

Haematological, Biochemical and Histopathological effects of anticoagulant rodenticides on white mice (*Mus musculus* var. *albus*)

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ABSTRACT

Three anticoagulant rodenticides (Bromadiolone, Coumachlor and Coumatetralyl) were tested at sublethal doses for their haematological; biochemical and histological effects on white mice (*Mus musculus* var. *albus*). Haemoglobin (Hb) content extremely reduced by bromadiolone with effective dose (ED_{50}) value equal 0.9 mg / kg body weight. Haematocrit (Hc) was weakly affected by the tested anticoagulant rodenticides. White blood cells (WBC's) counts in males mice were extremely reduced by coumatetralyl, coumachlor and bromadiolone with ED_{50} values equal 0.56, 0.8 and 0.9 mg / kg body weight, respectively; whereas Red blood cells (RBC's) counts were very sensitive to be reduced with 0.05, 0.1 and 0.5 mg / kg body weight by bromadiolone, coumachlor and coumatetralyl, respectively. WBC's and RBC's in females mice were also strongly affected by the tested anticoagulants with ED_{50} values ranged between 1.1- 4.0 mg / kg body weight. RBC's of females were more sensitive to the tested compounds than WBC's. Bromadiolone was strongly effective to elevate the activities of both serum alanine transaminase (s-ALT) and serum aspartate transaminase (s-AST) (at 1.5 and 2.0 mg / kg) and coumachlor against s-ALT (1.0 - 2.0 mg / kg) which produced hepatic necrosis and injury of liver. s-ALT enzyme was also sensitive to coumatetralyl (0.1 - 2.0 mg / kg), whereas coumatetralyl and coumachlor were less effective against s-AST enzyme. Hepatic necrosis, blood vessels destruction and increased lymphocyte cells were seen in liver treated with bromadiolone (0.1 mg / kg) and coumatetralyl (2.0 mg / kg), coumatetralyl and coumachlor at 0.1 mg / kg were less effective in this respect. Coumachlor (0.1 mg / kg), coumatetralyl (2.0 mg / kg) and bromadiolone (0.1 mg / kg) strongly decreased the spermatogenesis and destroyed the seminiferous tubules and sperms, as compared with untreatment.

INTRODUCTION

Anticoagulant rodenticides have been used in different areas for controlling rats and mice. These anticoagulants have many effects on certain sites of action on cumulative sublethal doses causing different problems for human and domestic animals. However, coumachlor and dicoumarol strongly inhibited s-ALT and s-AST enzymes in both male and female rats. Also, RBC's and WBC's were found to be highly sensitive to both compounds, which also moderately affected the haemoglobin content (Desheesh *et al.*, 2002). Sublethal doses of anticoagulant rodenticides (Bromadiolone, Coumachlor and Coumatet- ralyI at 0.1 mg / kg) were decreased the offspring litters and increased the stillborn and postnatal death fetuses on dosing during the first week of gestation period. Bromadiolone proved to be very effective to produce malformed fetuses and to interact with the initiation and development of embryos causing internal fetal hemorrhage inside the uterus of pregnant females during the three weeks of gestation period (Desheesh *et al.*, 1999). A case of intercerebral hematoma due to warfarin coagulopathy was seen during the examination of the 39-year old woman had spread a warfarin – type rat poison bait around her house using her bare hands, with no washing post application (Tomas *et al.*, 1994).

Coumarin derivatives and heparin has been proposed in different medical therapy as drugs and they were found to have number of potential harmful effects (Raivio *et al.*, 1977; Silvestein, 1979; Whitfield, 1980 and Harma *et al.*, 1993). The intratesticular injection of 0.1 mg heparin caused a significant and permanent decrease in motility of mouse spermatozoa (Sliwa, 1991).

Haemoglobin, haematocrit and RBC's count were significantly reduced whereas, mean corpuscular volume and corpuscular haemoglobin were increased in the swiss albino mice treated with brodifacoum (Kumar and Saxena, 1991). On the other hand, Hazelton (1956) and Evans *et al.*, (1989) studied the histological changes of some organs of rats treated with sublethal doses of coumarin derivatives. They recorded proliferation of ductal structures preceded by extensive damage to hepatocytes in centrilobular region of liver. Also, necrotic effects; spotted and mottled

liver; spotted kidneys and hyperanaemia of the intestines, stomach, testes and pancreas were recorded.

