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Research Article

TOXICITY OF SECOND GENERATION ANTICOAGULANT BROMADIOLONE AGAINST *RATTUS RATTUS*: INDIVIDUAL AND SEX SPECIFIC VARIATIONS

Nancy Garg and *Neena Singla
Department of Zoology, Punjab Agricultural University, Ludhiana 141004, India
**Author for Correspondence*

ABSTRACT

There are reports of development of resistance to the second generation anticoagulant, bromadiolone in rodents from several countries, but no systematic study has been carried out in India to record the status of bromadiolone resistance. To distinguish susceptible and resistant individuals of house rat, *Rattus rattus*, it was required to record their response towards toxicity of LD_{50} of bromadiolone. The Present study was carried out to determine LD_{50} value of 0.005% bromadiolone bait against *R. rattus* of both sexes. Different doses of cereal based bait of 0.005% bromadiolone were fed to different groups (5 rats per group) of male (1, 2, 4, 6, 8, 10, 16, 32 and 64 g/100g bw) and female (2, 4, 8, 16, 32 and 64 g/100g bw) rats. LD_{50} values were found to be 1.05 and 1.83 mg/kg bw of active ingredient corresponding to 2.10 and 3.67 g/100g bw of 0.005% bromadiolone bait for male and female rats, respectively. Female rats were found more tolerant to bromadiolone toxicity than male rats. In some cases, the mortality in rats of lower dose group was found more than those of higher dose group. Also, the groups which had consumed almost double the dose of bromadiolone from one another showed similar mortality rate. Within one group, days to death varied among individual rats. Present study therefore reports individual as well as sex specific variations in response towards toxicity of bromadiolone in *R. rattus.*

***Keywords**: Bromadiolone, Rattus Rattus, Toxicity, $LD_{50,}$ Individual Variations, Sex Specific Variations*

INTRODUCTION

The house rat, *Rattus rattus* (Linnaeus), one of the most common commensal rodent pest worldwide (Parshad, 1999), damages, contaminates and spoils packed food and non-food materials in transit and storage besides being involved in transmitting several diseases of man and livestock (Gratz, 1994; Singla *et al*., 2008, 2013). Studies have found *R. rattus* as the major and abundant species occupying poultry farms in India (Ahmad *et al.*, 1984; Parshad *et al.*, 1987; Soni and Rana, 1988). Loss estimates range from 0.57kg feed per day (Ahmad *et al.*, 1984), 0.39-10% eggs (Khatri and Veda, 1984; Soni and Rana, 1988), 0.95 egg trays and 1.07 gunny bags per day (Chopra, 1994) and approximately US$13.61 loss per 1,000 chicks (Ahmad *et al.*, 1984). The methods used for the management of rodent pests such as trapping, habitat manipulation, use of repellents/attractants/pathogenic agents, that induce mortality or migration of rodents have never produced consistent results (Buckle and Mullar, 2000).

Chemical control by acute and chronic rodenticides is the most commonly used method. Acute rodenticides lead to death of an animal within a few hours whereas in case of chronic rodenticides death delays for one to two weeks after ingestion of the lethal dose. Zinc phosphide, an acute rodenticide has been used predominantly over several years. However, the repeated use of acute rodenticides leads to rapidly recovering and bait shy rodent populations (Parshad, 1989; El-Deeb *et al.*, 2011). The development of chronic anticoagulant rodenticides in the early 1950s revolutionized rodent control and for the first time complete control of target rodent populations was possible and practical. The first of the anticoagulants to appear in the market was warfarin. With in a few years, additional anticoagulants collectively called as first generation anticoagulants such as diphacinone, chlorophacinone, pindone, coumafuryl, coumachlor and coumatetralyl were made available (Bentley, 1972). These all have broadly similar level of toxicity to the commensal rodents, although there is some variation between them in

toxicity to other species. The initial widespread success of these early anticogulants was, however, not maintained. Resistance not only to warfarin but to all first generation compounds was detected in UK in some Norway rat and house mouse populations (Boyle, 1960). Resistance to these anticoagulants was subsequently identified in many other countries including India (Deoras, 1966; Jackson and Kaukeinen, 1972; Mukthabai *et al*., 1981; Lam *et al.,* 1982; Lund, 1972). In the early 1970s, second generation anticoagulants i.e. difenacoum and bromadiolone were marketed and found effective against warfarin resistant rodent populations (Hadler and Shadbolt 1974). But after a very short time, the initial success of both of these compounds was shaken by reports indicating rodent populations showing cross resistance either to difenacoum or bromadiolone or both (Redfern and Gill, 1980; Rowe *et al.,* 1981; Lund, 1984).

