LETTER TO THE EDITOR

COMPARATIVE PERFORMANCE-BASED STUDIES OF SECOND GENERATION ANTICOAGULANT RODENTICIDES BROMADIOLONE AND FLOCOUMAFEN IN *MUS MUSCULUS*

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Received October 31, 2019 – Accepted December 12, 2019

To the Editor

Rodents not only destroy agricultural crops but can also spread several diseases in humans such as endemic rickettsiosis, plague, spirochetosis, leishmaniasis, leptospirosis, and tick-borne encephalitis (1). Chronic rodenticides, also called anticoagulant rodenticides, are generally preferred over acute rodenticides to control rats and mice populations (2). The active components of these pesticides are Indandione derivative and 4-Hydroxycoumarin. Only a small portion of these active components, ranging from 0.005 to 25%, is used in baits. Anticoagulants prevent blood clotting factor resulting from enzymatic lack of vitamin K which helps in clotting. Anticoagulants were discovered in 1940s, their quick results and efficacy make them a dominant method of rodent control (3). To date, two types of anticoagulant-based rodenticides have been introduced: first generation and second-generation (4). First generation anticoagulants include warfarin, diphacinone and chlorophacinone while second generation are brodifacoum, difenacoum, bromadiolone and difethialone. Rodents developed resistance against first generation anticoagulants after the latter were used frequently, hence second-generation anticoagulants were introduced as a more effective alternative (5, 6). Bromadiolone, $C_{30}H_{23}BrO_{4,}$ is a secondary rodenticide commercially available under the trade name of "MooshMoosh" and is greenish blue in color. It is also available with alternative names such as Pest-off and Talon. Bromadiolone was discovered in 1967 for commensal rat and mice population. Its solubility is 20mg/L in water, 8mg/L in ethanol and 730g/L in dimethyl formamide (7). Flocoumafen is another secondary anticoagulant rodenticide available under the common name of Storm and is an off-white color and has a molecular formula of $C_{33}H_{25}F_3O_4$-coumarin with a mechanism of action akin to that of warfarin. The presence of 40-coumarin on side chain makes flocoumafen 100-fold more effective compared to warfarin (5). The

Key words: rodenticides; complete blood count; efficacy

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0393-974X (2020)

current study was designed to compare and contrast bromadiolone and flocoumafen rodenticides and evaluate their efficacy and utility.

MATERIALS AND METHODS

A total of 50 healthy albino mice (*Mus musculus*) aged four weeks and weighing 25-30 g were included in this study. The mice were raised in sterile stainless-steel cages in a ventilated animal room under experimentally controlled conditions (temperature: 24±2°C; humidity; 60±10% and light: 12 h/dark cycle). Twelve mice were selected for testing their bromadiolone and flocoumafen tolerance. Because 100% survival was required, the animals were placed in different cages and acclimatized for a few days before bromadiolone or flocoumafen treatment. To assess the level of tolerance, acute toxicity test was carried out by the "no choice method". Two group of animals were selected for testing the acute toxicity test; one group consisting of 6 mice for bromadiolone tolerance and another group of 6 mice for flocoumafen tolerance. Oral toxicity study was conducted to determine the effects of bromadiolone and flocoumafen on albino mice at various intervals. New groups were assigned to

Table I. *Survival rate of mice after bromadiolone acute intoxication at different time intervals.*

BROMADIOLONE ACUTE INTOXICATION					
Exposure time	Bait consumption (g)	Dose ingested (mg)	Total number of mice	Number of alive mice	Number of dead mice
6 hours	0.8±0.09	4±0.47	6	6	-
12 hours	1.5±0.21	7.7±1.07	6	6	-
24 hours	3.5±0.41	17.3±2.05	6	6	-
48 hours	8±0.58	39.8±2.9	6	6	-
72 hours	12.1±0.52	60.5±2.59	6	5	1
96 hours	16.1±0.54	80.6±2.71	5	4	1
120 hours	20.7±0.5	103.7±2.52	4	2	2
144 hours	24.4±0.31	122.1±1.57	2	-	2

Table II. *Survival rate of mice after flocoumafen acute intoxication at various time intervals.*