However, these different problems directed our attention to investigate the side effects of anticoagulant rodenticides; bromadiolone, coumachlor and coumatetralyl at sublethal doses for their haematological (haemoglobin, haematocrit, RBC's and WBC's counts), biochemical (ALT and AST enzymes) and histological side effects (on liver and testis), on white mice (*Mus musculus* var. *albus*).

MATERIALS AND METHODS

I. Tested Anticoagulant Rodenticides :

1- **Bromadiolone:** 3- [3- (4-bromo- [1,1- biphenyl] -4-yl) 3-hydroxy -1-phenylpropyl]-4-hydroxycoumarin. Insoluble in water but soluble in acetone, ethanol and dimethylsulfoxide.

Oral LD₅₀ for rats 1.125 and for mice 1.75 mg / kg .

Technical grade: 25 %. Company: Lipha-French.

2- **Coumachlor:** 3-[1-(4-chlorophenyl) butanoyl] -4- hydroxycoumarin. Insoluble in water but soluble in acetone and ethanol.

Oral LD₅₀ : 900 mg / kg , 50 mg / kg if applied on 5 Consecutive days (Brooks and Rows, 1974).

Technical grade: 99.9 %. Company: Ciba Gige.

3- **Coumatetralyl:** 3-(α -telralyl) butanoyl]-4-hydroxycoumarin. Insoluble in water but soluble in acetone and organic solvents.

Sub chronic oral LD₅₀ (5 d) for rats 0.3 mg / kg .

Technical grade: 99.9 %. Company: Bayer, Leverkusen, Germany. (Anonymous, 1994).

II. Experimental Animals :

Males and females of Swiss albino mice (*Mus musculus* var. *albus*) weighing (20-25 gm) were taken from a colony raised in rodent Laboratory of Pesticide Chemistry Department, Faculty of Agriculture, Alexandria University.

III. Methods of Experiments :

A- Animal treatments:

The male and female mice were divided into six groups for each rodenticide (bromadiolone, coumachlor or coumatetralyl). Five groups were

intubated orally with 0.1, 0.5, 1.0, 1.5 and 2 mg / kg body weight of rodenticide repeated three times a week. Group six was treated with corn oil and used as control. Each group includes five animals.

B- Haematological and Biochemical Measurements :

At the end of experiment, the animals were decapitated and the blood samples were divided into two parts; the first part were collected in anticoagulated tubes with EDTA (Ethylene diamine tetra acetic acid) for haematological parameters. The other part was collected in clean noncoated tube to separate serum which used to determine ALT and AST activities.

Haemoglobin determination as gm haemoglobin per 100 ml of the blood (Hb %) was conducted according to Wintrobe (1965) method, using Boehringer Mannheim Gm bH Diagnostic kits. To determine the haematocrit value (Hc %), The blood was prepared by special centrifuge and the centrifuged blood (Packed cell volume) was measured by a special measurement of haematocrit. White blood cells (WBC's) and red blood cells (RBC's) of prepared blood samples were counted by using a haemocytometer (Dacie and Lewis, 1991). Determination of serum alanine transaminase (s-ALT) and serum aspartate transaminase (s-AST) activities were based on the methods described by Reitman and Frankle (1957) using Boehringer Mannheim Gm bH Diagnostic kits. Activities of enzymes (U / L) were calculated as follows:

$$\text{Activities (U/L)} = \frac{A}{K}$$

Where: A = Absorbance at 546 nm.

K = Slope of standard curve for each ALT and AST enzymes.

Untreated samples were also concurrently examined under the same conditions. Data were calculated as means and statistically analyzed (Steel and Torrie, 1980). Generally, the results were measured as reduction or elevation percent of blood contents or enzymes activity according to the following equation:

$$\% \text{ Reduction or elevation} = \left[100 - \left(\frac{\text{Treatment activity}}{\text{Control activity}} \times 100 \right) \right]$$

Effective dose caused 50% mortality (ED_{50}) values (calculated and expected) were obtained from the regression line on propit paper.

