

SUSCEPTIBILITY OF THE ALBINO NORWAY RAT, *Rattus norvegicus* TO CERTAIN ANTIOCOAGULANT RODENTICIDES.

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ABSTRACT

Laboratory studies were carried to investigate the susceptibility of three anticoagulant rodenticides, Brodifacoum, Chlorophacinone and Warfarin on both sexes of albino Norway rat, *R. norvegicus* which were fed on either equally mixture of maize and wheat or vegetables. The obtained results could be summarized in the following:-

A- Determination of LD values of three anticoagulant rodenticides:-

The final results proved that males of *R. norvegicus* which fed on either mixture of wheat and maize or vegetables were more tolerant than females approximately. In addition, the gained figures cleared that both sexes of *R. norvegicus* which fed on mixture of wheat and maize were more susceptible to Chlorophacinone than Brodifacoum and Warfarin. In spite of, the tested animals which fed on vegetables were more susceptible to Brodifacoum, they had tolerance to Warfarin.

B- Effect of $1/10$ of LD values of the tested anticoagulant rodenticides on bleeding time of both sexes of *R. norvegicus*:-

The results in case of feeding on maize and wheat showed a highly significant differences between rodenticides after 24, 48 and 72h at $1/10$ LD₉₀ and $1/10$ LD₅₀, while there was no any significant difference after 48 and 72h post-treatment.

The effect of $1/10$ LD₉₀ and $1/10$ LD₅₀ of the tested rodenticides on bleeding time of *R. norvegicus* which fed on maize and wheat could be arranged according to their effectiveness in descending order as follows; Brodifacoum > Chlorophacinone > Warfarin. Also, results showed no significant difference between replicates and sexes at the three periods of bleeding time whereas, in the case of feeding on vegetables, the results revealed a highly significant differences between treatments at the dose of $1/10$ LD₉₀. Also, the results showed a highly significant differences between the investigated doses during three periods in the two cases of feeding except for the Warfarin treatment after 72h.

INTRODUCTION

Despite the fact that chemical control of rodents has been practiced for more than 2000 years. It was only 35 years ago that the introduction of anticoagulant rodenticides revolutionised the efficacy and safety control of rodents (Dubock, 1979). The majority of anticoagulants chemically belong to coumarin group. Naturally coumarins are present in plants in free as well as conjugated group (Hagan *et al.*, 1967).

Many investigators gave their interests on their field such as Hazelton *et al.*, 1956, Feuer *et al.*, 1965, Patyza, *et al.*, 1965, Ophof and Langeveld, 1969, Grand, 1976 and Wang, 1978. Thus, the aim of this work was to investigate susceptibility of each of Chlorophacinone, Warfarin and Brodifacoum to the Norway rat, *Rattus norvegicus*, var. albino.

MATERIALS AND METHODES

Experimental design:-

1-Anticoagulants used:-

Three anticoagulants were tested during this study, i.e Warfarin, Chlorophacinone and Brodifacoum which were obtained from Glaxowellcome U.K., Beziers, France and ICI companies, respectively.

1-a- Warfarin:-

- **Common name:** Warfarin, coumafene.

- **Chemical name:** 3- (∞ - acetyl benzyl) – 4 hdroxy coumarin.

1-b- Chlorophacinone:-

- **Common name:** Chlorophacinone.

- **Chemical name:** 2- [2- (4 chlorophenyl) – 2- phenyl acetyl] indan – 1, 3-dione.

1-c- Brodifacoum:-

- **Common name:** Brodifacoum, klerat, and Talon.

- **Chemical name:** 3-[3-(4-bromo (1,1-biphenyl)-9 yl)-1, 2 – 3, 4 – tetrahydro – 1 naphthalenyl] – 4- hydroxyl – 2H - 1 – benzopyran – 2 – one.

