Journal of Animal Research: v.6 n.5, p. 955-959. October 2016

DOI: 10.5958/2277-940X.2016.00124.8

Intoxication Potential of Sublethal Doses of Bromadiolone in House Rat (*Rattus rattus)*

Nancy Garg[1] and Neena Singla[2*]

[1]Department of Zoology, P.I.G. Government College for Women, Jind, INDIA
[2]Department of Zoology, Punjab Agricultural University, Ludhiana, INDIA

**Corresponding author: N Singla; Email: neenasingla1@gmail.com*

Received: 09 March, 2016 **Accepted:** 28 May, 2016

ABSTRACT

Broamadiolone is an anticoagulant, recommended as 0.005% bait for controlling rodent pests. Present study was conducted to investigate the toxicity potential of sublethal doses of bromadiolone (0.001% bait) along with blood clotting response in house rat, *Rattus rattus*. Mature and healthy rats of both sexes were divided into three groups. Rats of group I were fed on bait containing 0.001% bromadiolone for 24 hours. In group II, male rats were fed on 0.001% bromadiolone bait @ 2.1g/100g b.wt and female rats @ 3.7 g/100g b.wt. (equivalent to LD_{50} of recommended bait of bromadiolone (0.005%) for male and female rats). In group III, male rats were fed on 0.001% bromadiolone bait @ 4.2g/100g b. wt. and female rats @ 7.4 g/100g b.wt. (equivalent to double the LD_{50} of 0.005% bromadiolone bait for male and female rats). Data was analysed using Student's t-test. Complete mortality was observed in rats of group I and group III within 2-8 days and 4-14 days, respectively. Rats of group I had ingested 13.5-14.3 g/100g b.wt. of bait in 24 hours. However, only 20-30% mortality was observed in rats of group II within 7-12 days. A significant ($P < 0.05$) increase in blood clotting response parameters i.e. PT (Prothrombin Time), R (Prothrombin Ratio) and INR (International Normalized Ratio) was recorded in both male and female rats after 48 hours of treatment. The study suggests the use of 0.001% bromadiolone bait for control of *R. rattus*.

Keywords: Bromadiolone, toxicity, sublethal dose, *Rattus rattus*

Rodents are the most destructive vertebrate pests (Sinha, 2014). They cause economic problems because of the damage they inflict on agricultural systems (Singla and Babbar, 2010), environmental problems due to the chemicals used for their control (Singleton and Redhead, 1989) and health problems as carriers of zoonoses (Singla *et al.*, 2008; Singla *et al.*, 2013).

House rat, *Rattus rattus* is the most notorious rodent pest species in agricultural and commensal situations (Awan and Hussain, 2015). Use of anticoagulant rodenticides is the most effective method to control undesired rodent species. With all the anticoagulants, death occurs within 5-7 days of the initial ingestion of a lethal dose (Buckle and Smith, 1994; Littin *et al.*, 2000). Bromadiolone is the second generation anticoagulant having single dose effect (Singh and Saxena, 1989). It was effective against rodents which had developed resistance to first generation anticoagulants (Greaves, 1994) and those which had developed shyness to zinc phosphide bait (Sheikher and Jain, 1996). However, the broad-scale field use of anticoagulants has raised concern regarding their potential to cause environmental damage through poisoning and bioaccumulation in wildlife fauna that feed on the baits or on rodents (Fisher *et al.*, 2003). Present study was thus conducted to record the toxicity potential of sublethal doses of bromadiolone in bait (0.001%) to *R. rattus* along with blood clotting response to treatment.

MATERIALS AND METHODS

Collection and maintenance of animals

Mature and healthy *R. rattus* of both sexes (127g to 152g body weight) were trapped live from poultry farms in Ludhiana, Punjab (India) with the help of multi catch rat traps. In the laboratory, rats were acclimatized individually

in cages for 15-20 days before the commencement of experiment. Food and water were provided *ad libitum*. Food consisted of a 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. After acclimatization, rats were weighed and divided into three groups (n = 10 of each sex per group). Animals were maintained at Animal House facility, Department of Zoology, Punjab Agricultural University, Ludhiana, India as per the guidelines of CPCSEA under the supervision of Institutional Animal Ethics Committee.

Treatment

Bait of 0.001% bromadiolone was prepared afresh by mixing 4 g of 0.25% bromadiolone concentrate in WSO bait containing 956g of cracked wheat, 20g of powdered sugar and 20 ml of groundnut oil. Rats of each group were fed on plain WSO bait before and after the treatment. During treatment, rats of group I were fed on 0.001% bromadiolone bait in no-choice for 24 hours, after which the unconsumed bait was weighed to record the consumption (g/100g b.wt.). In group II, male rats were fed on 0.001% bromadiolone bait @ 2.1 g/100g b.wt. and female rats @ 3.7 g/100g b.wt. in no-choice. These doses were equivalent to Median Lethal Doses (LD_{50}) of 0.005% bromadiolone bait as determined by us previously (Garg and Singla, 2014). In group III, male rats were fed on 0.001% bromadiolone bait @ 4.2 g/100g b.wt. and female rats @ 7.4 g/100g b.wt. in no-choice (doses equivalent to double the LD_{50} of 0.005% bromadiolone bait).