Exposure time	Bait consumption (g)	Dose ingested mg/kg	Total number of mice	No of alive mice	Number of dead mice
6 hours	0.8±0.12	4±0.58	6	6	-
12 hours	1.4±0.25	7.2±1.27	6	6	-
24 hours	3.7±0.29	18.6±1.43	6	6	-
48 hours	6.7±0.35	33.7±1.77	6	6	-
72 hours	13.7±0.33	68.3±1.63	6	6	-
96 hours	18.3±0.34	91.5±1.71	6	5	1
120 hours	23.3±0.34	116.7±1.7	5	3	2
144 hours	28±0.47	140.1±2.35	3	-	3

animals. The animals of both categories i.e. bromadiolone and flocoumafen were divided into 4 groups, named G1 to G5, each consisting of 3 mice. G1 was the control group which received no anticoagulant doses and were fed on normal feed. G2 was treated for 6 hours, G3 for 12 hours, G4 for 24 hours and G5 for 48 hours in either category. Blood samples were taken from mice in both categories through sinus puncture for hematology analysis. The blood was drawn into clean sterile vials containing EDTA as anticoagulant. After every single treatment, mice in both categories (bromadiolone and flocoumafen) and control group were sacrificed, after which their kidneys and livers were dissected for comparative analysis. Weight of the kidney and liver was determined and compared for mice in both categories along with blood count i.e. RBCs, WBCs (neutrophils, basophils etc.), platelets.

RESULTS

Survival rate against Bromadiolone remained 100% up to 48 hours but declined subsequently with time. The survival rate was 83% at 72 hours, 66% at 96 hours and 50% at 120 hours. After 144 hours all the mice were dead (Table I). Flocoumafen toxicity

Table III. *Effects of bromadiolone on complete blood count of Mus musculus at different time intervals.*

Parameters	Group 1 (control)	Group 2 (6 hours)	Group 3 (12 hours)	Group 4 (24 hours)	Group 5 (48 hours)
WBCs ($x10^3/\mu L$)	10.26 ± 0.15^{D}	10.4 ± 0.1^{D}	13.23 ± 0.68^{C}	16.13 ± 0.41^{B}	21.80 ± 0.69^{A}
RBCs ($10^{12}/L$)	7.96 ± 0.15^{A}	8.06 ± 0.05^{A}	7.86 ± 0.22^{A}	7.80 ± 0.26^{A}	7.50 ± 0.86^{A}
Hgb (g/dL)	14.6 ± 0.55^{A}	14.2 ± 0.32^{AB}	14.16 ± 0.05^{ABC}	13.53 ± 0.47^{BC}	13.26 ± 0.75^{C}
HCT (Vol%)	38.1 ± 0.15^{A}	37.2 ± 0.26^{A}	36.06 ± 3.87^{AB}	31.8 ± 0.15^{BC}	33.46 ± 0.89^{C}
MCV (fl)	49.3 ± 0.55^{A}	45.4 ± 0.53^{B}	46.13 ± 0.41^{B}	45.4 ± 0.53^{B}	37.1 ± 1.22^{C}
MCHC (g/dL)	38.2 ± 0.17^{B}	40.5 ± 0.50^{A}	38.36 ± 0.40^{B}	36.7 ± 0.64^{C}	35.84 ± 0.20^{D}
MCH (pg)	17.51 ± 0.5^{C}	18.27 ± 0.11^{B}	19.3 ± 0.32^{A}	19.16 ± 0.15^{A}	18.53 ± 0.40^{B}
Neutrophils (%)	20 ± 2.0^{A}	21.26 ± 1.1^{B}	21.8 ± 1.04^{C}	22.16 ± 1.04^{C}	22.06 ± 0.60^{C}
Lymphocytes (%)	55 ± 5.0^{C}	68.6 ± 3.51^{B}	68.53 ± 0.50^{B}	71.40 ± 1.21^{B}	76.8 ± 0.91^{A}
Monocytes (%)	2.6 ± 1.52^{B}	03 ± 1.0^{B}	3.66 ± 0.57^{AB}	4.16 ± 0.15^{AB}	5.2 ± 1.31^{A}
Eosinophils (%)	2.6 ± 2.08^{A}	4.3 ± 0.57^{A}	4.16 ± 0.29^{A}	5.33 ± 0.57^{A}	04 ± 2^{A}
PLT (/cmm)	384.3 ± 51.0^{A}	216 ± 76.5^{AB}	317 ± 55.0^{AB}	192.66 ± 51.5^{B}	194 ± 59.22^{B}

Table IV. *Effects of flocoumafen on complete blood count of Mus musculus at different time intervals.*