2- Tested animals:-

Albino adults of Norway rats *Rattus norvegicus* were obtained from culture of experimental animals in Helwan, (Egyptian Organization for Biological Products and Vaccine). Rats were individually reared, acclimatized under laboratory conditions. Active and healthy rats of both sexes were chosen for mating to obtain a healthy offspring. The resultant offspring were fed on a standard laboratory ration till maturity stage. The active, healthy and similar weight as possible of Norway rats of males and virgin females were randomly chosen and separated into two groups. The first group was fed on equally mixture of wheat and maize (1:1), while, the second one was fed on vegetables for two weeks, then weighed before treatments.

3- Determination of various lethal values (LD₃₀, LD₅₀ and LD₉₀):-

Serial different doses of the tested rodenticides, Warfarin, Chlorophacinone and Brodifacoum active ingredient were calculated as mg/kg body weight were prepared. For each dose, four adults of each of males and females were administrated by oral intubation and were caged individually. A parallel control test was conducted using a solvent for the tested rodenticides. Mortality percentages were recorded up to 28 days post treatment.

4- Determination of bleeding time:-

Bleeding time (B.T), was measured to animals (fed on wheat and maize) which treated with $1/10LD_{50}$ and $1/10LD_{90}$ of certain rodenticides (Brodifacoum, Chlorophacinone and Warfarin) according to Duke (1910). Animal's tail was pricked sharply with a needle, the oozing blood was consequently blotted with filter paper until bleeding stopped. The bleeding time was recorded in seconds by using a stopwatch. The difference percentage was calculated according to the following equation:

$$\% \text{ Difference} = \frac{(\text{Post-treatment}) - (\text{Pre-treatment})}{\text{Pre-treatment}} \times 100$$

5- Statistical analysis:-

LD₃₀, LD₅₀ and LD₉₀ values were calculated by probit regression analysis. Also analysis of variance and T test were calculated by using Finney 1971 and slide write and COSTAT programs.

RESULTS AND DISCUSSION

1- Acute oral toxicity determination of certain rodenticides:-

LD₃₀, LD₅₀ and LD₉₀ of each of Brodifacoum, Chlorophacinone and Warfarin were detected to the males and females of the albino Norway rat *Rattus norvegicus* which were fed on either (maize and wheat) or vegetables for two weeks.

1-A- Brodifacoum toxicity on albino rat *R. norvegicus*:-

Data present in Table (1) showed that the LD₃₀, LD₅₀ and LD₉₀ of Brodifacoum for males of *R. norvegicus* which fed on mixture of maize and wheat were 0.11, 0.23 and 1.64 mg/kg b.w. while for females were 0.26, 0.35 and 2.70 mg/kg b.w. . On the other side the LD₃₀, LD₅₀ and LD₉₀ of Brodifacoum for males and females which were fed on vegetables were (0.13, 0.23 and 0.95 mg/kg) and (0.12, 0.23 and 1.2 mg/kg body weight) respectively Table (2).

1-B- Chlorophacinone toxicity on albino rat, *R. norvegicus*:-

LD₃₀, LD₅₀ and LD₉₀ which tabulated in Table (1) were 6.87, 12.81 and 58.61 mg/kg b.w. for males, but they were 7.18, 16.043 and 114.84 for females which were fed on mixture of maize and wheat. While they were 13.33, 24.53 and 108.88 mg/kg b.w. for males, but they were 14.56, 26.52 and 113.72 mg/kg b.w. for females which they fed on vegetables, respectively, in Table (2).

1-C- Warfarin toxicity on albino rat, *R. norvegicus*:-

Data illustrated in Table (1) showed that LD₃₀, LD₅₀ and LD₉₀ of Warfarin they were 84.14, 151.19 and 633.1 mg/kg b.w. for males, but they were 85.29, 196.75 and 1517.28 mg/kg b.w. for females which were fed on mixture of maize and wheat. While those were 146.87, 333.03 and 2483.03 mg/kg b.w. for males, but in case of females they were 210.59, 435.94 and 2583.63 mg/kg b.w. which were fed on vegetables respectively Table (2).