C- Histopathological Studies :

Histopathological studies were conducted on liver and testis of both control mice and mice treated with 0.1 or 2.0 mg / kg body weight of bromadiolone, coumachlor or coumatetralyl. The dosages were orally administered three times a week, then the mice decapitated and the organs removed, cleaned of extraneous materials, and fixed in neutral buffered 10 % formalin (Liver) or Bowin's solution (testis). The fixed organs were embedded in paraffin, sectioned at 4 micron, transferred to glass slides and stained with haematoxylin and eosin by standard techniques. The stained sections were prepared at Pathology Department, Faculty of Medicine, University of Alexandria. The sections were microscopically examined and photographed.

RESULTS AND DISCUSSION

A- Haematological and Biochemical Effects :

The effects of the tested three anticoagulant rodenticides on different parameters of blood content (haemoglobin, haematocrit, WBC's and RBC's content) as well as s-ALT and s-AST enzymes activities of mice males and females (*Mus musculus* var. *albus*) were recorded in Tables (1, 2, 3, 4, 5 and 6). The categories of toxicity on the basis of amounts of the chemicals necessary to produce harm have been proposed according to (Loomis, 1976).

Haemoglobin content of males mice (*Mus musculus* var. *albus*) was extremely reduced with ED_{50} equal 0.9 mg / kg of bromadiolone whereas, haem - oglobin of females was less affected with bromadiolone which needed to increase its highest dose to reach the expected ED_{50} value (3.0 mg / kg) (Table 1).

Bromadiolone exhibited some effects on male hemoglobin specially at the high tested doses (Kumar and Saxena, 1991).

Table (1): Effect of different doses of three anticoagulant rodenticides on haemoglobin content of mice males and females (*Mus musculus* var. *albus*) indicated as % reduction and ED₅₀ (calculated and expected).

Compounds	Sex	Doses, mg / kg					Calculated ED ₅₀ mg / kg	Expected ED ₅₀ mg / kg
		0.1	0.5	1.0	1.5	2.0		
Bromadiolone	♂	37.95	38.65	51.92	54.49	55.64	0.9	---
	♀	14.41	16.85	19.23	36.22	37.06	> 2.0	3.0
Coumachlor	♂	18.71	19.35	25.42	30.19	40.58	> 2.0	4.0
	♀	27.04	29.86	35.14	38.87	39.29	> 2.0	3.5
Coumatetralyl	♂	12.26	18.32	30.00	38.06	38.58	> 2.0	3.0
	♀	10.69	18.61	26.04	45.00	45.00	> 2.0	2.5

Coumachlor and coumatetralyl were less effective to affect the haemoglobin content in both males and females of mice since it needed to increase the higher dose to be more than 2.0 mg / kg body weight to obtain the expected ED₅₀ values.

All the tested doses (0.1 – 2.0 mg / kg) of the three anticoagulants were non significantly affected the haematocrit value (Packed cell volume) in comparison with untreated mice (Table 2).

Table(2): Effect of different doses of anticoagulant rodenticides on haematocrit volume/100 ml blood of mice males and females (*Mus musculus* var. *albus*).

Compounds	Sex	Doses, mg / kg						L.S.D
		0.0	0.1	0.5	1.0	1.5	2.0	
Bromadiolone	♂	36.2	35.0	34.4	29.4	28.6	26.6	1.37
	♀	34.6	33.2	31.4	29.4	29.0	28.0	1.93
Coumachlor	♂	36.2	33.6	32.4	30.2	29.2	28.4	1.51
	♀	34.6	34.4	33.0	32.4	31.8	29.2	1.34
Coumatetralyl	♂	36.2	32.4	30.4	28.8	28.6	25.6	1.68
	♀	34.6	33.6	33.0	30.2	29.0	28.2	1.89

F tab. at 0.05 = 4.56
at 0.01 = 9.55

The data obtained showed no significant differences between the two sexes in this respect.

Table (3) showed that coumatetralyl, coumachlor and bromadiolone were extremely reduced the WBC's counts of mice males with ED₅₀ values equal 0.56, 0.8 and 0.9 mg / kg, respectively. On the other hand, coumachlor highly reduced the WBC's counts of females with ED₅₀ value equal 1.3 mg / kg body weight, whereas both bromadiolone and coumatetralyl were needed to increase the highest tested doses to cause higher effect with expected ED₅₀ values equal 4.0 and 4.5 mg / kg, respectively. So, males WBC's counts were decreased by coumatetralyl, coumachlor and bromadiolone, in descending order whereas, coumachlor was more affected WBC's of females. However, the range of the effect between the lowest and highest doses was wide.

