

## EFFICIENCY OF CERTAIN ANTICOAGULANT RODENTICIDES ON ALBINO NORWAY RAT, *Rattus norvegicus*

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### ABSTRACT

Laboratory studies were applied to investigate the efficiency of three anticoagulant rodenticides, Brodifacoum, Chlorophacinone and Warfarin on the both sexes of albino Norway rat, *Rattus norvegicus* which were fed on either maize and wheat or vegetables.

The obtained results could be summarized in the following :-

The results proved that, all three rodenticides induced a considerable reduction in numbers of implantation sites in both left and right horn uterus and these reduction widely differed according to the rodenticides type. The maximum reduction was observed in both cases with percentage of 100% when females were treated with Brodifacoum and Chlorophacinone at 1/10 LD90, while minimum reduction was noticed when females were feeding on maize and wheat treated with Warfarin at 1/10 LD30, where the percentage of reduction was 12.5%. On the other hand, the data appeared that the sublethal dose 1/10 LD30 of all anticoagulants have non-significant effect on both gestation period and weight of fetuses in the two groups. But they have a highly significant effect on the mean number of fetuses when females which were feeding on maize and wheat treated with Brodifacoum and Chlorophacinone. Also, the same results were obtained when females fed on vegetable treated with Brodifacoum. In addition, the obtained results cleared that the sublethal dose 1/10 LD30 from Brodifacoum in the two cases of feeding have a significantly increase on the mean time of weaning per days. While, there were non-significant increase on the mean time of weaning per days with other treatment in two cases of feeding. Also, there was non-significant increase on the mean of opening eyes in all treatment with the three rodenticides in two groups of feeding.

### INTRODUCTION

Rodents cause an economic loss to farmers, food manufacturers and processors as well as causing damage to the structure and buildings fabric. Undoubtedly the economic loss due to rodents is enormous particularly in the tropics, but it is impossible to exact monetary values on the damage caused. The World Health Organization estimates that about 33 million tons of food is destroyed world-wide each year.

The use of toxicants of chemical pesticides had been relatively ineffective, due to poison shyness, or behavioral resistance, developing as results of the rapid onset of poisoning symptoms before ingestion of a lethal dose had occurred (Grand, 1976). (Ophof and Langeveld, 1969) and (Wang, 1978). 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 (Hagen *et al.*, 1967).

Thus, the aim of this work was to investigate Chlorophacinone, Warfarin and the toxicity of Brodifacoum against the albino Norway rat, *Rattus norvegicus*, var. albino

## MATERIAL AND METHODS

### Experimental design:

#### 1-Anticoagulants used :

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

#### 1-a- Warfarin :-

##### - Chemical name:

3- (∞ - a cetonylbenzyl) - 4 hydroxy Coumarin.

#### 1-b-Chlorophacinone:

##### - Chemical name:

2- [ 2- (4 Chlorophenyl)-2- phenyl acetyl] indan - 1,3- dione.

#### 1-c- Brodifacoum:-

##### - Chemical name :

3- [ 3- (4- bromo (1,1 - biphenyl) - 4 yl) -1, 2- 3, 4 - tetrahydro - 1 naphthalenyl ] - 4- hdroxy - 2H - 1- benzopyran -2- one.

#### 2- Tested animals:-

Albino adults of Norway rat, *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's males and virgin females were randomly chosen and separated into two groups. The first group was fed on wheat and maize, while, the second was fed on vegetables for two weeks, then weighed before treatments.

#### 3- Parameters evaluated:-

##### 3-A- Determination of various leathal values (LD30, LD50 and LD90):-

Serial different doses of the various rodenticides, Warfarin, Chlorophacinone and Brodifacoum active ingredient calculated as mg/kg body weight were prepared. Four adults of each male and female rats, caged individually, were used for each dose administrated by oral incubation. A paralld control test was conducted using a solvent for the rodenticides tested. Mortality percentages were recorded up to 28 days post- treatment.

##### 3-C-Biologiagl effect of the three anticoagulants :-

The adult Norway rat, *R. norvegicus*, were divided males and females separately under laboratory conditions coded into two groups and



fed 2 weeks (wheat and maize for one group and vegetables for the other one). One male was paired with three females of each group were administered by the tested anticoagulants at the 1/10 LD90, 1/10 LD50 and 1/10 LD30 mg/kg body weight. After two weeks rats were desiccated. The gestation period, number of newly born, implantations, resorption, time weaning and opening eyes of the treated animals were recorded and compared with the control group.

