

## **THE HAEMATOTOXICITY OF COUMATETRALYL AND THE ANTIDOTAL EFFECT OF VITAMIN K IN ALBINO RATS**

BY

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### **ABSTRACT**

The haematotoxicity of coumatetralyl, and the antidotal effect of vitamin K repeated doses on blood parameters was evaluated in albino rats anticoagulated with  $1/2$  LD<sub>50</sub> of coumatetralyl. Anticoagulant administration resulted in haemorrhage accompanied by elevation in prothrombin time (PT) and partial thromboplastin time (PTT) and significant increases in white blood cells count (WBC's), while exhibited a significant reduction in haemoglobin content, haematocrit value, and red blood cells count (RBC's). The absolute values, the mean cell volume (MCV), the mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) represented macrocytic anemia. Vitamin K intake affected some haematological parameters as reduction of PT and PTT to almost control. There is a significant dose related reduction in clotting time. While other parameters as haematocrit value, haemoglobin content, RBC's, WBC's and the absolute values did not normalize to control values.

### **INTRODUCTION**

Coumatetralyl, 4-hydroxy-3-(1, 2, 3, 4-tetrahydro-1-naphthyl) coumarin (Racoumin 57) was first introduced in 1956, and is now one of the most widely used of the first generation of anticoagulants (Buckle, 1994). The anticoagulants became used to almost total exclusion of other

rodenticides. Because they are widely used as rodenticides, poisoning sometimes occurs in children, suicide in adult (Holmes and Love, 1952), occupational workers (Ahmed *et al.*, 1990), domestic farm and wild animals (Meehan, 1984). In developing countries the anticoagulant rodenticides are extensively used for the eradication of rodents. The misused of such compounds will lead to poisoning of non target animals. As in Egypt when an outbreak abortions and haemorrhages in sheep and goats in 1982 was traced to intoxication with the rodenticide brodifacoum. (Feinsod *et al.*, 1986).

The chronic mode of action of the anticoagulants is the key to their success, they act by interrupting the vitamin K cycle in liver microcosms leading to a lowered prothrombin concentration (Mac Nicoll, 1986). The supply of the active form of the vitamin K can be preserved, and the ability of the blood to clot maintained, by the administration of excess amounts of vitamin K<sub>1</sub>. (Buckle, 1994). Thus there were recorded incidents of vitamin K arresting were bleeding in pigs poisoned with coumatetralyl (Dobson, 1973) dogs and rabbits poisoned with bromadiolone (Grand, 1976) rodents given difenacoum (Bull, 1976) and it is effective against brodifacoum poisoning (Dubock & Kaukeinen, 1978). In warfarin-treated rats, blood clotting factors promptly return to normal after administration of vitamin K<sub>1</sub> (Owen and Bowie, 1978). Most previous researches did not show whether a dose or multidoses of vitamin K are better for ceasing anticoagulant symptoms.

This study is designed to address two goals. First the haematotoxicity of coumatetralyl in albino rats. Second, investigate frequent doses of vitamin K as antidote to recover from the haematotoxicity back to the control situation.

## MATERIALS AND METHODS

### Animals :

Male white albino rats (*Rattus norvegicus albinus*), each weighing  $168 \pm 19$  gm, were used in this study. They were obtained from the High Institute of Public Health, Alexandria University, Egypt, and housed four per cage, then were given food and water *ad libitum* for two weeks before the experiment.

**Rodenticide :**

The anticoagulant rodenticide, Coumatetralyl (Racoumin 57) (Bayer AG.) 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl) coumarin was used in its blue powder formulation (0.0375%). It was obtained from the Ministry of Agriculture.

**Antidote :**

Vitamin K (Konakion, 10 mg/ml) was injected intraperitoneal three times in a concentration of (4 mg / kg). This concentration were chosen according to Abou-El-Khear (1993).

All other chemicals were obtained from Sigma or BDH companies.

**Animal treatments :**

The animals were divided into three groups: The first was orally treated with  $1/2$  LD<sub>50</sub>, 0.15 mg/kg of coumatetralyl in corn oil for five days (Worthing and Hance, 1991). After 18 h of the last treatment the animals were injected intraperitoneally with 4 mg/kg of vitamin K for three times with 8-hours intervals. The second group was treated with corn oil only, while the last group received corn oil and vitamin K (4mg / kg). The second and third groups were used as control. Blood samples were collected once after 18 hr. of coumatetralyl administration and three times 8 hr. later after injection by vitamin K. Blood samples were taken from the control at respective schedule treatment.