Table (3): Effect of different doses of anticoagulant rodenticides on WBC's of mice males and females (*Mus musculus* var. *albus*) shown as % reduction, and ED₅₀ (calculated and expected values).

Compounds	Sex	Doses, mg / kg					Calculated ED ₅₀ mg / kg	Expected ED ₅₀ mg / kg
		0.1	0.5	1.0	1.5	2.0		
Bromadiolone	♂	7.46	35.82	52.24	53.73	53.73	0.9	---
	♀	6.02	9.77	13.53	32.95	38.69	> 2.0	4.0
Coumachlor	♂	11.76	38.24	51.47	67.65	69.12	0.8	---
	♀	3.29	7.16	26.49	49.71	51.64	1.3	---
Coumatetralyl	♂	26.47	41.18	44.12	73.53	88.24	0.56	---
	♀	2.11	4.03	23.22	42.42	42.42	> 2.0	4.5

RBC's counts of mice males were extremely reduced by bromadiolone, coumachlor and coumatetralyl with ED₅₀ values equal < 0.1, 0.1 and 0.5 mg / kg body weight, respectively. Bromadiolone was the strongest since it was needed to doses lower than the lowest tested doses to give an expected ED₅₀ value equal to 0.05 mg / kg body weight (Table 4).

Table (4): Effect of different doses of anticoagulant rodenticides on RBC's of mice males and females (*Mus musculus* var. *albus*) shown as % reduction, and ED₅₀ (calculated and expected values).

Compounds	Sex	Doses, mg / kg					Calculated ED ₅₀ mg / kg	Expected ED ₅₀ mg / kg
		0.1	0.5	1.0	1.5	2.0		
Bromadiolone	♂	60.57	60.57	71.43	72.00	73.71	< 0.1	0.05
	♀	36.50	39.70	43.40	52.80	54.10	1.2	---
Coumachlor	♂	51.14	52.30	55.70	61.40	64.80	0.1	---
	♀	43.30	46.30	48.10	52.20	52.20	1.1	---
Coumatetralyl	♂	47.70	49.90	63.30	65.30	65.50	0.5	---
	♀	34.30	37.35	43.17	62.21	67.29	1.2	---

Females RBC's counts were highly affected by coumachlor, bromadiolone and coumatetralyl with ED₅₀ values equal 1.1, 1.2 and 1.2, respectively. All the tested anticoagulants were very potent to reduce the counts of RBC's of males and females of mice. The range of the effect between the lowest and highest doses was wide.

ALT enzyme primarily present in liver but AST enzyme is present in many tissues including the heart, skeletal muscles, kidney and brain in addition to liver. Both enzymes are elevated to some extent in nearly all liver diseases. The highest level is found in associated with conditions causing extensive hepatic necrosis, toxin induced liver injury or prolonged circulatory collapse. Less elevations are encountered in diffuse and focal chronic liver diseases (e.g. chronic active hepatitis, cirrhosis, hepatic metastases) (Dacie and Lewis, 1991). Bromadiolone caused the lowest and highest elevation of s-ALT enzyme activity at 1.0 and 2.0 mg / kg body weight, equal to 20.0 and 346.7 % elevation, respectively, (Table 5). This reflected that bromadiolone may induce different effects such as diffuse and focal chronic liver disease in addition to extensive hepatic necrosis and liver injury in the treated mice. Coumachlor caused lower elevation of s-ALT enzyme activity ranged between 20.0 to 46.7 % at 0.1 to 1.0 mg / kg body weight whereas, the elevation jumped to 86.7 % at higher two doses.

So, coumachlor may cause extensive hepatic necrosis, liver injury in addition to diffuse and chronic diseases.

Coumatetralyl were slightly elevated the activity of s-ALT enzyme with narrow range between from 46.7 to 60.0 % elevation which may be caused by the initiation of some liver diseases or acted as a factor to show the symptoms.

Table (5): Effect of different doses of anticoagulant rodenticides on the activity of s-ALT enzyme (serum alanine transaminase) of mice females (*Mus musculus* var. *albus*) shown as (U/ L).

