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Antidoting Effect of Vitamin K_1 Supplementation on Difethialone Poisoned House Rat, *Rattus rattus* Linneaus

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Abstract: With the advent of second-generation anticoagulant rodenticides rodent management technology has substantially improved, since they provide sustainable control of rodents and are relatively safe to non-targets compared to acute rodenticides. Two dosages (1 and 2 mg kg^{-1}) of vitamin K_1, an effective antidote for such rodenticides, supplementation for 5 and 15 days were given to house rat *R. rattus* fed on difethialone bait (0.0025%) for one day for studying the effect of antidoting on poisoned rats. The results indicated that the lower dosage could not reverse the anticoagulation process, however the period of mortality was considerably increased from 3-9 days (in control) to 4-11 and 11-22 days (5 and 15 days supplementation regime, respectively). However, when the vitamin K_1 dosage was doubled and given for 15 days, antidoting was more pronounced due to reversal of anticoagulation process in 83% rats, which became normal within a month of difethialone poisoning.

Key words: *Rattus rattus,* anticoagulants, difethialone, baits, vitamin K_1 supplemented food, antidote.

Anticoagulant rodenticides have proved a landmark development in the field of rodent pest management. Their origin lies in an accidental observation on hemorrhagic condition in cattle due to feeding on spoiled sweet clover hay in USA. The active ingredient in the feed was later identified as dicoumarin. Subsequently compounds like warfarin, fumarin, coumachlor, diphacinone, chlorophacinone, etc., collectively referred as first generation anticoagulant rodenticides, were synthesized, which possessed greater potency against wide range of rodents and were able to overcome nearly all the negative aspects of their predecessors, the acute rodenticides (Hadler and Buckle, 1992). However, due to their extensive use in commensal and field rodent control, problem of resistance and cross-resistance was reported against many rodent pests in Europe and USA (Boyle, 1960; Greaves *et al.*, 1976; Hadler and Buckle, 1992). Resistance to warfarin in case of *R. rattus* was also reported in India (Deoras, 1967; Arora and Lal, 1979). These rodenticides could not gain much acceptance by the end users in India due to their multi-dose requirement extending upto 2-3 weeks of exposure for effective kill of target pests (Mathur and Prakash, 1984). Thus zinc phosphide, being a fast acting acute rodenticide, continued to dominate the chemical control of rodents despite several limitations in its usage viz., pre-baiting requirement, induction of bait/poison

shyness in target pests, extreme toxicity to non-target animals and lack of effective antidote.

Evolution of single dose anticoagulants in later years referred as second generation anticoagulant rodenticides included bromadiolone, brodifacoum, flocoumafen, difethialone, etc., have revolutionized the concept of rodent management all over the world. They are very potent rodenticides against a variety of rodent pests because of higher single dose toxicity (LD_{50} 0.08-2.5 mg kg^{-1}) (Mathur *et al.*, 1992) at lower dosages (0.0025-0.005% in baits) without pre-baiting requirements and having an effective antidote in the form of vitamin K_1. These rodenticides have been found to be highly effective against a variety of Indian rodent pest species in laboratory and field (Jain, 1980; Balasubramanyan *et al.*, 1984; Jain and Tripathi, 1988; Jain *et al.*, 1992; Parshad, 1999; Chaudhary and Tripathi, 2003).

The mode of action of the anticoagulants involves inhibition of blood coagulation in exposed animals, which ultimately die of internal bleeding. Both anticoagulant and its antidote vitamin K_1 are known to share the same site of action by blocking the epoxide reductase enzyme system in the blood (Silverman, 1980). Studies on safety aspects of vitamin K_1 in accidental ingestion of anti-coagulant rodenticides by higher vertebrates have been conducted by several authors (Mackintosh *et al.*, 1988; Woody *et al.*, 1992; Nee *et al.*, 1999; Reitemeyer *et al.*, 2001; Markussen *et al.*, 2003). However, such studies against rodents are very limited. Markussen *et al.* (2003) studied the vitamin K_1 requirement in anticoagulant resistant Norway rats and Chaudhary *et al.* (2004) reported the antidoting effect of vitamin K_1 supplementation on anticoagulant poisoned Indian gerbil (*Tatera indica*). Difethialone, a new introduction in the series of second-generation anticoagulants has also proved its potency against wide range of commensal as well as field rodents in India (Sheikhar and Sood, 2000; Sridhara *et al.*, 2000; Chaudhary and Tripathi, 2003, 2004; Chaudhary *et al.*, 2005). Present communication attempts to quantify the antidoting effect of vitamin K_1 supplementation in difethialone treated house rats, *Rattus rattus* in laboratory.