Determination of blood clotting response

Whole blood (up to 1 ml) from retro-orbital plexus of each rat was collected using capillary tube prior to treatment and 48 hours after treatment in an eppendorf tube containing 3.2% tri-sodium citrate solution as an anticoagulant. Blood was centrifuged at 3000 r.p.m. for 15 minutes. The supernatant plasma was collected into a separate tube and used immediately to determine Prothrombin Time (PT), International Normalized Ratio (INR) and Prothrombin Ratio (R) (ratio of mean PT of the treated rat to the mean PT of normal rat) as blood clotting response parameters using coagulation analyzer as per the manufacturers' instructions of rabbit brain thromboplastin reagent kit (Robonik India Pvt. Ltd., Mumbai).

Toxicity of bromadiolone

Toxicity of 0.001% bromadiolone bait against both male and female *R. rattus* was determined based on mortality data recorded daily during and after the treatment. Days to death for each rat and percent mortality in each group were determined.

Statistical analysis

Values were determined as Mean ± SD. The Significance of difference between PT, R and INR values before treatment and after 48 hours of treatment and consumption of treatment bait by rats of two sexes was determined using Student's t-test (Panse and Sukhatme, 1978). The statistical differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Feeding of rats of group I on bait containing 0.001% bromadiolone in no-choice for 24 hours resulted in ingestion of average 14.3 g/100g b.wt. and 13.5g/100g b.wt. of bait by male and female rats, respectively (Table 1). There was no significant difference in consumption of treatment bait between male and female rats. The bait resulted in complete mortality of rats of both sexes within 2-8 days. A significant ($P < 0.05$) increase was observed in PT from 15.8 sec before treatment to 186.4 sec after 48 hours of treatment in male rats and 13.9 sec before treatment to 125.1 sec after 48 hours of treatment in female rats. The R and INR of male rats were found increased significantly ($P < 0.05$) from 0.8 each before treatment to 9.8 and 10.6, respectively after 48 hours of treatment. In female rats also the R and INR were found increased significantly ($P < 0.05$) from 0.7 each before treatment to 6.6 and 7.0, respectively after 48 hours of treatment (Table 1).

Feeding of 2.1 g/100g b.wt. and 3.7 g/100g b.wt. of 0.001% bromadiolone bait to male and female rats of group II under no-choice resulted in 30 and 20% mortality, respectively within 8-12 days and 7-9 days of treatment, respectively. Feeding of 4.2 g/100g b.wt. and 7.4 g/100g

Table 1: Changes in blood clotting response parameters in *R. rattus* after feeding with 0.001% bromadiolone bait for 24 hours

Sex	Dose of 0.001% bromadiolone bait ingested (g/100g b.wt.)	Blood clotting response parameters						Percent mortality (days to death)
		PT (sec)		INR		R		
		At 0 h	At 48 h	At 0 h	At 48 h	At 0 h	At 48 h	
M	14.3±2.9	15.8±2.8	186.4±206.9*	0.8±0.1	10.6±12.2*	0.8±0.1	9.8±10.9*	100 (2-7)
F	13.5±3.3	13.88±3.23	125.1±129.1*	0.7±0.2	7.0±7.6*	0.7±0.2	6.6±6.8*	100 (2-8)

Values are Mean±SD, PT-Prothrombin Time, INR-International Normalized Ratio, R-Prothrombin Ratio

* Significant increase in PT, R and INR in after 48 hours of treatment at P <0.05

Table 2: Changes in blood clotting response parameters in *R. rattus* after feeding with specific doses of 0.001% bromadiolone bait

Group	Sex	Dose of 0.001% bromadiolone bait ingested (g/100g b.wt.)	Blood clotting response parameters						Percent mortality (days to death)
			PT (sec)		INR		R		
			At 0 h	At 48 h	At 0 h	At 48 h	At 0 h	At 48 h	
II	M	2.1	14.3±3.9	20.7±4.9	0.7±0.2	1.1±0.3	0.7±0.2	1.1±0.3	30 (8-12)
	F	3.7	16.3±3.7	27.6±8.3	0.9±0.2	1.5±0.5	0.9±0.2	1.4±0.4	20 (7-9)
III	M	4.2	28.2±5.7	136.9±63.2*	1.5±0.3	7.7±3.6*	1.5±0.3	7.2±3.3*	100 (4-12)
	F	7.4	25.3±4.8	69.8±28.9*	1.3±0.3	3.8±1.6*	1.3±0.2	3.7±1.5*	100 (5-14)

Values are Mean±SD, PT-Prothrombin Time, INR-International Normalized Ratio, R- Prothrombin Ratio

* Significant increase in PT, R, and INR after 48 hours of treatment at P< 0.05

b.wt. of 0.001% bromadiolone bait under no-choice to rats of group III resulted in complete mortality of rats within 4-12 days and 5-14 days of treatment, respectively (Table 2).