The previous data proved that the males are more sensitive than females of *R. norvegicus* in all doses used of the tested rodenticides and for the two types of food at LD₃₀, LD₅₀ and LD₉₀. The LD₃₀, LD₅₀ were higher in case of vegetables feeding than maize and wheat in both LD values. Also, the data revealed that LD₃₀, LD₅₀ were higher in case of vegetables feeding than in case of mixture of maize and wheat feeding in both sexes. In addition, the results reported that Brodifacoum was the most effective followed by Chlorophacinone and Warfarin in both sexes for the two types of food. These results agreed with several authors e.i, Bull, 1976 who evaluated the acute oral single dose LD₅₀ value to albino Norway rats *R. norvegicus* when treated with Warfarin was 186.0 mg/kg. Meanwhile, Thonison, 1976 found that LD₅₀

was 6.26 mg/kg for Chlorophacinone. Also March *et al.*, 1980 found that the LD₅₀ was 180 mg/kg for Warfarin on the previous rat. As well as, Mathur and Prakash, 1981 calculated LD₅₀ of *R. rattus* to Brodifacoum were 0.73 and 0.65 mg/kg for males and females respectively. There fore Johnson and Scott, 1986 evaluated the LD₅₀ of *R. norvegicus* was 0.26 mg/kg for Brodifacoum.

Table (1): Effect of three anticoagulant rodenticides at different doses against both sexes of albino Norway rat, *R. norvegicus* fed on maize and wheat.

Rodenticides	Sex	LD ₃₀ mg/kg	LD ₅₀ mg/kg	LD ₉₀ mg/kg
Brodifacoum	Male	0.11 (0.07 – 0.13)	0.23 (0.17 – 0.3)	1.64 (1.33 – 2.74)
	Female	0.26 (0.98 – 0.21)	0.35 (0.3 – 0.42)	2.70 (2.1 – 3.9)
Chlorophacinone	Male	6.87 (4.44 – 8.38)	12.81 (09.24 – 16.74)	58.61 (49.99 – 100.46)
	Female	7.18 (06.65 – 8.87)	16.04 (016.15 – 21.44)	114.84 (114.44 – 459.7)
Warfarin	Male	84.14 (65.76 – 100.14)	151.19 (113.62 – 183.05)	633.1 (531.12 – 870.28)
	Female	85.29 (38.69 – 110.52)	196.75 (113.52 – 307.77)	1517.28 (136393 - 4344.69)

Table (2): Effect of three anticoagulant rodenticides at different doses against both sexes of albino Norway rat, *R. norvegicus* fed on vegetables.

Rodenticides	Sex	LD ₃₀ mg/kg	LD ₅₀ mg/kg	LD ₉₀ mg/kg
Brodifacoum	Male	0.13 (0.095 – 0.15)	0.23 (0.18 – 0.27)	0.95 (0.8 – 1.27)
	Female	0.12 (0.095 – 0.14)	0.23 (0.2 – 0.27)	1.25 (1.07 – 1.58)
Chlorophacinone	Male	13.33 (10.11 – 15.75)	24.53 (19.71 – 29.76)	108.99 (93.06 – 152.63)
	Female	14.56 (12.28 – 16.64)	26.52 (23.03 – 30.07)	113.72 (98.06 – 139.6)
Warfarin	Male	146.87 (89.75–180.52)	333.03 (238.31– 436.01)	2483.03 (2058.87 – 4735.96)
	Female	210.59 (165.56–249.9)	435.95 (366.7 – 515.51)	2583.63 (2059.85– 3759.993)

2- Effect of Brodifacoum, Chlorophacinone and Warfarin rodenticides on bleeding time in both sexes of albino Norway rat, *R. norvegicus*:-

Data presented in Table (3) demonstrated the effect of sublethal doses, $1/10$ LD₉₀ and $1/10$ LD₅₀ of the three rodenticides, Brodifacoum, Chlorophacinone and Warfarin on the bleeding time during three periods, 24h, 48h and 72h, where albino Norway rat, *R. norvegicus* were fed on mixture of maize and wheat. The analysis of variance showed a highly significant difference between treatment, where (F) values were 60.7, 140.5 and 23.6 after 24, 48 and 72h at $1/10$ LD₉₀. whereas the (F) values were 64.4,