Parameters	Group 1 (control)	Group 2 (6 hours)	Group 3 (12 hours)	Group 4 (24 hours)	Group 5 (48 hours)
WBCs ($x10^3/\mu L$)	10.26 ± 0.15^{A}	8.43 ± 0.4^{B}	8.83 ± 0.28^{B}	10.4 ± 0.50^{A}	10.80 ± 0.26^{A}
RBCs ($10^{12}/L$)	7.96 ± 0.15^{A}	7.65 ± 0.56^{A}	6 ± 0.21^{B}	5.80 ± 0.26^{B}	4.76 ± 0.25^{C}
Hgb (g/dL)	14.6 ± 0.55^{A}	14.46 ± 0.41^{A}	11.4 ± 0.50^{B}	11.63 ± 0.55^{B}	11.96 ± 1.0^{B}
HCT (Vol%)	38.1 ± 0.15^{A}	33.3 ± 4.16^{AB}	36.06 ± 3.87^{A}	28.3 ± 0.36^{C}	29.76 ± 0.25^{BC}
MCV (fl)	49.3 ± 0.55^{B}	47.63 ± 0.47^{C}	47 ± 0.52^{D}	51.5 ± 0.51^{A}	51 ± 1.76^{AB}
MCHC (g/dL)	38.2 ± 0.17^{B}	38.5 ± 0.5^{B}	37.9 ± 0.51^{A}	37.5 ± 0.45^{A}	37.1 ± 1.10^{A}
MCH (pg)	17.51 ± 0.5^{C}	18.1 ± 0.17^{C}	19.56 ± 0.51^{B}	21.46 ± 0.50^{A}	21.2 ± 0.8^{A}
Neutrophils (%)	22 ± 2.0^{A}	22.23 ± 0.25^{B}	22.2 ± 2.17^{B}	22.5 ± 0.5^{B}	24.06 ± 0.60^{B}
Lymphocytes (%)	55 ± 5.0^{C}	72 ± 2.0^{B}	77 ± 3^{AB}	77 ± 3.0^{AB}	82.33 ± 3.21^{A}
Monocytes (%)	2.6 ± 1.52^{A}	3.67 ± 0.57^{AB}	04 ± 2.64^{AB}	4.66 ± 0.57^{B}	6.67 ± 2.51^{B}
Eosinophils (%)	2.6 ± 2.08^{B}	05 ± 1.0^{AB}	5.67 ± 1.52^{A}	5.33 ± 1.15^{AB}	4.3 ± 1.52^{AB}
PLT (/cmm)	384.3 ± 51.0^{A}	404 ± 177.07^{A}	353 ± 42.02^{A}	274 ± 75.0^{A}	253 ± 40.70^{A}

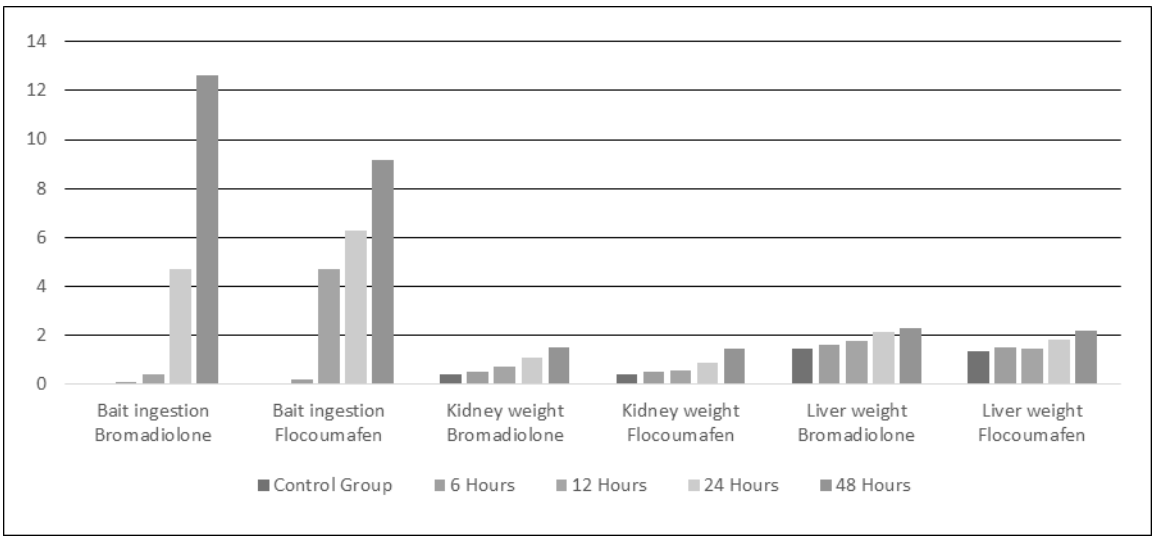

Fig. 1. *Comparative action of bromadiolone and flocoumafen on Mus musculus bait ingestion, kidney weight and liver weight.*