In rats of group II, no significant increase was observed in PT from 14.3 sec and 16.3 sec before treatment to 20.7 sec and 27.6 sec after 48 hours of treatment in male and female rats, respectively. Also no significant increase was observed in R and INR of male and female rats after 48 hours of treatment (Table 2). In rats of group III, a significant ($P < 0.05$) increase in PT was observed from 28.2 sec and 25.3 sec before treatment to 136.9 sec and 69.78 sec after 48 hours of treatment in male and female rats, respectively. The R and INR of male and female rats were also found increased significantly ($P < 0.05$) in both male and female rats after 48 hours of treatment (Table 2). In present study, female rats were found to be more tolerant to toxic effects of 0.001% bromadiolone bait. Similar observations were reported during our previous studies on determination of LD_{50} values of 0.005% bromadiolone bait (Garg and Singla, 2014). Scepovic *et al.* (2016) also reported higher toxicity of bromadiolone on males rather than females in house mice. Standard bait of bromadiolone (0.005%) has been found to cause complete mortality of commensal rodents within a period of 2-15 days (Revathi and Yogananda, 2006; Chaudhary and Tripathi, 2009; Borah and Bhattacharyya, 2015). Sahni and Prabha (2012) suggested that low lethal dose of bromadiolone should be used for killing rats. In present studies, a complete mortality of *R. rattus* was observed within 2-8 days after feeding on 0.001% bromadiolone for 24 hours. Similar to present study, a complete kill of *R. rattus* feeding on 0.001 and 0.002% bromadiolone in 4-day's was reported (Brooks *et al.*, 1990). Sublethal dose of bromadiolone (0.001%) in combination with sub lethal dose of a subacute poison such as cholecalciferol (0.005%)

also resulted in a complete mortality of rats (Singla *et al.*, 2015).

Anticoagulant toxicants interfere with normal synthesis of vitamin-K, a key protein required for the formation of blood clot, resulting in an increase in blood clotting time leading to internal bleeding and eventual death (Revathi and Yogananda, 2006). The blood clotting response is a test that provides a measure of the extrinsic blood coagulation system, and is commonly used to indicate clinical effects of anticoagulants (Poller and Hirsh, 1996). Blood clotting response test methods have been standardized for warfarin (MacNicoll and Gill, 1993) and second-generation anticoagulants such as difenacoum, bromadiolone, chlorophacinone and diphacinone (Gill *et al.*, 1994; Prescott and Buckle, 2000; Garg and Singla, 2015) for few rodent species like *Rattus norvegicus*, *Mus domesticus* and *R. rattus.* For these species, it was proposed that an INR equal to or greater than 5 be used as the indication of toxic effect of the anticoagulant (Prescott and Buckle, 2000). In present study, a significant increase in blood clotting factors was observed in rats fed on 0.001% bromadiolone for 24 hours and dose of 0.001% bromadiolone equivalent to twice the LD_{50} value of 0.005% bromadiolone. Thus the present study reveals the use of sublethal dose (0.001%) of bromadiolone bait in causing complete mortality of *R. rattus*.

ACKNOWLEDGEMENTS

The authors are thankful to University Grants Commission, New Delhi, India for providing financial assistance and Professor and Head of the Department of Zoology, Punjab Agricultural University, Ludhiana, India, for the facilities provided.

REFERENCES

Awan, D.S. and Hussain, I. 2015. The level of rodent problems in poultry farms of Rawalpindi-Islamabad, Pakistan. *Mammalia* DOI:10.1515/mammalia-2014-0046.

Borah, R.K. and Bhattacharyya, B. 2015. Toxicity of anticoagulant rodenticides against Rattus rattus. *Ann. Plant Prot. Sci.*, **23**(1): 135-139.

Brooks, J.E., Ahmad, E. and Hussain, I. 1990. The chronic oral toxicity of three anticoagulant rodenticides to *Rattus rattus*. *Pak. J. Sci. Ind. Res.*, **33(5):** 218-220.

Buckle, A.P. and Smith, R.H. (Eds.). 1994. Rodent Pests and their Control. CAB International, Wallingford, Oxon, UK, pp. 405.

Chaudhary, V. and Tripathi, R.S. 2009. Field efficacy of second generation anticoagulant rodenticides in managing rodents pests in poultry farms. *Pestology.*, **33(10):** 41-45.