**Statistical analysis :-**

1/10 LD90, 1/10 LD50 and 1/10 LD30 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

Laboratory studies were applied to investigate the efficiency of three anticoagulant rodenticides, Brodifacoum, Chlorophacinone and Warfarin on the both sexes of albino Norway rats, *R. norvegicus* which were fed on either maize and wheat or vegetables.

### 1- The toxicological effects of the three rodenticides acute oral toxicity Determinations, LD30, LD50 and LD90 :-

#### 1-A- Brodifacoum treatments on albino Norway rat, *R. norvegicus* :-

The presented data in tables 1-2 demonstrated the LD30, LD50 and LD90 in males which were fed on maize and wheat were; 0.11, 0.23 and 1.64 mg/kg b.w. while in females were 0.260, 0.354 and 2.70 mg/kg b.w. while the LD30, LD50 and LD90 in males which were fed on vegetables were; 0.13, 0.225 and 0.95 mg/kg but they were 0.12, 0.231 and 1.25 mg/kg b.w. respectively.

#### 1-B-Chlorophacinone treatments on albino Norway rat, *R. norvegicus*:-

The LD30, LD50 and LD90 were; 7.18, 16.043 and 114.84 in females which were fed on maize and wheat in males 1, 12.8 and 6.87 mg/kg b.w. while they were; 13.33, 24.532 and 108.88 mg/kg b.w. but they were; 14.56, 26.523 and 113.72 mg/kg b.w. in females which they fed on vegetables.

#### 1-C-Warfarin treatment on albino Norway rat, *R. norvegicus*:-

The LD30, LD50 and LD90 were; 48.14, 151.194 and 633.1 mg/kg b.w. in males. But they were; 85.29, 196.75 and 1517.28 mg/kg b.w. in females that were fed on maize and wheat. While those were; 146.87, 333.025 and 2483.63 mg/kg b.w. in females were fed on vegetables in males which were; 210.59, 435.945 and 2583.63.

These results agree with several authors e.i. Bull, 1976 who evaluated the acute oral single dose LD50 value to albino Norway rat, *R. norvegicus* when treated with Warfarin was 186.0 mg/kg. Thonison, 1976 found the LD50 was 6.26 mg/kg for Chlorophacinone, while, Marsh *et al.*, 1980 determined the LD50 was 180 mg/kg for Warfarin on the previous rat. Mathur and Prakash, 1981 calculated the LD50 at *R. rattus* to Brodifacoum were 0.73 and 0.65 mg/kg for males and females respectively, 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 <sub>30</sub> mg/kg	LD <sub>50</sub> mg/kg	LD <sub>90</sub> mg/kg
Brodifacoum	Male	0.11 (0.07-0.13)	0.234 (0.17-0.3)	1.64 (1.33-2.74)
	Female	0.260 (0.98-0.21)	0.354 (0.3-0.42)	2.70 (2.1-3.9)
Chlorophacinone	Male	6.87 (4.44-8.38)	12.805 (09.24-16.74)	58.61 (49.99-100.46)
	Female	7.18 (06.65-8.87)	16.043 (016.15-21.44)	114.84 (114.44-459.7)
Warfarin	Male	84.14 (65.76-100.14)	151.194 (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 (1363.93-4344.69)

**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 <sub>30</sub> mg/kg	LD <sub>50</sub> mg/kg	LD <sub>90</sub> mg/kg
Brodifacoum	Male	0.13 (0.095-0.15)	0.225 (0.18-0.27)	0.95 (0.8-1.27)
	Female	0.12 (0.095-0.14)	0.231 (0.2-0.27)	1.25 (1.07-1.58)
Chlorophacinone	Male	13.33 (10.11-15.75)	24.532 (19.71-29.76)	108.99 (93.06-152.63)
	Female	14.56 (12.28-16.64)	26.523 (23.03-30.07)	113.72 (98.06-139.6)
warfarin	Male	146.87 (89.75-180.52)	333.025 (238.31-436.01)	2483.03 (2058.87-4735.96)
	Female	210.59 (165.56-249.9)	435.945 (366.7-515.51)	2583.63 (2059.85-3759.993)

