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Rodenticidal effect of furosemide (drug) against black rat *Rattus rattus* (Rodentia: Muridae) under laboratory and animal production farm conditions

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Abstract:

Efficacy of furosemide (drug) 40 mg was evaluated against black rats *Rattus rattus* L. (Rodentia: Muridae) , under laboratory and animal production farm conditions. LD_{50} of furosemide was determined and its value was 4745 mg/kg b.w. Also, non and free choice feeding test were tested, the results revealed that furosemide (2.1%) bait mixed with crushed maize achieved complete mortality 100% after four days of treatment in the non choice feeding test. While it gave 80% mortality and 51% acceptance in free choice feeding test. furosemide drug caused increasing the extraction of the urine amount led to decrease in the body weight of the tested animals. The effect of furosemide was evaluated against *R. rattus* under animal production farm conditions at Kafr El-Zayat, Gharbiya Governorate. The results indicated that furosemide bait (2.1%) mixed with crushed maize achieved 89% in rats population reduction. It can be concluded that furosemide can be use in the integrated rodents management.

Introduction

Rodents are important pests in the Egypt and other countries of the world such as black rats *Rattus rattus* L. (Rodentia: Muridae), the common and harmful rat. They feed on agricultural crops and storage food, as well as transmitting diseases to human and domestic animals causing great economic losses for the agriculture sector (Meehan, 1984).

Chemical rodenticides play important role in controlling rodents. The use of these of chemicals hazards to the environment. They may cause secondary poisoning to humans, farm and domestic animals (Vipin and Tripathi, 2006). Rodents may also acquire resistance to repeated and / or wrong use of certain rodenticides (Witmer *et al.*, 2007). Therefore, researches start to look for other compounds that could alleviate these problems and control rodent populations effectively with no harm to the environment. Some drugs were used to reduce the rodent population such as warfarin which use as anticoagulant. So in this study furosemide

drug was used to reduce the population of black rat *R. rattus*.

Furosemide drug is sensitive to light induced decomposition (Ambrogi *et al.,* 2012). Furosemide inhibits tubular reabsorption of sodium and chloride in the proximal and distal tubules, as well as in the thick ascending loop of Henle by inhibiting sodium chloride co. transport system resulting in excessive excretion of water a long with sodium chloride, magnesium and calcium (Chang and Winkelmayer , 2010). Furosemide causes hepatic necrosis and increase of Alanine Amino Transferase (ALT) activity in mice (Williams *et al*., 2007). The oral LD_{50} of Furosemide values were 2700 to 7537 mg/kg in rat (EMA,2000). Therefore, the aim of this work is to study the LD_{50} determination of Furosemide on *R. rattus* toxicity and palatability of this compound under laboratory condition and to integrate in the information rodent pest management programs in agricultural fields in order to reduce population densities of rodents.

Materials and methods

1. Tested compounds:

Furosemide (drug, 40 mg) Chemical name: 5- (amino sulfonyl) – 4- chloro-2 [(2-furanyl methyl) amino]. It used for fluid accumulation in tissue following heart or liver disease. It was obtained from Sanofi Co., as tablets each of 40mg. It was powdered and used at 2.1% mixed with crushed maize or soluble in water to determine LD_{50}.

2. Tested animals:

Adult black rats *R. rattus* caught by rat traps from fields and stores, located in Abu-Roash, Giza. Rats were acclimatized individually and fed on a free diet and water for 15 days. The weight of rats ranged about 150 to 200g. Animals were divided into groups for tests (five rats for each) and another one for control.

3. Experimental laboratory:

3.1. LD_{50} determination of furosemide:

Serial four doses of furosemide as mg/kg b.w., were measured using oral dose technique. furosemide was soluble in water and administrated by oral intubation. Animals were fasted for 12 hr. before treatment. The mortality was recorded after 4 days of treatment and LD_{50} value was calculated according to Weil (1952).

3.2. Non choice feeding test:

Animals were fed on 50 g standard diet (65% crushed maize + 25% ground wheat + 5% sugar + 5% corn oil) and water for four successive days. The amount of consumed bait was weighted daily. Then animals were fed on treated crushed maize mixed with furosemide powder (2.1%). The consumed amount of bait was weighted for four successive days. The treated bait was removed, and survivor rats were fed on standard diet and water (Shefte *et al*., 1982) and observation for 28 days. During this period the mortality was recorded and the body weight of rats was recorded.

3.3. Free choice feeding test:

The test is very important to determine the acceptability of furosemide bait, comparing with challenge diet. The treated bait and diet were offered to each rat (50g of each) in small separate dishes. Their position was daily altered to a void feeding preference for certain location. The consumed amount of bait and diet were recorded daily for four successive days (Palmateer, 1974). Bait acceptance was recorded using the following equation (Mason *et al*., 1989).

$$\text{Acceptance \%} = \frac{\text{Consumed amount of treatment bait}}{\text{Consumed amount of treatment bait} + \text{challenge diet}} \times 100$$

4. Field experiment.

Field evaluation of furosemide (2.1%) mixed with crushed maize was carried out under animals production farm condition of Kafour Belshay Village, Kafr El-Zayat Center, El-Gharbiya Governorate. The area was $350m^2$ and infected with black rat *R. rattus.* Pretreated with diet 3200 gm (small black plastic sacks100 gm of each) was put

inside bait station distributed inside farm. The consumed amount were weighted daily for four days and removed. After that treated bait was applied as the previous method and weighted daily for four days. The consumed amount was recorded and the population reduction of rats was calculated as follows:

$$\text{Population reduction \%} = \frac{\text{Pre treat. cons.} - \text{Post treat. Cons.}}{\text{Pre treat. Cons.}} \times 100$$

The population density of rodent was estimated pre and post treatment according to Dubock (1984).