Compounds	Doses, mg / kg						LSD
	0.0	0.1	0.5	1.0	1.5	2.0	
Bromadiolone	7.5	7.5	7.5	9.0	12.0	33.5	1.7
Coumachlor	7.5	9.0	9.0	11.0	14.0	14.0	2.8
Coumatetralyl	7.5	11.0	11.0	11.5	12.0	12.0	3.6

F tab. at 0.05 = 3.11 at 0.01 = 5.06

s-AST enzyme activity (Table 6) was highly elevated with higher two tested doses of bromadiolone (1.5 and 2.0 mg / kg body weight) to 72.7 and 86.4 % elevation, respectively. So, bromadiolone may cause extensive hepatic necrosis, liver injury or prolonged circulatory collapse. Coumatetralyl weakly increased the s-AST activity at the highest two tested doses to (59.1 % elevation), which means that it was acted as a factor to initiate or show the liver disease symptoms. However all the tested anticoagulants were poorly affected the activity of s-AST enzyme of treated mice at the lower tested doses. It was found that, bromadiolone was strongly effective to elevate the activities of s-ALT and s-AST enzymes and coumachlor against s-ALT to produce hepatic necrosis and injury of liver specially at the higher tested doses, s-ALT enzyme was also sensitive to the anticoagulant coumatetralyl whereas, coumatetralyl and coumachlor were less effective against s-AST enzyme.

Table (6): Effect of three anticoagulant rodenticides on the activity of s-AST enzyme (serum aspartate transaminase) of mice females (*Mus musculus* var. *albus*) shown as (U/L).

Compounds	Doses, mg / kg						LSD
	0.0	0.1	0.5	1.0	1.5	2.0	
Bromadiolone	22.0	19.5	19.5	21.0	38.0	41.0	2.1
Coumachlor	22.0	21.0	21.0	22.0	25.0	27.0	3.8
Coumatetralyl	22.0	19.0	19.5	21.0	30.0	35.0	7.5

F tab. at 0.05 = 3.11

at 0.01 = 5.06

B- Histopathological Studies :

The histological changes of liver and testes tissues of mice were examined microscopically and photography recorded in Figs. (1 to 10), from which it could be deduce :

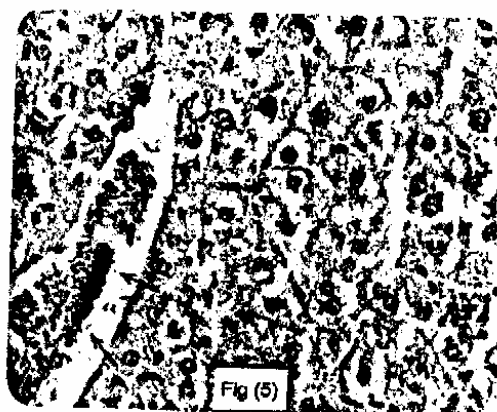
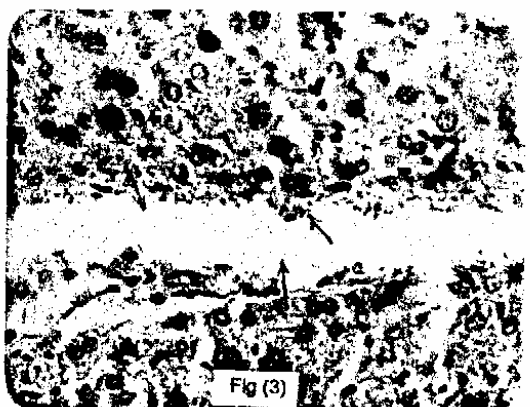
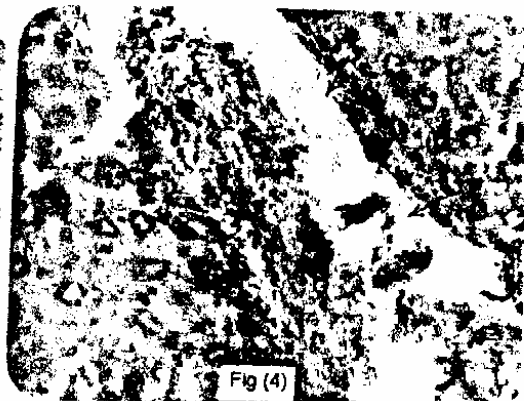
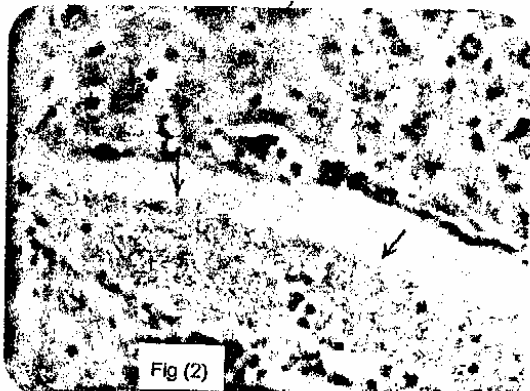
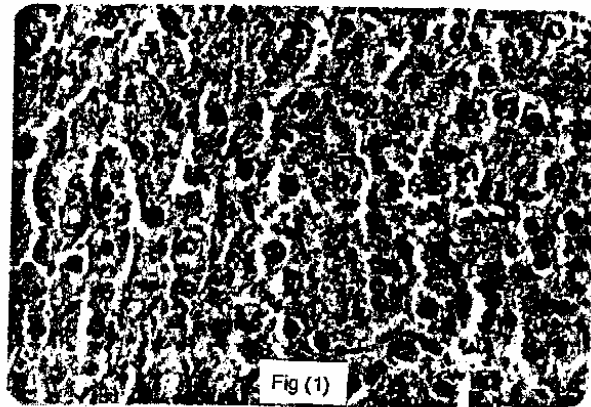
1- Effect of anticoagulants on liver tissues :