Resistance to second generation anticoagulants has not become as wide spread as to the first generation compounds (Buckle, 1994). Resistance to anticoagulants can develop in a population after 5-10 years of sustained use of anticoagulant rodenticides and is inheritable (Greaves and Ayres, 1967). Efficacy of control measures may be lost by physiological resistance to anticoagulant rodenticides and by behavioural reactions of the target rodent population (Greaves and Ayres, 1967; Pelz and Klemann, 2004). The broad-scale field use of anticoagulants has also raised concern regarding their tendency to bio-accumulate in non-target species (Giraudoux *et al*., 2006; Giorgi and Mengozzi, 2010). In India, bromadiolone has been made commercially available since 1988 for the control of agricultural and commensal rodents. Since then it is the only second generation anticoagulant being used commonly as 0.005% bait. As resistance to difenacoum and bromadiolone has become a problem of practical importance in some countries shortly after their introduction, this must call for attention in those countries still fighting resistance only to the first-generation anticoagulants. Inspite of bromadiolone still being effective, there are certain unpublished reports of decreased efficacy of this compound. No systemic study has been carried out in India to determine the current status of bromadiolone resistance in rodent populations. To distinguish susceptible and resistant individuals of house rat, *Rattus rattus*, it was required to record their response towards toxicity of LD_{50} and twice the LD_{50} doses of bromadiolone.

Earlier studies on toxicity of bromadiolone revealed oral LD_{50} value of bromadiolone in house mouse, house rat and Norway rats but there is no report regarding LD_{50} value of cereal based formulation of 0.005% bromadiolone against house rat. Mortality and post mortem picture of anticoagulant rodenticides fed in bait was, however, studied by Mlynareikova *et al.* (1999) in *Rattus norvegicus*. Present study was hence carried out to determine LD_{50} value of 0.005% bromadiolone bait against *R. rattus* of both sexes.

MATERIALS AND METHODS

Collection and maintenance of animals

For present studies, *R. rattus* of both sexes were trapped live from poultry farms in Ludhiana, India with the help of multi catch rat traps. In the laboratory, rats were acclimatized individually in cages of size 36×23×23 cm each for 15–20 days before the commencement of experiment. Food and water were provided *ad libitum.* Food consisted of freshly prepared mixture of cracked wheat, powdered sugar and groundnut oil (WSO bait) in ratio 96:2:2. Metallic trays were kept under each cage for the collection and disposal of urine and faeces. Approval of Institutional Animal Ethics committee (IAEC) was obtained for the usage of animals. After acclimatization, mature and healthy rats of both sexes were selected and weighed.

Treatment

During treatment, a total of 75 rats (45 males, 30 females) were weighed and divided into groups of five rats each. The number of rats kept in each group was as per the approval of IAEC. The body weight of rats in different groups of male and female rats did not differ significantly. Each rat was kept separately in a laboratory cage. Before treatment rats were deprived of food for about 12 hours. Freshly prepared cereal based formulation (cracked wheat, powdered sugar, groundnut oil and bromadiolone in 94: 2: 2: 2) of 0.005% bromadiolone bait was fed to each rat in no-choice feeding test. 0.25% bromadiolone powder

procured from Pest Control of India, Pvt. Ltd., New Delhi, India was used for the preparation of bait. Nine different doses of 0.005% bromadiolone bait i.e. 1, 2, 4, 6, 8, 10, 16, 32 and 64 g/100g body weight (bw) were fed to nine different groups of male *R. rattus* whereas, six different doses of bromadiolone bait (2, 4, 8, 16, 32 and 64 g/100g bw) were fed to six different groups of female rats. These values corresponded to 0.5 to 32.0 mg/kg bw of active ingredient in male rats and 1.0 to 32.0 mg/kg bw of active ingredient in female rats. After the consumption of bromadiolone bait, each rat was fed on WSO bait. During and after the treatment, animals were observed daily for mortality. For determination of LD_{50} values, mortality of rats up to 15 days was considered. LD_{50} values were determined separately for male and female rats through Probit Analysis using Polo Software. Percent mortality of rats in each group was determined using the formula given below:

$$\text{Percent mortality} = \frac{\text{No. of rats died after treatment in a group}}{\text{Total no. of rats treated in a group}} \times 100$$

RESULTS AND DISCUSSION

Data on observed mortalities for both male and female rats are summarized in Tables 1 and 2. The LD_{50} values of active ingredient were found to be 1.05 and 1.83 mg/kg bw with corresponding values of 2.10 and 3.67 g/100g bw for male and female rats, respectively (Table 3). Bromadiolone was hence somewhat more toxic to males than females or in other words the females were more tolerant to bromadiolone than males. Complete mortality was achieved at higher doses of 0.005% bromadiolone i.e 10, 16, 32 and 64g/100g bw in male rats and at 32 and 64g/100g bw in case of female rats.