Fisher, P., Eason, C.T., O'connor, C.E., Lee, C.H., Smith, G.B. and Endepols, S. 2003. Coumatetralyl Residues in Rats and Hazard to Barn Owls. In: Singleton, G., Hinds, L., Krebs, C.J., Spratt, D.M., (Eds.), Rats, Mice and People: Rodent Biology and Management. Australian Centre for International Agricultural Research, Canberra, pp. 457-460.

Garg, N. and Singla, N. 2014. Toxicity of second generation anticoagulant bromadiolone against *Rattus rattus*: individual and sex specific variations. *CIB Tech. J. Zool.*, **3(2):** 43-48.

Garg, N. and Singla, N. 2015. Blood clotting response test for detecting resistance to second generation anticoagulant bromadiolone in house rat, *Rattus rattus. Indian J. Anim. Res.*, **49**: 607-611.

Gill, J.E., Kerins, G.M., Langton, S.D. and MacNicoll, A.D. 1994. Blood clotting response test for bromadiolone resistance in Norway rats. *J. Wildl. Manage.*, **58**: 454-461.

Greaves, J.H. 1994. Resistance to anticoagulant rodenticides. In: Buckle, A.P., Smith, R.H., (Eds.), Rodent Pests and their Control. CAB International. Wallingford, Oxon, UK, pp. 197-217.

Littin, K.E., O'Connor, C.E. and Eason, C.T. 2000. Comparative effects of brodifacoum on rats and possums. *N. Z. Plant Prot.*, **53:** 310-315.

MacNicoll, A.D. and Gill, J.E. 1993. Revised methodology for a blood-clotting response test for identification of warfarin resistant Norway rats (*Rattus norvegicus*). *Bull. OEPP,* **23**: 701-707.

Panse, V.G. and Sukhatme, P.V. 1978. Statistical Methods for Research Workers. Indian Council of Agricultural Research, New Delhi, pp. 1-347.

Poller, L. and Hirsh, J. 1996. Laboratory monitoring of anticoagulants. In: Poller, L., Hirsh, J. (Eds), Oral Anticoagulants. Hodder Headline Group, New York, pp. 49-64.

Prescott, C. and Buckle, A. 2000. Blood-clotting response tests for resistance to diphacinone and chlorophacinone in the Norway Rat (*Rattus norvegicus*, Berk). *Crop Prot.*, **19**: 291-296.

Revathi, K. and Yogananda, M. 2006. Effect of bromadiolone on haematology, liver and kidney in *Mus musculus*. *J. Env. Biol.*, **27(1):** 135-140.

Sahni, K. and Prabha, C. 2012. Comparative efficacy of zinc phosphide and bromadiolone against rats in houses. *J. Life Sci.*, **4(1):** 67-68.

Scepovic, T., Jokic, G., Esther, A., Kataranovski, D., Vuksa, P., Dedovic, S. and Vuksa, M. 2016. VKOR variant and sex are the

main influencing factors on bromadiolone tolerance of the house mouse (*Mus musculus* L.) *Pest Manage. Sci.,* **72(3):** 574-9.

Sheikher, C. and Jain, S.D. 1996. Mode of application and performance of rodenticides in vegetable crops. *Int. J. Agric. Sci.,* **66(7):** 437-440.

Singh, R. and Saxena, Y. 1989. The susceptibility of house rat to some anticoagulant rodenticides. *Pesticides,* **23(3):** 27-28.

Singla, L.D., Singla, N., Parshad, V.R., Juyal, P.D. and Sood, N.K. 2008. Rodents as reservoir of parasites in India. *Integr. Zool*., **3**: 21-26.

Singla, N. and Babbar, B.K. 2010. Rodent damage and infestation in wheat and rice crop fields: District wise analysis in Punjab State. *Indian J. Ecol*., **37**: 184-188.

Singla, N., Kaur, S. and Javed, M. 2015. Rodenticidal potential of bromadiolone and cholecalciferol in synergism against *Bandicota bengalensis*. *Crop Prot.,* **72**: 163-168.

Singla, N., Singla, L.D., Gupta, K. and Sood, N.K. 2013. Pathological alterations in natural cases of *Capillaria hepatica* infection alone and in concurrence with *Cysticercus fasciolaris* in *Bandicota bengalensis*. *J. Parasit. Dis*., **37:** 16-20.

Singleton, G.R. and Redhead, T.D. 1989. House mouse plagues. In: Noble, J.C., Bradstock, R.A. (Eds.), Mediterranean Landscapes in Australia - Mallee Ecosystems and their Management, CSIRO, Melbourne, Australia, pp. 418-433.

Sinha, B. 2014. Non-empirical validation of indigenous rodent control methods practiced in Northeastern India. *Proc. Nat. Acad. Sci. India*, **80(2)**: 235-245.