Results and discussion

1. Laboratory studies:

1.1. LD_{50} of furosemide (drug) determination:

The LD_{50} of furosemide (drug) was determined against *R. rattus* to know the dose for the follow experiments. Table (1) indicated that the oral doses of furosemide were 3094, 3681, 4381 and 5200 mg/ kg b.w. , gave 50, 100, 100, and 100% mortality after 4 days of treatment, respectively. Also, the results revealed that LD_{50} value was 4745 mg/kg b.w. These results agree with (EMA, 2000 and Dunn *et al*., 2012) which indicated that the LD_{50} value dose of Furosemide in rat was 2700 to 7537 mg/ kg b. w. The mortality may be due to disorder of liver function resulted from the over dose of drug, there is a marked dieresis with the danger of fluid loss and electrolytes which has been seen to lead to somnolence, confusion, hypotension, hyponatremia, hypochloremic, alkalosis, hemo- concentration dehydration and circulatory collapse. This result agreed with Williams *et al*. (2007) who recorded that metabolism and toxicity of furosemide in the wistar rat and mouse which caused hepatotoxicity of liver after 24h of treatment.

Table (1): LD_{50} determination of furosemide (drug) against *Rattus rattus* after 4 days of treatment.

Dose mg/kg b.w.	Mortality %	LD_{50} (mg/kg b. w.)
3094	50	4745
3681	100	
4381	100	
5200	100	

1.2. Effect of furosemide 2.1% against *Rattus rattus* for four successive days:

Data in Table (2) indicated that the non choice feeding test on furosemide bait (2.1% mixed with crushed maize) against *R. rattus.* The average of consumption bait was 17 gm which gave 100% mortality and the time of death ranged between one to four days with average 0.5 days.Regarding the free choice feeding test, furosemide bait achieved high acceptance percent 51% and 80% mortality with time to death ranged between 2-4 days.

From the previous results, it could be concluded that furosemide bait gave good and encouraging results as a rodenticide against black rats. In non choice test Kandil *et al.* (2015) recorded that abamectin bait achieved 80% mortality while diphacenone anticoagulant rodenticide caused 73.8% mortality after 3 days of treatment. Nakagawa *et al.* (2015) revealed that the bromodiolone anticoagulant rodenticide caused 75% mortality against *R. rattus* this mortality considered satisfactory in the free feeding test.

The high palatability of the bait may be resulted in repeated feeding for several days, nevertheless, exclusive feeding on the bait is uncommon under natural condition, as mice are diffuse and sporadic feeders regularly visiting many feeding points during their feeding activities (Crowcroft, 1959). Moreover, recently showed that anticoagulant bait has potential to substantially decrease food intake shortly after the initial consumption of the bait, which shortens the activity period of the over dosed individuals (Frankova *et al.*, 2017).

Table (2): Effect of furosemide (Bait 2.1%) against *Rattus rattus* for four successive days using non and free choice test.

Feeding method	Average treated bait consumption(g)	Average challenge bait consumption (g)	Acceptance %	Mortality %	Time of death (days)	
					Range	Mean
Non- choice	17	-	-	100	1-4	0.5
Free choice	4.93	4.70	51	80	2-4	1.5

1.3. Effect of Furosemide bait 2.1% the animal body weight:

Furosemide bait affected on the body weight of tested animals *R. rattus*, during the feeding test for four days. Data in Table (3) indicated that the furosemide bait caused loss in body weight of tested animals whereas the reduction in body weight between (10 to 30 gm b. w.), whereas the body weight of treated rats was reduced from (180. 200. 200. 200 and 170 gm to 150, 180, 190, 170 and 140gm) represented 16.6, 20, 10, 15 and 30%. This result may be due to severe dieresis through the four days of treatment when rats feeding on excessive amount of furosemide bait. This is may be resulted to the toxic in renal. The same results occurred with Felker *et al*. (2011) and Inomata *et al.* (2017) who used higher doses of furosemide causing higher net fluid and weight loss at the cost of an increased incidence of rising serum creatinine.

Table (3): Effect of furosemide (drug) bait on body weight of *Rattus rattus* during the feeding test four days.

No. of treated rats	Body weight before treatment (g b.w.)	Body weight after treatment (g. b.w.)	Reduction of body weight %
1	180	150	16.6
2	200	180	20
3	200	190	10
4	200	170	15
5	170	140	30

2. Field studies:

The efficiency of furosemide (drug 2.1%) bait mixed with crushed maize was tested against *R. rattus* (black rats) under animal production farm conditions. Data in Table (4) explained that the average consumption of crushed maize in pre-treatment was 3190 g from 5200g and the consumption of treated bait was 3156g while the average consumption of post-treatment was 352g. Results revealed that the Furosemide bait achieved 89% population reduction of rats as the consumption was reduced from 3190g before treatment to 352g after treatment. So this means that the furosemide bait has high efficacy against *R. rattus* while abamectin biocide gave 70.5% population reduction (Kandil *et al*., 2015). Also, Frankova *et al.* (2019) recorded that the low dose bait 25ppm brodifacoum was tested under field conditions in two populations of *R. rattus* showing 95.7% and 99.8% efficacy. So furosemide caused high mortality comparing with the previous results with other authors so, it can be concluded that furosemide (drug) may be can use to reduce the rodents in animal's farm or stores and it can be use in the integrated rodent management. But with taking into consideration that furosemide (drug) should be placed in black bags inside bait station to be obscured from the light in order to avoid it being broken by light in addition to not being targeted by the non target animals.

Table (4): Field evaluated of furosemide bait (2.1) on *Rattus rattus* for four days under animal production farm.

Bait consumption (g)			Population reduction %
Pre - treatment	Treatment	Post - treatment	
3190	3156	352	89

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