The tabulated results in Tables (3-4) cleared that the effect of the 3 sublethal doses (1/10 LD<sub>90</sub>, 1/10 LD<sub>50</sub> and 1/10 LD<sub>30</sub>) from the 3 rodenticides on the pregnant females of albino Norway rat. All the rodenticides induced a considerable reduction in numbers of implantation sites in both left and right horn uterus. Obtained results showed that Brodifacoum, Chlorophacinone and Warfarin at 1/10 LD<sub>90</sub> in the two cases of feeding were more effective than the other sublethal doses, all implantation sites formed fetuses without any resorbed ones. A significant reduces in the mean of implantation when females were treated with Brodifacoum and Chlorophacinone at 1/10 LD<sub>90</sub> in both cases of fed. A



significant reduce in the mean of implantation when females were fed on maize and wheat treated with Brodifacoum at 1/10 LD50, while, there was non-significant in the mean of implantation in the other rodenticides.

The maximum reduction reached 100% when females were treated with Brodifacoum and Chlorophacinone at 1/10 LD90, while the minimum reduction was 12.5% when females were fed on Maize and Wheat treated with Warfarin at 1/10 LD30.

Therefore the gained figures in the same tables illustrated the effect of 1/10 LD30 of the 3 rodenticides on females and fetuses of albino Norway rat. For gestation period and number and weight of fetuses. The obtained results showed that the sublethal doses 1/10 LD30 of all rodenticides used has non-significant effect on both gestation period and weight of fetuses in the two groups but they have a highly significant effect on the mean number of fetuses when females were fed on maize and wheat with Brodifacoum and Chlorophacinone, also, the same results were obtained when females were on vegetables treated with Brodifacoum, while, the other treatments gave a significant effect on the number of fetuses in the two groups of feeding.

On the other hand, the weaning time and opening eyes the obtained results showed that the sublethal dose 1/10 LD30 from Brodifacoum in the 2 cases of feeding has a significant increase in the mean time of weaning per days, while, there were non-significant increase on the mean time of weaning per days of other treatments in the 2 cases of fed, also, there were non-significant increase on the mean of opening eyes in all treatments with the 3 rodenticides in the two groups of feeding. All the 3 rodenticides were very active and causing increase in the percentages of absorbed fetuses in all the sublethal doses.

Youssef (1994), showed that the results obtained indicated that the sublethal does of the tested rodenticides caused abortion and resorption fetuses to the treated pregnant females during the differewnt weeks. Also, Ibrahim (2001), stated the effect of  $\frac{1}{4}$  LD50 of same plant extracts on pregnancy of albino rat. The author was remarkable the drastic potential harmful effects on the fetuses when females treated with plant extracts at the progressed pregnancy stages. Also, he was observed the resorption effect as internal hamorrhage inside the uterus of the pregnant females when compared with those of the untreated pregnant females. Finally, El-Essely, (2002), proved that the pregnant female rats treated with the sublethal doses of Chlorophacinone and Warfain at the 3rd day of pregnancy the percentage of resorbed fetuses increased in all the doses depending on the dose level. The author, also, showed that tested anticoagulants have an embeyototoxicity effect on the pregnant female rats during the first week of gestation period. Also, he was found non-significant effect for the two tested anticouglulant neither on the gestation period of the treated pregnant females, nor on the weight of the results fetuses from treated mothers.

Table (3): Biological effect of the anticoagulants on numbers of implantation and resorption of embryos of albino Norway rat *R. norvegicus* fed on vegetables.