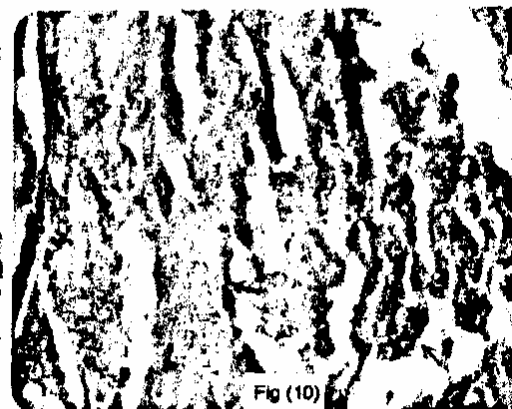
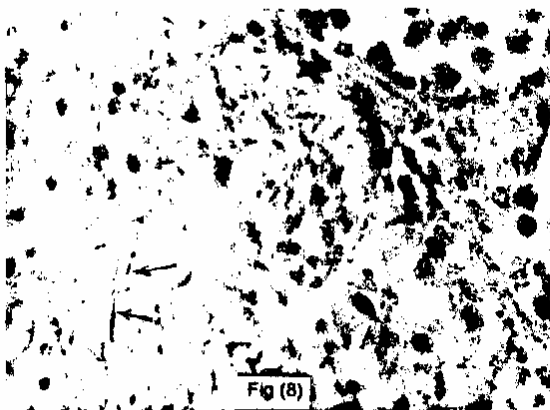
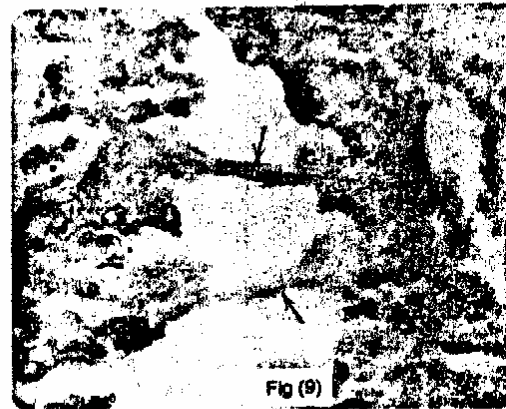
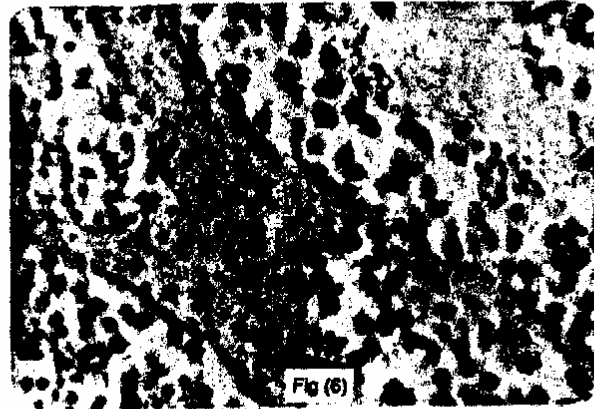
The section of liver of untreated mouse (Fig. 1) served as base line departure point for the histologic assessment of treated groups. Bromadiolone at 0.1 mg / kg body weight (Fig. 2) caused hepatic necrosis in liver cells which observed as necrotic cells; it has a destructive effects on the blood vessels which increased the lymphocyte cells, as compared with control. These results proved to be in agreement with the effects on haemoglobin as well as on s-ALT and s-AST enzymes and explain the reason of internal haemorrhage leading to death (Hazleton *et al.*, 1956).

