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Abstract

The efficacy of four compounds was tested against Norway rat, Rattus norvegicus and roof rat, Rattus rattus alexandrines, under laboratory and field conditions. In both non-choice and free choice feeding tests, results proved that Oshar crude plant extract (10ml/ kg crushed maize) was the most effective one when extracted by chloroform or ethanol against both rat species followed by alphachloralose 2% (anaethetic) while L. histidine 0.005% (an amino acid) was the lowest effective one. In addition the data illustrated that Norway rat was more susceptible than roof rat for the all cases. Concerning the acceptance, L. histidine achieved the highest value followed by Oshar extracted by chloroform and ethanol, while the lowest value of acceptance was in case of alphachloralose for both tested species. On the other way, the pathological changes on the different organs of the treated animals were studied. All the tested compounds caused obvious reduction for the body weight at different levels. In contrast, there were obvious increasing for the internal organs weight for the all cases. Sever cloudy and congestion were shown in liver and heart for all treatments except for L. histidine who caused similar symptom slightly. Also, noticeable congestion was observed in kidney. Darkness of color occurred in spleen specially with Oshar treatment. Also, obvious congestic... and bleeding were observed on the lung in all cases. The field results were in harmony with the laboratory results as Oshar plant ethanolic extract was the most effective one. Whereas it gave the highest value of population reduction of rodent followed by Oshar extracted by chloroform while alphachloralose failed to achieve good reduction and the field performance of L. histidine was the less effective one.

INTRODUCTION

Anticoagulant rodenticides are the most widely used in the control of rodents in Egypt and all over the world. All the anticoagulant rodenticides currently available are of two chemical types, hydroxicoumarin or indan-dione derivatives. Although differing in chemical structure, their physiological effect on animals is the same (Meehan, 1984). There are some potentiators have been introduced to increase the action of these rodenticides (Gabr, 2006). Also, there are other compounds and natural products such as plant extracts were used as rodenticides (Gabr *et al.*, 2004).

Therefore, the present work was conducted to study the rodenticidal effect of certain compounds against Norway rat and roof rat, who are the most common species in Egypt under laboratory and field conditions.

MATERIALS AND METHODS

1- Chemicals Used:-

1-1- Oshar crude plant extract:-

Oshar plant *Calotropis procera* was obtained from Wadi Hoof desert. The plant leaves were dried and grounded. 150g of powder were successive extracted with two solvents varied in their polarity i.e. ethanol and chloroform according to procedure of **Freedman** *et al.* (1979). Each crude extract was mixed with crushed maize 10 ml/kg.

1-2- L. Histidine:-

An amino acid L-histidine (Monohydrochloride) obtained from Loba Chemic LTD. India. It was used at 0.005% concentration.

1-3- Alphachloralose:-

Alphachloralose is an anaethetic W.P samples was obtained from societ chimque, France. It was used at level 2%.

2- Tested Animals:-

Two rat species were used in this study i.e., Norway rat, *Rattus norvegicus*, and Roof rat, *Rattus rattus*. Animals of both species were trapped from different locations of Quesna district, Menofia Governorate. The trapped animals were transported to laboratory and caged individually for at least two weeks for acclimatization and fed on standard diet composed of (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. Ten animals of each species were used for each test.

3- Laboratory Experiments:-

The rodenticidal efficiency and the pathological changes of the tested compounds were studied against the two rat species using non and free choice feeding methods.

3-1- Non-choice method:-

Animals of each species were divided into groups (each of 10 rats). One group for each tested compound and another one as a check control test. Each animal was offered 50g of the treated bait in small clay dishes and free access of water for 4 successive days. The consumed amount of bait was daily estimated. The poisoned bait

was removed and the survivor animals were fed on standard diet and observed for 28 days. During this period, mortality and time to death were recorded. Animals were autopsied to observe the clinical symptoms of poisoning for each compound. Different internal organs of each animal were weight and the pathological changes for each organ were investigated and recorded.