Dose mg/kg	Uterus	Brodifacoum						Anticoagulant						Warfarin			
		Mean + S.E. implantation		Mean + S.E. resorption	% resorption	Computed T implantation	Mean + S.E. implantation	Mean + S.E. resorption	% resorption	% reduction	Computed T implantation	Mean + S.E. implantation	Mean + S.E. resorption	% resorption	% reduction	Computed T implantation	
		Mean + S.E. implantation	Mean + S.E. resorption	% resorption	% reduction	Computed T implantation	Mean + S.E. implantation	Mean + S.E. resorption	% resorption	% reduction	Computed T implantation	Mean + S.E. implantation	Mean + S.E. resorption	% resorption	% reduction	Computed T implantation	
1/10 LD <sub>50</sub>	Left	0	0	0	0	0	0	0	0	0	2 + 0.35	1 + 0.35	50%				
	Right	0	0	0	0		0	0	0		3 + 0	3 + 0	100%				
	Total	0	0	0	100%	7.5**	0	0	100%	7.5**	5 + 0.35	4 + 0.35	80%	42.9%	2.01		
1/10 LD <sub>50</sub>	Left	3 + 0.61	2 + 0.35	66.6%			3 + 0.35	1 + 0.35	33.3%		3 + 0.5	1 + 0.5	33.3%				
	Right	2 + 0.61	1 + 0	50%			3 + 0.5	3 + 0.5	100%		3 + 0.0	2 + 0	66.6%				
	Total	5 + 1.1	3 + 0.35	60%	28.6%	1.39	6 + 0.61	4 + 0.61	66.6%	14.3%	0.9	4 + 0.5	4 + 0.5	50%	14.3%	0.95	
1/10 LD <sub>30</sub>	Left	3 + 0	2 + 0.35	66.6%			3 + 0.61	0	0%		3 + 0.5	0	0%				
	Right	3 + 0.35	0	0%			4 + 0.61	0	0%		4 + 0.79	1 + 0.79	25%				
	Total	6 + 0.35	2 + 0.35	33.6%	14.3%	1.01	7 + 0.0	0	0%	0%	0	1 + 0.5	1 + 0.5	14.3%	0		
Control	Left	4 + 0.93	0	0%													
	Right	3 + 0.5	0	0%													
	Total	7 + 0.93	0	0%													



Table (4): Biological effect of 1/10 LD<sub>50</sub> of the rodenticides on female and fetuses of albino Norway rat *R. norvegicus* fed on maize and wheat.

Treatments	Mean of gestation period / day	Mean + S.E. No. of fetuses			Computed T	Mean weight of fetuses (gm)	Computed T	Mean of time weaning (days)	Computed T	Mean of opening eyes (days)	Computed T
		Alive	Mortality	% Mortality							
Brodifacoum	21.5 ± 0.29	1 ± 0.35	3 ± 0.4	75	6.84**	4.2 ± 0.45	2.37	27 ± 0.79	3.5*	12 ± 0.61	2.39
Chlorophacinone	21.5 ± 0.35	3 ± 0.45	2 ± 0.82	40	4.71**	4.3 ± 0.61	1.97	25 ± 0.41	2.18	11 ± 0.41	1.92
Warfarin	22 ± 0.41	4 ± 0.61	1 ± 0.4	20	3.51*	4.7 ± 0.56	1.57	25 ± 0.61	1.96	11 ± 0.81	1.59
Control	21 ± 0.91	8 ± 0.96	1 ± 0.61	11.11		6.0 ± 0.61		23 ± 0.82		9 ± 0.96	

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## كفاءة بعض المبيدات المسيلة للدم ضد الفأر النرويجي (الألبينو)

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أجريت دراسات معملياً لدراسة بعض التأثيرات السمية والبيولوجية لثلاث مبيدات مسيلة للدم وهي بروديفاكوم، كلورفاسينون، ووارفارين. وذلك علي ذكور وإناث الفأر الألبينو المتسلق النرويجي وفؤيرة المنازل الألبينو الذي تم تغذيتهم إما علي القمح والذرة أو علي الخضار لمدة أسبوعين قبل المعاملة وعلي نفس التغذية بعد المعاملة.