Coumatetralyl (2.0 mg / kg) produced many necrotic cells (Fig. 4), which means that the normal shape of nuclear chromatin structure and DNA of cell as well as the blood vessels in liver were destroyed leading to death of cells. This compound at lower dose (0.1 mg / kg) caused the same effects but to less extent (Fig. 3). Coumachlor (0.1 mg / kg) slightly affected the liver, it weakly showed necrotic cells and lymphocyte cells (Fig. 5) as compared with control.

2- Effect of anticoagulants on testis tissues :

The testis section of corn oil treated mice (Fig. 6) were used as a baseline departure point for assessment of treated groups.





Key of Histological Figures

Fig (1): Section of Liver from untreated mouse.

Fig (2): Section of Liver from a mouse treated with 0.1mg / kg of bromadiolone three times a week (Hepatic necrosis cells).

Fig (3): Section of Liver from a mouse treated with 0.1mg / kg of coumatetralyl three times a week (Hepatic necrosis cells).

Fig (4): Section of Liver from a mouse treated with 2.0mg / kg of coumatetralyl three times a week (Destroyed blood vessels).

Fig (5): Section of Liver from a mouse treated with 0.1mg / kg of coumachlor three times a week (Hepatic necrosis cells).

Fig (6): L.S. of Testis from untreated mouse.

Fig (7): L.S. of Testis from a mouse treated with 0.1mg / kg of Bromadiolone three times a week (Spermatogonia).

Fig (8): L.S. of Testis from a mouse treated with 0.1mg / kg of coumatetralyl three times a week. (Sperms).

Fig (9): L.S. of Testis from a mouse treated with 2.0 mg / kg of coumatetralyl three times a week (Seminiferous tubules destructive effect).

Fig (10): L.S. of Testis from a mouse treated with 0.1mg / kg of coumachlor three times a week (Seminiferous tubules destruction).

Bromadiolone (0.1 mg / kg) completely destroyed the spermatogenesis and sperms were absolutely absent (Fig. 7). Coumatetralyl (2.0 mg / kg) strongly destroyed the seminiferous tubules and completely prevented the spermatogenesis, so the sperms were not seen (Fig. 9). However, coumatetralyl (0.1 mg / kg) slightly decreased the spermatogenesis and complete grown sperms were seen (Fig. 8), as compared with untreated mice. Coumachlor (0.1 mg / kg) strongly decreased the spermatogenesis and it had destructive effects on the seminiferous tubules and sperms (Fig. 10). However, bromadiolone (0.1 mg / kg) were very effective against liver and testes of treated mice but the changes in liver were more than in the testicle tissues. Coumatetralyl at the highest tested dose (2.0 mg / kg) was very effective against both liver and testicle tissues of treated mice, whereas, coumatetralyl at (0.1 mg / kg) was less effective in this respect. Coumachlor (0.1 mg / kg) was effective on testicle than liver tissues.

It could be concluded that bromadiolone was very effective against haemoglobin. Coumachlor, coumatetralyl and bromadiolone were very potent to decrease the count of WBC's in treated mice males in descending order whereas, RBC's of males mice were extremely reduced by bromadiolone, coumachlor and coumatetralyl. Haematocrit weakly affected by the three tested anticoagulants. Bromadiolone and coumachlor were very active to cause harmful changes in both liver and testicle tissues but bromadiolone was the strongest on liver and coumachlor on testis. Coumatetralyl based on the applied dose levels, its effects on both liver and testis were increased with increasing the dose levels.

