

EFFICIENCY OF WARFARIN ANTICOAGULANT ON THE BLACK RAT POPULATIONS TRAPPED FROM DIFFERENT DISTRICTS OF SHARKIA GOVERNORATE

GABR, W.M.

Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt

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Abstract

Efficiency of warfarin anticoagulant was studied against different populations of the black rat, *Rattus rattus*, caught from 4 districts at Sharkia governorate (Belbies, Kafr-Sakr, Menia El-Khamh and Hehya districts) using biological method (lethal feeding periods "LFP") and biochemical technique (prothrombin time "P.T" determination). The results showed that the highest values of LFP50 and LBP99 were recorded with rats trapped from Hehya (3.4 and 9.9 days), while the lowest values were in case of rats trapped from Belbies (2.6 and 6.9 days). Data obtained from biochemical test were in the same trend whereas effect of warfarin (5mg/kg) on P.T. values was obviously noticed in animals caught from Belbies, while the lowest effect was observed on the animals of Hehya. Results indicated that rats trapped from Hehya showed a high tolerance to warfarin, while those caught from Belbies were relatively susceptible followed by those from Kafr-Sakr and Menia El-Khamh districts.

INTRODUCTION

There are several anticoagulant rodenticides in use today which have a similar chemical structure to warfarin and others which are not related chemically, but have similar physiological action. Thus it is not surprising that rodent resistance to warfarin may also be cross-resistant to other commonly used anticoagulants. The first indication that cross-resistance could occur was noted in the Scottish rat population where resistance was first described (Boyle, 1960 and 1965).

In Egypt, the anticoagulant rodenticides have been used on a large scale to control rodents either in agriculture or for public health purposes. However, there are very little studies carried out to determine the susceptibility level of the Egyptian commensal rodents after this long and extensive use of anticoagulants (Kandil et al., 1994).

In this study an attempt had been conducted to check the present status of the

susceptibility level of black rat, *Rattus rattus*. It is considered as one of the most common and harmful rodent species in Sharkia governorate in which anticoagulant rodenticides had been early used with high amounts (General Department for Rodent Control, Ministry of Agriculture, Egypt).

MATERIAL AND METHODS

1. Rodenticides Backhistory: A survey of previously used anticoagulant rodenticides was conducted in the field of Belbies, Kafr-Sakr, Menia El-Khamh and Hehya districts at Sharkia governorate during the period from 1990 to 1999. Total amount of each rodenticide was recorded and the amount per feddan was calculated and summarized in Table 1. Such data were obtained from the General Department for rodent Control, Ministry of Agriculture, Egypt.

Data showed that five different anticoagulants were used, i.e. brodifacoum, bromadiolone, chlorophacinon, difenacoum and coumatetralyl. The greater amount of rodenticides applied per feddan was recorded in Hehya district (0.903kg), followed by Kafr-Sakr (0.572kg), and Menia El-Khamh (0.375kg), while the lowest amount was used in Belbies district (0.236kg).

2. Chemicals Used: Warfarin anticoagulant: 3-(α -acetylbenzyl)-4-hydroxycoumarin, (98%) as water insoluble powder was supplied by KZ pesticides Company, Egypt. It was used either as a stock stable suspension in saline solution (0.85% NaCl) using tween 80 for oral intubation or mixed with crushed maize at a concentration of 0.025% as a feeding bait.

3. Tested Animals: Black rat individuals, *Rattus rattus*, were trapped from 4 different districts (Belbies, Kafr-Sakr, Menia El-Khamh and Hehya) at Sharkia governorate. Trapped animals were transferred to the laboratory, caged individually for two weeks for acclimatization and fed on a free standard diet (65% crushed maize + 25% ground wheat + 5% sugar + 5% corn oil) and water. The unhealthy and pregnant animals were excluded. A few days before the test, animals were sexed, weighed and given a reference number for each animal.

4. Methods

4.1. Biological Method: The lethal feeding periods (LFP) method as recommended by *WHO (1982)* was used. Animals were fed on warfarin bait (0.025%) for various fixed periods and the linear relationship between feeding periods (log. days) and probit mortality was established. The feeding periods produced 50 and 99% mortality were derived and used as a checking periods in the subsequent checks.

4.2. Biochemical Method: Ten animals of each district mentioned before were fasted for 12h with free water, then orally intubated with warfarin dose (5mg/kg). Blood samples were collected pre and 24h post-treatment by retro-orbital sinus puncture in trisodium citrate solution 3.8%, mixed and centrifuged at 3000 r.p.m. for 15 minutes. The supernatant plasma were removed and prothrombin time (P.T.) was determined according to (*Dacie and Lewis, 1984*) using the fibrometer and commercial reagents obtained from Hoechst Company.

RESULTS AND DISCUSSION

Determination of The Susceptibility Level to Warfarin

1. Lethal Feeding Periods (LFP): This method measures the susceptibility level of population of a particular rodent species to a given anticoagulant rodenticide. The technique is designed to detect the emergence of an anticoagulant resistance if and when it appears which determined the median lethal time of warfarin to population of *R. rattus* trapped from different areas where anticoagulants had been used before in rodent control programs.