ويمكن تلخيص النتائج المتحصل عليها فيما يلي:-

- ١- كانت الجرعات المميتة (ج.م.) ٠.٠٠٠، (ج.م.) ٠.٠٠٠، (ج.م.) ٠.٠٠٠، لذكور الفأر النرويجي الذين تم تغذيتهم علي الذرة والقمح المعامل بالبروديفاكوم كانت ٠.٠١١، ٠.٠٢٢٤، ١.٦٤ ملجم/كجم من وزن الجسم بينما كانت للإناث ٠.٠٢٦، ٠.٠٣٥٤، ٢.٧ ملجم/كجم من وزن الجسم علي التوالي وكانت (ج.م.) ٠.٠٠٠، (ج.م.) ٠.٠٠٠، (ج.م.) ٠.٠٠٠، للذكور التي تم تغذيتهم علي الخضار ٠.٠١٣، ٠.٠٢٢٥، ٠.٠٩٥ ملجم/كجم من وزن الجسم وللإناث ٠.٠١٢، ٠.٠٢٣١، ١.٢٥ ملجم/كجم من وزن الجسم علي التوالي.
- ٢- بينما كانت (ج.م.) ٠.٠٠٠، (ج.م.) ٠.٠٠٠، (ج.م.) ٠.٠٠٠، لإناث الفأر النرويجي الذين تم تغذيتهم علي القمح والذرة المعامل بالكلوروفاسينون، بينما كانت للذكور ١.٦٨٧، ١٢.٨٠٥، ٥٨.٦١ ملجم/كجم من وزن الجسم وكانت ١٤.٥٦، ٢٦.٥٢٣، ١١٣.٧٢ ملجم/كجم من وزن الجسم وللإناث الذين تم تغذيتهم علي الخضار وللذكور 13.33، ٢٤.٥٢٢، ١٠٨.٩٩ ملجم/كجم من وزن الجسم علي التوالي.
- ٣- أما بالنسبة لمبيد الوارفارين كانت قيم (ج.م.) ٠.٠٠٠، (ج.م.) ٠.٠٠٠، (ج.م.) ٠.٠٠٠، للذكور كانت ٤٨.١٤، ١٥١.١٩٤، ٦٣٣.١ ملجم/كجم من وزن الجسم بينما للإناث كانت ٨٥.٢٩، ١٩٦.٧٥، ١٥١٧.٢٨ ملجم/كجم من وزن الجسم الذي تم تغذيتهم علي القمح والذرة علي التوالي. وكانت ١٤٦.٨٧، ٣٣٣.٠٢٥، ٢٤٨٣.٠٣ ملجم/كجم من وزن الجسم للإناث التي تم تغذيتهم علي الخضار والذكور ٢١٠.٥٩، ٤٣٥.٩٤٥، ٢٥٨٣.٦٣ ملجم/كجم من وزن الجسم علي التوالي.
- ٤- النتائج المتحصل عليها أظهرت أن البروديفاكوم والكلوروفاسينون والوارفارين عند الجرعة ١/١٠ الجرعة التحت مميتة القاتلة لـ ٩٠% في حالي التغذية كانت أكثر فاعلية عن الجرعتين الأخرتين. جميع أماكن زرع الأجنة أنتجت أجنة بدون أمتصاص لأي منها. كان هناك نقص معنوي في متوسط عدد أماكن زرع الأجنة عند معاملة الإناث بكل من البروديفاكوم والكلوروفاسينون عند الجرعة ١/١٠ الجرعة التحت مميتة القاتلة لـ ٩٠% من الحيوانات المختبرة. وكان هناك نقص معنوي في متوسط عدد أماكن زرع الأجنة عندما تغذت الإناث علي الذرة والقمح المعاملة بالبروديفاكوم بالجرعة ١/١٠ الجرعة القاتلة لـ ٥٠% بينما كان هناك فرق غير معنوي في متوسط أماكن زرع الأجنة وذلك عند استخدام المبيدين الأخرين.
- وكان الحد الأعلى للنقص قد وصل إلي ١٠٠% عند معاملة الإناث بالبروديفاكوم والكلوروفاسينون عند الجرعة ١/١٠ الجرعة التحت مميتة القاتلة لـ ٩٠% بينما كان الحد الأدنى للنقص كان ١٢.٥% عند تغذية الإناث علي القمح والذرة ومعاملة بالوارفارين عند ١/١٠ الجرعة التحت مميتة القاتلة لـ ٣٠%.
- ٥- أظهرت النتائج المتحصل عليها أن الجرعة ١/١٠ الجرعة التحت مميتة القاتلة لـ ٣٠% وذلك لمبيدات القوارض الثلاثة أن هناك تأثير غير معنوي علي كلا من فترة الحمل ووزن الأجنة في مجموعتي التغذية لكن التأثير كان معنوياً علي متوسط أعداد الأجنة عند تغذية الإناث علي القمح والذرة وذلك باستخدام البروديفاكوم والكلوروفاسينون.
- ٦- أوضحت النتائج المتحصل عليها أن الجرعة تحت مميتة ١/١٠ الجرعة القاتلة لـ ٣٠% لمبيد البروديفاكوم كان لها زيادة معنوية في فترة الفطام بينما كان هناك زيادة معنوية في متوسط فترة الفطام للمعاملات الأخرى وأيضاً كان هناك زيادة غير معنوية في متوسط تفتح الأعين في كل المعاملات وفي كل من مبيدات القوارض الثلاثة.