From the obtained results, it gives the guide light for the precaution during the application and handling of these tested anticoagulant rodenticides.

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Received 18 / 10 / 2004
Accepted 20 / 11 / 2004

التأثيرات الهيماتولوجية والبيوكيميائية والهستوباثولوجية لمبيدات القوارض مانعة التجلط على فئران التجارب السويسرية البيضاء

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اختبرت ثلاثة من مبيدات القوارض المسيلة للدم وهي البروموديلون، الكوماكلور، والكوماتتراليل بالجرعات تحت المميته على بعض الأنظمة الحيوية مثل مكونات الدم (الهيموجلوبين، الهيماتوكريت، أعداد كرات الدم الحمراء والبيضاء) كما اختبرت على بعض أنزيمات الدم (الأنيز، ترانس أمينيز وأسبرتات ترانس أمينيز) كما درست تأثيراتها المرضية على بعض الأنسجة مثل خلايا الكبد والحوصلات المنوية والأسبرمات وذلك على فئران التجارب الصغيرة البيضاء (*Mus musculus var. albus*).

وقد أوضحت النتائج إنخفاض نسبة الهيموجلوبين بشدة من تأثير البروموديلون بجرعة فعالة (ED_{50}) (٠,٩ مجم / كجم من وزن الجسم) ولكن تأثير هذه المبيدات على الهيماتوكريت (حجم كرات الدم المنضغطة) كان ضعيفاً .

إنخفضت بشدة أعداد كرات الدم البيضاء في ذكور الفئران المعاملة بمبيدات الكوماتتراليل ، الكوماكلور ، والبروموديلون بجرعات فعالة (ED_{50}) بلغت ٠,٥٦ ، ٠,٨ ، ٠,٩ (مجم / كجم من وزن الجسم) على التوالي. كما إنخفضت أعداد كرات الدم الحمراء بشدة نتيجة المعاملة بمبيدات البروموديلون، الكوماكلور، والكوماتتراليل بجرعات بلغت ٠,٠٥ ، ٠,١ ، ٠,٥ (مجم / كجم من وزن الجسم) على التوالي ، ودلت النتائج أيضاً أن كرات الدم البيضاء والحمراء في إناث الفئران المعاملة قد تأثرت بقوة بهذه المبيدات بجرعات تراوحت ما بين (١,١ - ٤ مجم / كجم من وزن الجسم) ولكن كرات الدم الحمراء في الإناث المعاملة كانت أكثر حساسية لهذه المبيدات المختبرة عن كرات الدم البيضاء .

سبب مبيد البروموديلون زيادة في نشاط كلا الإنزيمين المختبرين s-AST ، s-ALT (الأنيز ترانس أمينيز والأسبرتات ترانس أمينيز) بجرعات ١,٥ و ٢ مجم / كجم من وزن الجسم بينما الكوماكلور رفع نشاط إنزيم s-ALT بجرعات بلغت ١ - ٢ مجم/كجم من وزن الجسم والذي نتج عنه تقرحات كبدية وإصابة الكبد ، وأيضاً أثر مبيد الكوماتتراليل على إنزيم s-ALT بجرعات ٠,١ - ٢ مجم / كجم من وزن الجسم، ولكن الكوماتتراليل والكوماكلور كانا أقل فعالية وتأثيراً على نشاط إنزيم s-AST .

أظهرت الدراسات التشريحية على خلايا الكبد تقرحات كبدية وتحطم للأوعية الدموية في أنسجة الكبد نتيجة المعاملة بمبيد البروموديلون (٠,١ مجم / كجم) بينما سبب مبيد الكوماتتراليل نفس التأثيرات بجرعة أعلى بلغت ٢ مجم/كجم كما حدث تأثير شديد على الحويصلات المنوية والقنوات المنوية والأسبرمات بالمعاملة بمبيدات الكوماكلور (٠,١ مجم/كجم) و الكوماتتراليل (٢ مجم/كجم) والبروموديلون (٠,١ مجم / كجم) وذلك بالمقارنة بالفئران الغير معاملة .