demonstrated a 100% survival rate till 72 hours and then gradually declined, falling to 0% after 144 hours (Table II). The blood count of mice in both categories was then compared. Mice which ingested bromadiolone bait showed significance variation for different parameters in blood of different groups (Table III). WBC increased from 10.26±0.15 to 21.80±0.69 from group 1 to group 6, respectively, whereas RBCs decreased from 7.96±0.15 to 7.50±0.86 from group 1 to group 6, respectively. While all other parameter, such as Hgb (14.6±0.55 to 13.26±0.75), HCT (38.1±0.15 to 33.46±0.89), MCHC 38.2±0.17 to 35.84±0.20) decreased with time, neutrophils (20±2.0 to 22.06±0.60), monocytes (2.6±1.52 to 5.2±1.31) lymphocytes (55±5.0 to 76.8±0.91) and eosinophils (2.6±2.08 to 4.0±2) showed increase in number. Platelets decreased drastically.

Mice treated with Flocoumafen bait showed significant variation in different parameters of different groups (Table IV). Similar trends of blood cell counts were observed in the case of bromadiolone. There was an increase in WBC, and a decrease in RBCs and platelets after bromadiolone ingestion in mice.

Bait consumption was more for Flocoumafen compared to bromadiolone. Decreasing trend in liver and kidney weight was observed in both groups with the passage of time compared to the control group. Moreover, Flocoumafen showed more of a decrease in organ weight compared to bromadiolone (Fig. 1).

DISCUSSION

The main component of bromadiolone is dicoumarol. Dicoumarol delays prothrombin formation and thus anti-coagulates the blood. Delay in clotting has also been observed in a 40-year-old female who accidently ingested bromadiolone (8). Bromadiolone is also involved in the degeneration of liver and kidney, resulting in the delay of excretion and reduction in platelet count. The dicoumarol acts as an inhibitor to block the vitamin K cycle (3).

In this study, baiting with bromadiolone induced mortality after 48 hours whereas in the cases of flocoumafen, mortality began after 72 hours of baiting. Similar results have been reported by Bhattacharyya and Borah (2), who studied efficacy of four SGARs as bromadiolone, brodifacoum, difenacoum and Flocoumafena on *Mus musculus*. They found bromadiolone more efficient in action than flocoumafen; therefore, our results are in agreement with theirs.

Based on oral toxicity test results, it is concluded

that bromadiolone is more efficient than flocoumafen. Bromadiolone degrades cofactors required for blood clotting more efficiently than flocoumafen. It can be assumed that due to availability of antidote and minimal non-target attractions, second generation rodenticides can be considered more useful in homes, offices, educational institutes, shops and agricultural fields.

REFERENCES

1. Slankard KG, Gaskill CL, Lynne M, Cassone LM, Rhoden CM. Changes in detected anticoagulant rodenticide exposure in barn owls (Tyto alba) in Kentucky, USA, in 2012–16. J Wildl Dis 2019; 55(2):432-37.
2. Bhattacharyya B, Borah RK. Comparative evaluation of second-generation anticoagulant rodenticides against Mus musculus. Ind J Agric Res 2015; 50(1):1-5.
3. Shore RF, Henrys PA, Walker LA. Power analysis of liver second generation anticoagulant rodenticide (SGAR) residue data in barn owls from Britain. 2017. http://nora.nerc.ac.uk/id/eprint/508401/.
4. Meehan AP. Rodenticidal activity of bromadiolone—a new anticoagulant. Proceedings of the 8th Vertebrate Pest Conference 1978; 31:122-26.
5. Lo VM, Ching CK, Chan AYM, Mak TW. Bromadiolone toxicokinetics: Diagnosis and treatment implications. Clinical Toxicology 2008; 46:703-10.
6. Huckle KR, Hutson DH, Logan CJ, Morrison BJ, Warburton PA. The fate of the rodenticide flocoumafen in the rat: retention and elimination of a single oral dose. PesticSc 1989; 25:297-312.
7. Fisher P. Review of house mouse (Mus musculus*)* susceptibility to anticoagulant poisons. DOC Sci Internal Series 2005; 198:5-17.
8. Revathi K, Yogananda M. Effect of Bromadiolone on hematology, liver and kidney in Mus musculus. J Environ Biol 2004; 27(1):135-40.