10.5 and 10.2 after 24, 48 and 72h at $1/10LD_{50}$. Also, it noticed that, the effect of those rodenticides on bleeding time, could be arranged according to their effectiveness in a descending order as follows: Brodifacoum > Chlorophacinone > Warfarin. The analysed data cleared that there is no any significant difference between replicates and both sexes at three periods of bleeding time. Exceptionally the case of 24h of bleeding time at $1/10LD_{50}$ which has a significant differences between males and females for the whole investigated rodenticides.

Table (3): Mean of bleeding time of the Norway rat, *R. norvegicus* (Fed on wheat and maize) which treated with $1/10LD_{50}$ and $1/10LD_{90}$ of certain rodenticides.

Treatment	Sexes	$1/10 LD_{90}$			Tabulated F	$1/10 LD_{50}$			Tabulated F
		Mean of bleeding time (in Sec.) after				Mean of bleeding time after			
		24h	48h	72h		24h	48h	72h	
Brodifa-coum	Male	167.5	162.5	127.5		167.5	157.5	126.25	
	Female	167.5	161.25	146.25		136.25	130	113.75	
	Total Mean	335	323.75	273.75		303.75	287.5	240	
Chloropha-cinone	Male	171.25	161.25	122.5		120	721.25	101.25	
	Female	153.75	132.5	120		120	113.75	100	
	Total Mean	325	293.75	242.5		240	235	201.25	
Warfarin	Male	116.25	106.25	83.75		115	111.25	97.5	
	Female	120	111.25	107.25		115	107.5	91.25	
	Total Mean	236.25	217.5	191.25		230	218.75	188.75	
Calculated (F) within	Treatment	60.7	140.5	23.6	5.14	64.4	10.5	10.2	5.14
	Rep.	1.12	1.24	0.12	4.76	0.78	0.93	1.38	4.76
	Sexes	0.7	3.55	1.13	4	4.02	0.49	0.36	4
L.S.D.	Treatment	17.1	11.32	20.99		11.99	27.13	20.54	
	Rep.	-	-	-					
	Sexes	-	-	-		11.99			

On the other hand, when albino Norway rat, *R., norvegicus* were fed on vegetables, the results revealed that the effect of sublethal doses ($1/10LD_{90}$ and $1/10LD_{50}$) of the same rodenticides on the bleeding time during the same periods. Highly significant difference between treatments at the dose $1/10LD_{90}$ were noticed during the three periods, where (F) values were 7.9, 19.4 and 98.7 after 24, 48 and 72h at $1/10LD_{90}$, respectively table (4). Also, there was a clear difference in bleeding time at the doses $1/10LD_{50}$ after 48h, while there is no considerable difference between treatments at the doses $1/10LD_{50}$ after 24h, 72h. Also, data in Table (4) showed that there were no significant differences between replicated and sexes at the two doses during three periods. Data obtained are similarly those findings by Gabr, (1997) who found that, there is no considerable difference between treated rats and control in the first day after treatment, which at the second and third day there was a clear difference in bleeding time between the treated and untreated rats when studied the effectiveness of different doses of investigated anticoagulant on the bleeding time of rats. In contrast El-Mahrouky, (1984) found that bleeding

time was greatly increased to about 15 time when albino Norway rat, *R. norvegicus* treated with LD₅₀ from Brodifacoum in comparison with untreated.

Table (4): Mean of bleeding time of the Norway rat, *R. norvegicus* vegetables which treated with $1/10$ LD₅₀ and $1/10$ LD₉₀ of certain rodenticides.