**Haematological Studies :**

Fresh blood samples were used without delay. Haemoglobin assay were undertaken according to method of (Drabkin and Austin 1932) using kits of Bio-Merieux company. The percent of haematocrit value was determined using microhaematocrit centrifuge MLW TH21. The plasma coagulation time (PT) in the presence of excess tissue thromboplastin with calcium was recorded according to the method of (Quick, 1935) using kits from Bio-Merieux company. The determination of partial thromboplastin time (PTT) was carried out using pathromtin kits from Behring Company, according to the manufacturer's instructions Munteen et al.,(1992). The red and white blood cells count was carried out using the haemocytometer.

The absolute values, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated according to (Dacie and Lewis, 1991).

**Statistical analysis :**

The data were calculated as mean  $\pm$  SD and analysed using analysis of variance (ANOVA). The p value of  $\leq 0.05$  was considered significant.

## RESULTS AND DISCUSSION

The Haematotoxicity of coumatetralyl and the antidotal effect of vitamin K repeated doses on blood parameters were studied in albino rats anticoagulated with coumatetralyl.

Fig.(1) demonstrates the prothrombin time (PT) and partial thromboplastin time (PTT) in control and treated animals with repeated doses of vitamin K after being anticoagulated with  $1/2$  LD<sub>50</sub> of coumatetralyl. PT was highly increased in animals which were treated with

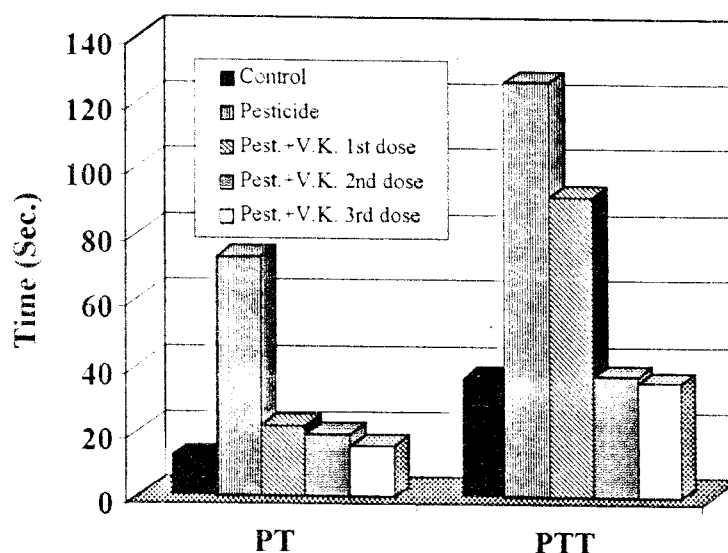


Fig.(1): Effect of repeated dose (4 mg/kg) of V.K on prothrombin time (PT) and partial thromboplastin time (PTT) in albino rat anticoagulated with  $1/2$  LD<sub>50</sub> of coumatetralyl.

coumatetralyl only and before vitamin K effect takes place. The time of clotting was prolonged from 12.8 sec in control to 73.0 sec in treated animals with anticoagulant. PT were gradually decreased after the three doses of vitamin K. to  $22 \pm 3.5$ ,  $19.25 \pm 1$  and  $15.5 \pm 0.58$  sec. It is clear that the higher effect of vitamin K was found after repeated doses of vitamin K and prolongation in clotting time normalize to control group after three doses of it.

PTT values highly significantly increased after coumatetralyl treatment, (Fig. 1) . The PTT value was extended to 126.4 sec in the anticoagulated animals with coumatetralyl compared with control animals, 36.7 sec. The effect of repeated doses of vitamin K in the anticoagulated animals with coumatetralyl was appeared by reduction the PTT values to 91.75 sec after a single dose 8 hr later than coumatetralyl administration. This reduction was not significantly returned to the control value, while after two and three doses the values recovered to  $37 \pm 4.2$  sec and  $35 \pm 4.0$  sec., respectively. Thus three doses of vitamin K normalized the PT and PTT values to control value better than a dose or two doses did. There was a significant dose-related reduction in clotting time. The present study is coincident with many investigators who noticed the prolongation in PT and PTT values after anticoagulants administration as (Ahmed *et al* 1989, 1990, Shimaila 1989, Said 1990, Huckle *et al.*, 1989 and El-Mahrouki 1984.)