In some cases, the mortality in rats of lower dose group was found more than that in higher dose group. The mortality was only 60% in male rats of group ingesting 8g/100g bw of 0.005% bromadiolone bait, whereas, it was 80% in rats of groups fed on 2 and 6 g/100g bw of bromadiolone bait and 100%, in rats of group ingesting 4 and 10g/100g bw of bromadiolone bait (Figure 1). In female rats, percent mortality was same in the groups fed on 8 and 16g/100g bw of 0.005% bromadiolone bait although group fed on 16g/100g bw had consumed almost double the dose of the active ingredient ingested by group fed on 8g/100g bw of 0.005% bromadiolone bait (Figure 2).

In present studies, individual variations were observed in days to death after bromadiolone ingestion by both male and female rats. In male rats, mortality was delayed up to day 15 in rats of groups ingesting by 2, 3, 5 and 16 mg/kg bw of active ingredient and up to day 14 in rats of group ingesting 32 mg/kg bw of active ingredient. Mortality of male rats started by day 8 in rats of groups ingesting both 1 and 5 mg/kg bw of active ingredient. In group of rats ingesting 2 and 4 mg/kg bw of active ingredient, mortality started by day 5 and 4, respectively whereas in rats of group ingesting 3 mg/kg bw of active ingredient, mortality started by day 11 of treatment (Table 1). In female rats, mortality was delayed up to day 11 in rats of group ingesting 4 mg/kg bw of active ingredient, whereas in rats of groups ingesting 2 and 8 mg/kg bw of active ingredient, mortality was delayed up to days 15 and 14, respectively (Table 2). Within the same group of rats also a lot of variation was observed in individual rats with respect to the day on which mortality was observed. Similar trend was observed in both male and female rats. (Tables 1 and 2).

Different LD_{50} values for the two sexes indicate sex specific variations. Meehan (1978) reported acute total LD_{50} values of 0.57 and 0.75 mg/kg bw of active ingredient for male and female Wistar rats, respectively. The LD50 values reported by Meehan (1978) are also in agreement with those reported by Erickson and Urban (2002), who reported LD_{50} values of 0.56-0.84 mg/kg bw of active ingredient in laboratory rats. In a study by Kohn and Pelz (1999) similar pattern has been shown in the Norway rats. Similar to present studies, Meehan (1978) and Kohn and Pelz (1999) have also observed that bromadiolone was somewhat more toxic to males than females or females are more tolerant to anticoagulant poison than males.

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Table 1: Percentage mortality in male *R.rattus* after administration of different doses of bromadiolone

Body weight (g)(n = 5 each)	Dose of 0.005% bromadiolone bait ingested (g/100g bw)	Dose of active ingredient ingested (mg/kg bw)	Number of animals died/treated	Mortality (%)	Days to death (range)
142.6015.76	0.0	0.0	0/5	0	-
169.80±15.40	1.0	0.5	0/5	0	-
169.60±18.30	2.0	1.0	4/5	80	8-13
169.60±31.19	4.0	2.0	5/5	00	5-15
169.60±12.99	6.0	3.0	4/5	80	11-15
169.80±21.03	8.0	4.0	3/5	60	4-10
169.80±23.88	10.0	5.0	5/5	100	8-15
152.40±25.30	16.0	8.0	5/5	100	6-9
142.60±22.70	32.0	16.0	5/5	100	7-15
129.60±18.17	64.0	32.0	5/5	100	5-14

Table 2: Percentage mortality in female *R. rattus* after administration of different doses of bromadiolone

Body weight (g) (n = 5 each)	Dose of 0.005% bromadiolone bait ingested (g/100g bw)	Dose of active ingredient ingested (mg/kg bw)	Number of animals died/treated	Mortality (%)	Days to death (range)
140.40±25.66	0.0	0.0	0/5	0	-
127.60±16.35	2.0	1.0	2/5	40	8
128.00±17.39	4.0	2.0	3/5	60	10-15
132.80±18.69	8.0	4.0	4/5	80	6-11
131.00±12.77	16.0	8.0	4/5	80	3-14
131.20±19.95	32.0	16.0	5/5	100	3-9
136.80±35.99	64.0	32.0	5/5	100	1-6