The response of different *R. rattus* populations to warfarin using lethal feeding periods is shown in Table 2 which summarized the LFP₅₀ and LFP₉₉ values of warfarin for different groups of *R. rattus* trapped from Belbies, Kafr-Sakr, Menia El-Khamh and Hehya districts at Sharkia governorate. Data indicate that rats of Hehya dist., showed a noticeable tolerance to warfarin (LFP₅₀ and LFP₉₉ values were 3.4 and 9.9 days), followed by those from Menia El-Khamh (3.1 and 9.2 days) and Kafr-Sakr district (2.8 and 8.5 days), while rats of Belbies district exhibited more susceptibility to warfarin (2.6 and 6.9 days), respectively. *Kandil et al. (1995)* found that lethal feeding periods (LFP₅₀ & LFP₉₉) were 2.0 and 6.9 days feeding on warfarin 250 ppm for *R. rattus* in-

dividuals trapped from untreated areas of Abu-Rawash, Giza, Egypt.

Said and Kandil (1995), Gabr (1997) and El-Deeb et al (1999) studied the relative susceptibility of rodent populations to anticoagulant rodenticides and they found that this phenomenon obviously differed according the location from which animals trapped and rats caught from areas treated with anticoagulant rodenticides for long period, exhibited a comparative tolerance.

2. Biochemical Response: The susceptibility or resistance of further samples of the wild-caught roof rats was determined using biochemical methods utilize the changes in blood coagulation activity following anticoagulant administration.

Data in Table 3 indicated that the effect of 5mg/kg warfarin on the prothrombin time (P.T.) was obviously noticed in the case of rats caught from Belbies district, whereas P.T. values were considerably increased 3.5 times post-treatment than pre-treatment. On the other hand, the lowest effect was observed on the animals of Hehya district. P.T. values increased to 24.7 sec. post-treatment with increase of 2.2 time compared with 11.0 sec. pre-treatment. From the previous results it is clear that animals of Hehya exhibited noticeable tolerance to warfarin, while animals of Belbies dist., were more susceptible followed by Kafr-Sakr and Menia El-Khamh districts.

Bell and Galdwell (1973) revealed that in susceptible rats, warfarin acts by inhibiting vitamin K epoxide-reductase thereby blocking the synthesis of the blood clotting factors of the prothrombin complex and causing internal bleeding. In resistant rats the biochemical conversion of vit. K takes place even in the presence of anticoagulant. They also reported that resistance is due to a mutation, which alters the enzyme system responsible for the conversion of phyloquinone oxide to vit. K1, so that it is no longer inhibited by warfarin.

Reviewing the aforementioned results, it is clear that rats trapped from areas exposed to anticoagulants for long periods exhibited some tolerance to warfarin.

Table 1. Survey the amount of rodenticides used in different districts of Sharkia governorate during 1990-1999.

District Name	Rodenticides used amount (Kg)					Total Amount (Kg)	Land area (feddan)	Amount Kg/feddan
	Brodifacoum	Bromadiolone	Chlorophacinon	Difenacoum	Coumateiralyl			
Belbies	4250	2610	1250	1050	3180	12340	52312	0.236
Karf-Sakr	5100	3910	2500	3500	1650	16660	29152	0.572
Menia EL-Khamh	8150	3415	2640	2180	3650	20035	53494	0.375
Hehya	2280	4180	4300	2500	1350	14610	16178	0.903

Table 2. Lethal feeding periods (LFP) of Warfarin 0.025% to *Rattus rattus* trapped from different districts of Sharkia governorate.

District	LFP ₅₀ (day)	Confidence Limits	LFP ₉₉ (day)	Confidence Limits
Belbies	2.6	2.5 - 3.1	6.9	5.4 - 7.2
Kafr-Sakr	2.8	1.2 - 4.1	8.5	7.7 - 11.4
Menia El-Khamh	3.1	2.7 - 3.5	9.2	6.2 - 10.7
Hehya	3.4	2.9 - 3.6	9.9	8.0 - 11.1

Table 3. Prothrombin time (P.T.) of *Rattus rattus* trapped from different districts of Sharkia governorate and given a single oral dose (5mg/kg) of Warfarin.

District	P.T. (sec.)		
	Pre-treatment	Post-treatment	Ratio
Belbies	11.5	40.1	3.5
Kafr-Sakr	11.1	32.5	2.9
Menia El-Khamh	11.2	27.6	2.5
Hehya	11.0	24.7	2.2
L.S.D.	N.S	0.0	-

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فعالية الوارفارين المضاد للتجلط على مجتمعات الفأر المتسلق المصاد من مراكز مختلفة بمحافظة الشرقية

وحيد محمد جبر

معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - الجيزة

تم دراسة فعالية الوارفارين المضاد للتجلط على مجتمعات الفأر المتسلق الذي تم إصطياده من أربعة مراكز مختلفة بمحافظة الشرقية وهي (مراكز بلبيس، كفر صقر، منيا القمح وههيا) وذلك باستخدام الطرق البيولوجية (حساب فترات التغذية المميته) والطرق البيوكيميائية (تقدير زمن البروثرومين).

أوضحت النتائج أن أعلى قيمة لفترات التغذية المميته LFP_{99} ، LFP_{50} كانت للفئران المصادة من مركز ههيا حيث كانت (٩،٩،٢،٤) يوم، بينما كانت أقل قيم في حالة الفئران المصادة من مركز بلبيس (٦،٩،٢،٦) يوم). كانت النتائج المتحصل عليها من الاختبارات البيوكيميائية تسير في نفس الاتجاه حيث كان تأثير جرعة الوارفارين ٥ مجم/كجم على زمن البروثرومين واضح جداً على الحيوانات المصادة من مركز بلبيس بينما كانت أقل تأثيراً على الحيوانات المصادة من مركز ههيا. أشارت النتائج أن الفئران المصادة من مركز ههيا أظهرت تحمل عالي لمبيد الوارفارين بينما الفئران المصادة من مركز بلبيس كانت الأعلى حساسية يليها فئران كفر صقر ثم منيا القمح.