3-2- Free choice method:-

Free choice feeding test is important to determine the acceptability of each tested compound by comparing its consumption with that of challenge standard diet according to the method of Palmateer (1974). One of each tested compound and challenge diet (50g of each) were offered to each rat in small separated dishes. Their position was daily altered to avoid feeding preference for a certain location. The consumed amount of both bait and diet was daily estimated. This procedure was repeated daily for 4 successive days. After this period, the poison bait was removed and the survivor animals were fed on a standard diet only and observed until the 28th day. Mortality and time to death were recorded. Bait acceptance rate was calculated by the following equation:

4- Field Performance:-

The performance of the previous tested compounds was evaluated under the field conditions of Kafr El-Shiekh Ibrahim village, Quesna district, Menofia Governorate. After winter crops harvest, an infested area was chosen and divided into plots represent the number of the tested compounds (each of 2 feddans). One plot was left untreated as a check control. The population density of rodents was estimated pre and post treatment using food (crushed maize) consumption method. Two kg from crushed maize were divided and distributed inside 10 clay bait stations in each plot for 5 successive days. The average of food consumption in the last two days was determined. 200g from each tested compound were placed in each bait station for one week. The consumed amount of each tested compound was estimated. One week after the poison bait was removed, the population reduction of rodents was calculated using the same method mentioned above as the following equation:

RESULTS AND DISCUSSION

1- Laboratory Studies:

1-1- Non-choice test:

Data in Table (1) show the response of Norway rat, *R. norvegicus* and roof rat, *R. rattus*, to certain compounds using non-choice feeding method. Results indicate that alphachloralose achieved complete mortality against both rat species. Also, both extracts of Oshar plant gave complete killed for Norway rat while they caused 90% mortality in case of roof rat. L. histidine was the lowest effective one as it gave only 20% mortality for Norway rat and failed to achieve any mortality for roof rat. Regarding the time required to death, alphachloralose recorded the shorter time 3.5 and 4.5 days for Norway rat and roof rat, respectively, followed by 5.9 and 6.2 days for Oshar extracted by ethanol and 6.3 and 6.4 days for Oshar extracted by chloroform while this time prolonged to reach 9.5 days in case of L. histidine for Norway rat only. On the other hand, the range of days to death was very narrow for alphachloralose (3-4) and (3-6) days for Norway rat and roof rat, respectively, while it was wide in case of Oshar plant when extracted by ethanol (4-10) days or chloroform (4-11) days and (5-10) days for the two species, consecutively. This range was morderate (7-12) days for L. hisidine against Norway rat only.

1-2- Free choice test:-

The efficacy of certain compounds was tested against both rat species using free choice feeding method. Data in Table (2) indicated that Oshar plant induced the same results when extracted by ethanol or chloroform as they gave 90 and 80% mortality for Norway rat and roof rat with similar time to death (6.5 and 6.8 days) for Norway rat and (6.7 and 6.9 days) for roof rat for both tested extracts, respectively. Alphachloralose caused 60% mortality for Norway rat while it gave 40% for of roof rat. The time required to death was 4.0 and 4.8 days for the two species, respectively. No effect was observed on the animals of both species by L. histidine treatment. Concerning the acceptance, the highest values were 66.3 and 66.2% in case of L. histidine for Norway rat and roof rat, consecutively, followed by Oshar extracted by chloroform and ethanol (44.8 and 44.7%) for Norway rat and (41.3 and 37.9%) for roof rat, while the lowest value of acceptance were 30.8 and 28.7% in case of alphachloralose for the two tested species, respectively.

The previous results (Tables 1, 2), proved that Oshar plant extract was the most effective one against both rodent species followed by alphachloralose while L. histidine had the lowest effect. Also, for the all cases, Norway rat was more susceptible than roof rat. The efficacy of the tested compounds is differed from one

to another according to the type of compound, its chemical structure and concentration in addition to the rat species.

The present results are in agreement with those obtained by Ibrahim (2001) who found that Oshar leaves extract was the most effective one between different plant extracts against albino rat. Also, Gabr et al. (2004) recorded that in both non and free choice feeding tests, Oshar plant when extracted by ethanol was more effective against albino rat followed by hexane and petroleum ether extracts, while the water extract was the lowest effective one. Also, they observed that the ethanolic extract was the most acceptable followed by hexane and petroleum ether. Hussien (1991) and Khidr (2001) recorded many bioactive compounds in Oshar leaves extracted with ethanol. Regarding, alphachloralose, Saini and Parshed (1993) found that 0.5% alphachloralose caused 63.7 to 82.9% mortality within 72h for three rodent species. Meehan (1984) mentioned that alphachloralose acts on the nervous system causing a depression in brain activity showing the heart and respiratory. Concerning L. histidine, Feinburg et al. (1950) indicated that L. histidine acts as a vasoconstrictor in rats, while Muktha (1979) noticed that L. histidine at level 40 mg/kg of bait did not cause any mortality when administrate alone, El-Deeb et al. (1999) found that the mixture of L. histidine 0.004% to Warfarin enhanced the mortality from 60 to 80% against roof rat which exhibited a high tolerance to Warfarin.

