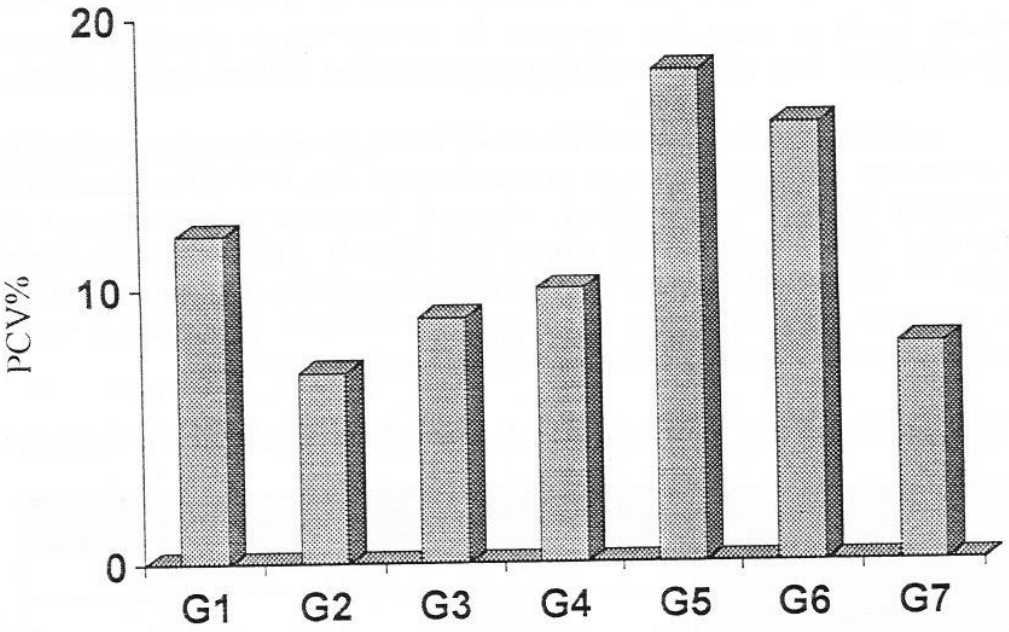

Figure (2): Effect of *H. perforatum* , vitamin C and E on PCV concentration (n = 6 in each group)

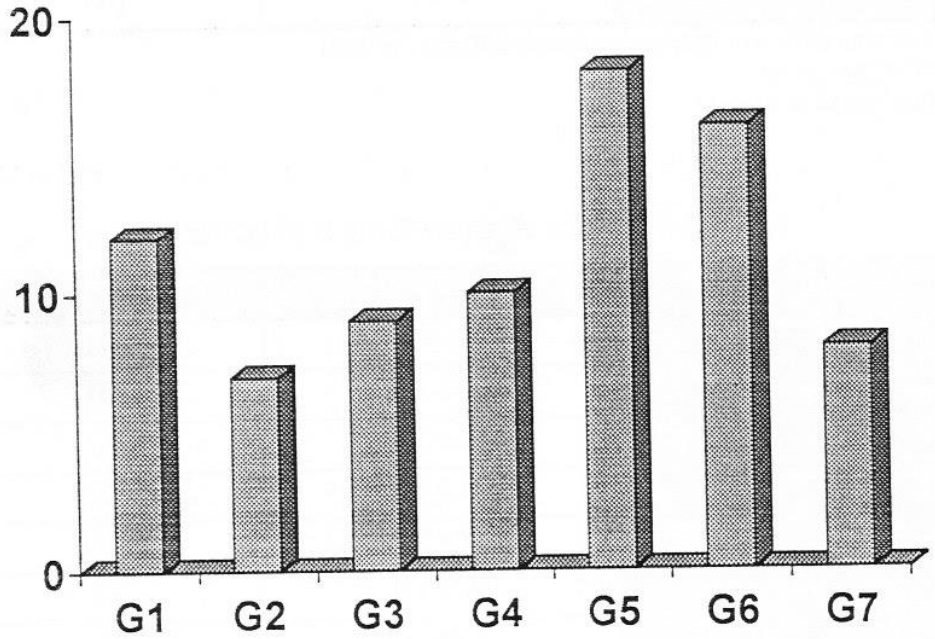

Figure (3): Effect of *H. perforatum*, vitamin C and E on haemoglobin concentration in chicken (n = 6 in each group)

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