Vitamin K either single dose or repeated doses as antidotes to anticoagulants are recommended by many investigators (Park and Leck, 1982, Sadowski and Suttie, 1974 and Ross *et al.*, 1992). These conclusions are in harmony with the present results which showed that vitamin K should be injected at different periods to induce animal recovery to the normal level of PT and PTT. This recommendation has its importance in using vitamin K successive dosing to resume normal blood clotting characteristics.

Table (1) shows the haematocrit values in control and treated groups with the same three doses, 4 mg/kg each of vitamin K, at 8 hr. time intervals after being incubated with coumatetralyl for five days. Haematocrit values were significantly decreased than control after treatment by coumatetralyl or even after vitamin K administration in a dose or two. These reductions were 37%, 39% and 37%, respectively, while it was 23.6% of control after the third dose of vitamin K.

Table (1): Effect of repeated doses (4 mg / kg) of vitamin K (vit. K) on blood parameters in albino rat anticoagulated with 1/2 LD<sub>50</sub> of coumatetralyl.

| Parameters                  | Mean ± SD                      |                          |                                  |                                  |                                  |
|-----------------------------|--------------------------------|--------------------------|----------------------------------|----------------------------------|----------------------------------|
|                             | Control<br>(corn oil + Vit.K ) | Coumatetralyl<br>alone   | Coumatetralyl + vitamin K        |                                  |                                  |
|                             |                                |                          | 1 <sup>st</sup> dose<br>of vit.K | 2 <sup>nd</sup> dose<br>of vit.K | 3 <sup>rd</sup> dose<br>of vit.K |
| Haematocrit value (%)       | 24.6 ± 0.99 <sup>c</sup>       | 15.3 ± 2.8 <sup>a</sup>  | 15.0 ± 0.0 <sup>a</sup>          | 15.5 ± 0.7 <sup>a</sup>          | 18.8 ± 3.2 <sup>b</sup>          |
| Haemoglobin (g/dl)          | 10.52 ± 3.9 <sup>c</sup>       | 6.0 ± 0.78 <sup>a</sup>  | 6.63 ± 0.3 <sup>a</sup>          | 7.48 ± 0.47 <sup>b</sup>         | 6.8 ± 0.53 <sup>a</sup>          |
| RBC's (10 <sup>6</sup> /μl) | 4.99 ± 0.32 <sup>b</sup>       | 3.23 ± 1.1 <sup>a</sup>  | 2.3 ± 2.8 <sup>a</sup>           | 2.57 ± 0.5 <sup>a</sup>          | 2.55 ± 0.2 <sup>a</sup>          |
| WBC's (10 <sup>3</sup> /μl) | 7.26 ± 1.6 <sup>a</sup>        | 10.86 ± 1.9 <sup>b</sup> | 15.5 ± 2.1 <sup>c</sup>          | 16.7 ± 0.6 <sup>c</sup>          | 16.0 ± 0.65 <sup>c</sup>         |
| MCV (fem. l/RBC)            | 56.5 ± 9.3 <sup>b</sup>        | 59.9 ± 12.3 <sup>b</sup> | 73.2 ± 9.0 <sup>c</sup>          | 66.2 ± 4.3 <sup>bc</sup>         | 38.1 ± 3.1 <sup>a</sup>          |
| MCH (Pg)                    | 25.4 ± 5.8 <sup>ab</sup>       | 22.3 ± 7.7 <sup>a</sup>  | 36.4 ± 3.1 <sup>d</sup>          | 32.3 ± 2.7 <sup>cd</sup>         | 28.5 ± 0.21 <sup>bc</sup>        |
| MCHC (g/dl)                 | 44.7 ± 4.8 <sup>b</sup>        | 34.9 ± 3.3 <sup>a</sup>  | 48.2 ± 1.9 <sup>b</sup>          | 49.5 ± 3.2 <sup>b</sup>          | 36.4 ± 3.2 <sup>a</sup>          |

\* Means having the same letter are not significantly different from each other, P value < 0.05 was considered significant.