1-3- Pathological changes:-

The clinical symptoms and pathological changes of the different investigated compounds against Norway rat which exhibited high susceptibility were compiled in Table (3). Data showed that the all tested compounds reduced the body weight of Norway rat at different levels. Alphachloralose treatment caused the highest value of reducing the body weight (140g) while the lowest level of decreased the body weight was 180g in case of L. histidine comparatively with 210g for untreated animals. Regarding the internal organs examination, severe cloudy and congestion were shown in liver and heart for all tested compounds except in case of L. histidine as it was less degree. Also, the weight of both organs increased to (10.5 and 1.8g), (11.6 and 2.0g) and (12.4 and 2.1g) for alphachloralose, L. histidine and Oshar extract, respectively, comparing with (7.3 and 1.0g) for control of the two organs, consecutively. A noticeable congestion was observed in kidney with obvious increasing of its weight for all tested compounds comparing with control. Concerning spleen, it exhibited a darkness of color with different degrees, as it was very dark in case of Oshar treatment while it was dark only for both alphachloralose and L. histidine in comparison with untreated animals. Also, the weight increase from 0.5g in control to 0.7 and 1.1g for alphachloralose and L. histidine, respectively, while that increasing

reached to the maximum (1.3g) for Oshar plant extract treatment. For lung, obvious congestion and bleeding were observed with increasing of its weight in all cases.

Similar pathological changes were observed in albino rats and mice treated with Oshar plant extract by Faye (1985), Sebaii (1996), Gabr *et al.* (2004) and Rezk (2006).

1-4- Field Studies:-

The efficiency of the investigated compounds was tested against rodents to examine their performance under field conditions as shown in Table (4).

Data cleared that Oshar plant ethanolic extract was the most effective one followed by Oshar extracted by chloroform as the first achieved 84.6% rodent population reduction and the second gave 83.3%. The field performance of both alphachloralose and L. histidine was weak as they failed to achieve good results whereas they caused 26.1 and 2.3% only population reduction of rodents for both compound, respectively. The lowest amount of bait consumption per feddan was 285g for alphachloralose while the highest amount was 1280g for L. histidine. This observation may be due to alphachloralose killed animals in a short time (Meehan 1984) comparatively with the rest compounds. Narrow variation was observed between the bait consumption of Oshar extracted by ethanol (1150g) and when extracted with chloroform (1080g).

Discussing the aforemtioned data, it could be noticed that the field performance was in harmony and confirmed the laboratory results. Similar observations in fields were recorded by Muktha (1979), Gabr *et al.* (2004) and Gabr (2006).

Table 1. Effect of certain compounds against *Rattus norvegicus* and *Rattus rattus alexandrines* using non-choice feeding method for 4 successive days under laboratory conditions

	% Mortality		Time to death days				
Tested compound			Range	e	Mean		
	R. norvegicus	R. rattus	R. norvegicus	R. rattus	R. norvegicus	R. rattus	
Alphachloralose 2%	100	100	3 – 4	3 – 6	3.5	4.5	
L. Histidine 0.005%	20	0.0	7 – 12	0.0	9.5	0.0	
Oshar extracted by	100	90	4 – 10	4 – 10	5.9	6.2	
ethanol (10mg/kg)							
Oshar extracted by	100	90	4 – 11	5 – 10	6.3	6.4	
chloroform (10mg/kg)							

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Table 2. Effect of certain compounds against *Rattus norvegicus* and *Rattus rattus alexandrines* using free choice feeding method for 4 successive days under laboratory conditions

	% Acceptance		% Mortality		Time to death (day)			
Tested compound					Range		Mean	
	R. norvegicus	R. rattus	R. norvegicus	R. rattus	R. norvegicus	R. rattus	R. norvegicus	R. rattus
Alphachloralose 2%	30.8	28.7	60	40	3 – 5	3 – 7	4.0	4.8
L. Histidine 0.005%	66.3	66.2	0.0	0.0	0.0	0.0	0.0	0.0
Oshar extracted by ethanol (10mg/kg)	44.7	37.9	90	80	4 – 10	4 – 10	6.5	6.7
Oshar extracted by chloroform (10mg/kg)	44.8	41.3	90	80	4 – 12	4 – 11	6.8	6.9

Table 3. Effect of certain compounds on the different organs of Norway rat, *Rattus norvegicus*.