Treatment	Sexes	$1/10$ LD ₉₀			Tabulated F	$1/10$ LD ₅₀			Tabulated F
		Mean of bleeding time (in Sec.) after				Mean of bleeding time after			
		24h	48h	72h		24h	48h	72h	
Brodifa-coum	Male	108.75	92.5	92.5		85	75	180	
	Female	101.25	90	90		98.75	68.75	60	
Total	Mean	210	182.5	182.5		183.75	143.75	120	
Chlorophacinone	Male	108	84	60		94.5	71.25	60	
	Female	102	84	60		71.5	67.5	60	
Total	Mean	210	168	120		166	138.75	120	
Warfarin	Male	102.5	74.25	60		80	67.5	58.75	
	Female	92.5	76	60		72.5	63.25	58.75	
Total	Mean	195	150.25	120		152.5	130.75	117.5	
Calculated (F) within	Treatment	7.99	19.4	98.7	5.14	3.7	9.79	3	5.14
	Rep.	1.88	1.92	1	4.76	0.69	2.49	1	4.76
	Sexes	7.87	0.85	0.41	4	1.42	2.75	1.75	4
L.S.D.	Treatment	75	8.99	8.9		-	5.13		
	Rep.	-	-	-		-	-	-	
	Sexes	-	-	-		-	-	-	

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حساسية الفأر النرويجي (السلالة الألبينو) لبعض مبيدات القوارض المسيلة للدم عبد المقصود عبد المقصود محمد أبو هاشم معهد بحوث وقاية النباتات - مركز البحوث الزراعية - وزارة الزراعة - الدقي - الجيزة

تم اختبار حساسية ذكور وأنثى الفأر النرويجي (سلالة الألبينو) لبعض المبيدات المسيلة للدم (البرودايفاكوم - الكلوروفاسينون - الوارفارين) والتي تغذيها علي مخلوط من القمح والذرة الشامية (١ : ١) ومجموعة أخرى تم تغذيها قبل معاملةها بالمبيدات علي الخضروات. ويمكن تلخيص النتائج المتحصل عليها فيما يلي:-

١- تقدير قيم الجرعات المميتة (LD_{30} , LD_{50} and LD_{90}) الثلاثة أنواع من المبيدات المسيلة:-
أوضحت النتائج أن ذكور الفأر النرويجي والمتغذية علي كل من مخلوط القمح والذرة أو الخضار كانت أكثر تحملاً من الإناث. وبالإضافة الي ذلك فقد أكدت النتائج أن كلا من الذكور والإناث كانت أكثر حساسية للكلوروفاسينون عن البرودايفاكوم والوارفارين. بينما مجموعة الحيوانات التي تم تغذيها علي الخضروات كانت أكثر حساسية للبرودايفاكوم وأكثر تحملاً للوارفارين.

٢- تأثير ١٠/١ الجرعات المميتة للمبيدات المختبرة علي وقت التجلط:-
أظهرت النتائج وجود فروق معنوية عالية بين فترات المعاملة (٢٤، ٤٨، ٧٢ ساعة) بـ ١٠/١ الجرعات المميتة (٩٠، ٥٠) للمبيدات الثلاثة المختبرة. بينما لم يوجد أي فرق معنوي في فترتي ٤٨، ٧٢ ساعة بالنسبة لمجموعة الفئران التي تغذت علي مخلوط القمح والذرة. هذا وأمكن ترتيب المبيدات المختبرة تنازلياً كما يلي برودايفاكوم < كلوروفاسينون < الوارفارين وذلك وفقاً لتأثير ١٠/١ الجرعات المميتة (٩٠، ٥٠) التي تم معاملة الفئران المختبرة والمتغذية علي الخضروات. كما أوضحت النتائج وجود فروق معنوية عالية في الجرعة ١٠/١ (LD_{90}). هذا وأكدت النتائج علي وجود فروق معنوية عالية بين فترات الإختبار الثلاثة (٢٤، ٤٨، ٧٢ ساعة) بالنسبة للجرعات المختبرة ١٠/١ (LD_{30} , LD_{50} and LD_{90}) لكلا حالتي التغذية ماعدا معاملة الوارفارين بعد ٧٢ ساعة.