\* LD<sub>50</sub> value was 0.3 mg/kg/day for five days (Worthing and Hance, 1991).

\* The control values for each parameter were calculated from the average of both control (oil only and oil with vit.K) since there was no significant differences between them or even between the time intervals.

The haemoglobin contents illustrate in Table (1) after treatment by coumatetralyl only or plus three doses of vitamin K (4 mg/ kg). The haemoglobin content was reduced to 42.96% of control after anticoagulant alone, and to 36.97%, 28.89%, and 35.36% after administration with 3 doses of vitamin K, respectively. The haemoglobin contents were not significantly return to the normal values after three doses of vitamin K.

RBC's counts of the control and treated animals are shown in Table (1). The data indicate that RBC's counts were highly significantly decreased after coumatetralyl treatment ( $1/2 LD_{50}$ ) and continued to decrease even after vitamin K injection by three doses at the different time intervals. The decreased values were 35.27, 53.9, 48.49 and 48.89% of control, respectively.

Inversely leukocytes counts were highly increased in treated animals with coumatetralyl before vitamin K effect takes place and after three doses of vitamin K at different time intervals. The increases values were one and half higher than control at zero time and two fold after vitamin K treatments.

The side effect of using anticoagulants under field conditions are expected to take place in blood parameters and other biochemical target on humans and non target organisms as shown in this study. These results were supported by many investigators., (Ahmed *et al.*, 1990 and Shimaila 1989) who found that the haemoglobin content and haematocrit values were highly decreased while the white blood cells increased after flocoumafen and coumatetralyl treatment. Also, El-Mahrouki (1984) reached the same data but after oral administration of  $LD_{50}$  brodifacoum in rats, and after subchronic doses of hydroxy coumarin compound by El-Nagar (1983).

From Table (1) the absolute values of MCV and MCH in animals which were anticoagulated with coumatetralyl ( $1/2 LD_{50}$ ) were not significantly change than control, and started to show higher significant after 8 hr. from the 1<sup>st</sup> dose of vitamin K and 2<sup>nd</sup> dose of the same drugs 8 hr. later, and they diminished after the 3<sup>rd</sup> dose of vitamin K. While the MCHC value was not affected much after treatment by coumatetralyl alone or plus vitamin K. The increases of MCV and MCH, while MCHC normal or diminished referred as macrocytic anaemia (Dacie & Lewis, 1991). Some investigators agree that the MCV, MCH, MCH values decreased as (Robbins, 1957) and some found it increased as (El-Hendi, 1986). These contradictions between investigators may be due to type of anticoagulant and /or the dose.

It can be recommended that after exposure to anticoagulant, it is better to take the antidote (vitamin K) in repeated doses with time intervals to resume normal blood clotting characteristics. But this will not include all the Haematological affected parameters, which might need other treatments to help blood cells to normalization to control.

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## الملخص العربى

### سمية مبيد الكوماتتراليل على دم فئران الالبينو والتأثير لفيتامين K

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تم دراسة التأثير السام لمبيد الكوماتتراليل بجرعات متكررة من الجرعة نصف المميته (٥٠٪ من الافراد المعاملة لمدة خمس ايام على دم فئران الالبينو) وكذلك التأثير المضاد لفيتامين K.

لقد وجد ان الكوماتتراليل بالجرعات المتكررة يحدث زيادة معنوية فى زمن البروثرومبين وزمن الثرومبوبلاستين الجزئى وعدد كرات الدم البيضاء. وعلى العكس فقد احدثت نفس الجرعات انخفاض معنوى فى كل من محتوى الهيموجلوبين وقيم الهيماتوكريت وعدد كرات الدم الحمراء، بالإضافة الى حدوث تغير فى القيم المطلقة (MCHC, MCH, MCV) عن الكنترول. وبدراسة تأثير اضافة جرعات متكررة ايضا من فيتامين K حدث انخفاض معنوى فى زمن البروثرومبين وزمن الثرومبوبلاستين الجزئى الى ان اقترب من الكنترول بعد الجرعة الثالثة من فيتامين K بينما لم تتأثر باقى الخصائص المدروسة باضافة فيتامين K.