Table 3: Calculated lethal dose of bromadiolone in male and female *R. rattus*

Lethal doses (mg/kg bw)	Male	Female
LD_{10}	0.22	0.38
LD_{50}	1.05	1.83
LD_{99}	17.99	31.77

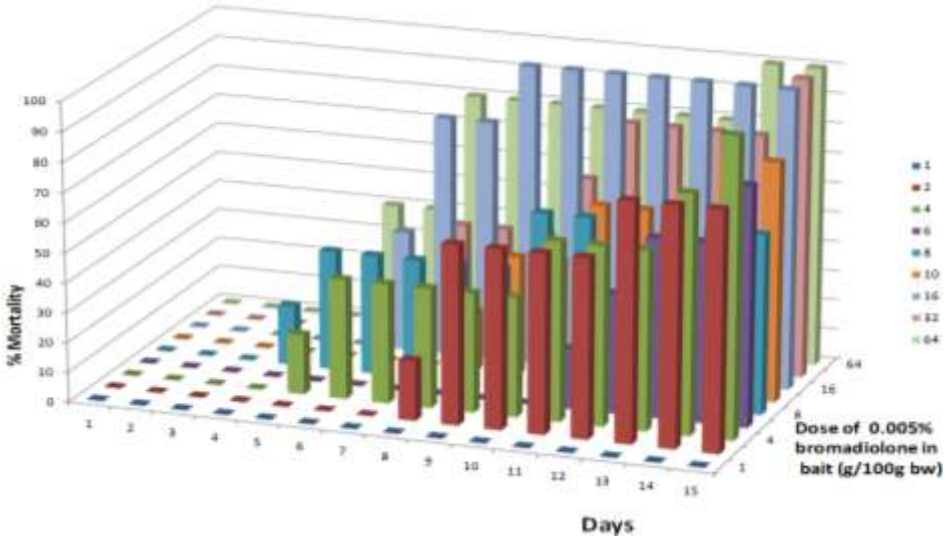

Figure 1: Response of male *R. rattus* towards toxicity of different doses of bromadiolone fed in bait

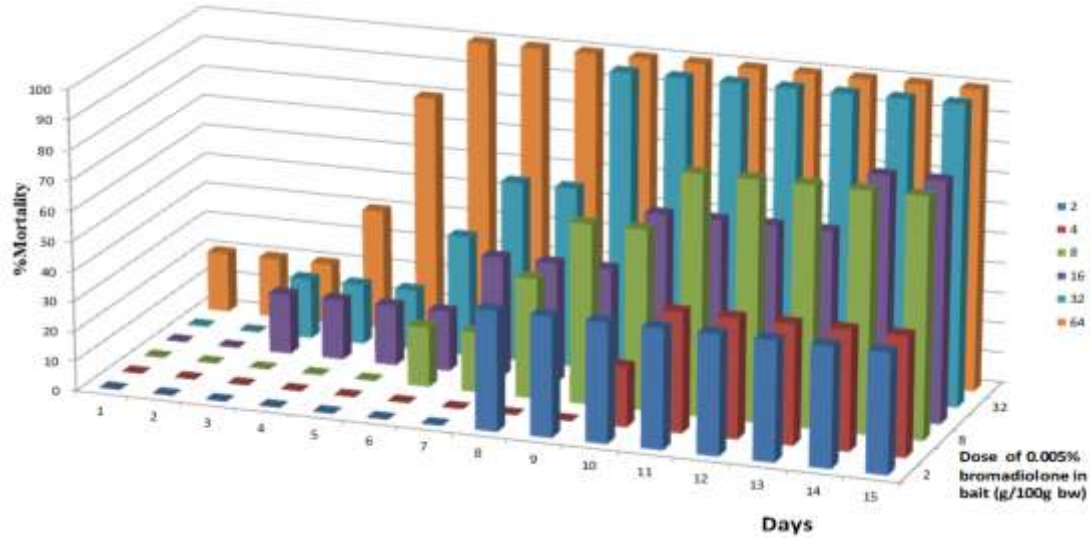

Figure 2: Response of female *R. rattus* towards toxicity of different doses of bromadiolone fed in bait

Present study reports individual as well as sex specific variations in response towards toxicity of cereal based formulation of 0.005% bromadiolone bait in *R. rattus*. Calculated sex specific LD_{50} values can be used in further studies to distinguish resistant and susceptible individuals of *R. rattus* to bromadiolone toxicity.

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