Organ	Treated	Alphachloralose		L. Histidine		Oshar plant extract	
	Average weight (g)	Average weight (g)	Pathological change	Average weight (g)	Pathological change	Average weight (g)	Pathological change
Body	210	140	Severe reduce in body weight	180	Reduce in body weight	155	Reduce in body weight
Liver	7.3	10.5	Severe cloudy and congestion	11.6	Cloudy and congestion	12.4	Severe cloudy and congestion
Kidney	1.4	1.9	Congestion	2.0	Congestion	2.1	Congestion
Heart	1.0	1.8	Cloudy and congestion	2.0	Cloudy and congestion	2.1	Cloudy and congestion
Spleen	0.5	0.7	Darkness color	1.1	Darkness color	1.3	Very darkness color
Lung	1.1	2.4	Bleeding and congestion	2.1	Bleeding and congestion	2.3	Bleeding and congestion

Table 4. Rodenticidal efficiency of certain compounds against field rodents under field conditions.

	Bait	Population		
Tested compound	Pre-treatment	Treatment	Post-treatment	reduction %
Alphachloralose 2%	1150	285	850	26.1
L. Histidine 0.005%	1300	1280	1270	2.3
Oshar extracted by	1300	1150	200	84.6
ethanol (10mg/kg)				
Oshar extracted by	1200	1080	200	83.3
chloroform (10mg/kg)				

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الكفاءة الإبادية لبعض المركبات ضد الفأر النرويجي وفأر السقف

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تم دراسة تأثير وفعالية أربعة مركبات ضد الفأر النرويجي والفأر المتسلق تحت الظروف المعملية والحقلية. أوضحت النتائج المعملية أن كلا من إختباري التغذية الإختيارية واللاختيارية كان مستخلص نبات العشار (١٠ مللي/كجم جريش ذرة) الأعلى تأثيراً عند إستخلاصه بالكلوروفورم أو بالإيثانول ضد كلا النوعين من الفئران تلاه الفاكلورالوز ٢% (مخدر)، بينما كان ل. هستيدين من المؤلل تأثيراً.

كما أكدت النتائج أن الفأر النرويجي كان الأكثر حساسية من الفأر المتسلق في جميع الحالات. وبالنسبة لإختبار نسبة الإستساغة فقد حقق ل. هستيدين أعلى معدل يليه مستخلص نبات العشار بالكلوروفورم ثم المستخلص الإيثانولي بينما كانت أقل نسبة إستساغة في حالة الفاكلورالوز لكلا النوعين من الفئران. أيضاً تم دراسة التغيرات المرضية علي الأعضاء المختلفة للفئران المعاملة حيث حدث إنخفاض في وزن الجسم بدرجات متفاوتة، وعلي النقيض كان هناك زيادة واضحة في وزن الأعضاء الداخلية في جميع الحالات، كما حدث تضخم وإحتقان شديد في الكبد والقلب في جميع المعاملات ما عدا ل. هستيدين حيث كانت الأعراض أقل حدة. كذلك لوحظ حدوث إحتقان في الكلية، كما حدث تغير في لون الطحال الي اللون الغامق وخاصة في حالة المعاملة بالعشار، ولوحظ أيضاً حدوث إحتقان ونزيف في الرئتين في جميع المعاملات.

أما بالنسبة للنتائج الحقلية فقد كانت متوافقة مع النتائج المعملية حيث أعطى مستخلص نبات العشار بالإيثانول أعلى نسبة خفض في تعداد القوارض تلاه مستخلص العشار بالكلوروفورم بينما الفاكلورالوز أخفق في تحقيق نتائج جيدة، كذلك كان الأداء الحقلي لــــل. هستيدين ضعيف للغاية.