Materials and Methods

Toxicity of difethialone baits vis-à-vis antidoting effect of vitamin K_1 was evaluated on house rat, *Rattus rattus* Linn. under caged conditions. The house rats were live trapped from houses and godowns of Jodhpur city (Lat. 26°18′N Long. 73°01′E) using live Sherman traps. It was ascertained that the captured rodents had no previous experience of feeding on any type of anticoagulant baits. Trapped juveniles, sub-adults, pregnant females and sick animals were discarded and only healthy adults (95-125 g) were subjected to these trials. The test rodents were weighed and lodged in iron mesh cages (24"x12"x12") for acclimatization. During this period the rats were fed pearl millet (*Pennisetum glaucum*) grain and water was available to them *ad libitum*.

Experimental design

The experiments were conducted in five sets representing five treatments. In each set six healthy house rats were kept individually in iron mesh cages at 28±4°C

with 40% RH. Two dosages of vitamin K_1 supplementation (1 and 2 mg kg^{-1}) at 2 feeding regimes (5 and 15 days) were evaluated for their role in affecting the anticoagulation in difethialone-poisoned rats. Four sets were used as treatment sets and the fifth one served as control. Prior to vitamin K_1 supplementation, each animal (30 nos.) was exposed to pearl millet based difethialone bait (0,0025%) for 24 hours under no-choice condition. Vitamin K_1 supplemented diet was provided by mixing its weighed quantities in arachis oil smeared peral millet grain so as to achieve a dossge of 1 mg and 2 mg kg^{-1}. Set-wise details of experimental design is briefed as under:

Sets I and II: Immediately after feeding on anticoagulant rodenticidal bait for 24 hours, the test animals of these two sets were provided vitamin K_1 supplemented diet @ 1 mg kg^{-1} body weight which was almost equal to the a.i. of difethialone ingested by test animals in one day exposure. In Set I the vitamin K_1 supplemented feed was given for 5 days whereas in Set II this period was extended for 15 days.

Sets III and IV: In these sets, the dosage of vitamin K_1 supplementation was doubled i.e., 2 mg kg^{-1}. This feed was also provided to the difethialone (0.0025%) fed rats under two feeding regimes i.e., for 5 days (Set III) and for 15 days (Set IV).

Set V: This set acted as control and the experimental rats were exposed to difethialone (0.0025%) in pearl millet grains for one day and only arachis oil (2%) smeared pearl millet grain (no vitamin K_1 supplement) was offered on subsequent occasions till death of the animals.

The symptoms of difethialone poisoning appears in the house rats after 3-4 days of poisoning and lasts upto 14 days (Chaudhary and Tripathi, 2003), therefore, two feeding regimes of 5 and 15 days were experimented for this study. Consumption pattern and symptoms of poisoning, such as blood discharge from nose, eyes, anus, ear, etc., dullness in activity and paralyses of limbs and death of experimental rodents were monitored in all the sets for 30 days immediately after feeding on difethialone baits.

Results and Discussion

Mean consumption of difethialone treated baits was significantly at par ranging between 3.73 and 4.44 g/100 g b. wt. in all the treatment sets and control indicating fairly good acceptability of pearl millet based difethialone baits by the house rats. Studies of Sheikher and Sood (2000), Sridhara *et al.* (2000) and Chaudhary and Tripathi (2003) conducted on Indian rodents indicated that it is fairly effective at half the dosages (0.0025%) as compared to other anticoagulant rodenticides viz., bromadiolone, brodifacoum, flocumafen, etc., of the same generation, which are effective at 0.005% in baits (Jain *et al.*, 1992; Mathur *et al.*, 1992). In the present investigation also, one-day exposure of difethialone (0.0025%) yielded 100% mortality of house rat (Control set V) (Table 1). Hadler and Buckle (1992) have included difethialone in five highly potent anticoagulants of second generation due to its lower LD_{50} values in the range of 0.2-0.4 mg kg^{-1} against Norway rats and thus its capability to kill the rats in single feed.

Results of the vitamin K_1 supplementation in diets of the poisoned rats are discussed below:

Table 1. Effect of supplementary feeding of vitamin K_1 on difethialone fed Rattus rattus (Values expressed in g/100 g body weight are mean±SE)

Experiment Set	Difethialone bait (0.0025%) consumption (g/100 g b. wt.) (Mean±SE)	Post treatment food consumption				% mortality (n/N)	Mean days to death (%)	Survival (n/N)
		Vitamin K_1 supplement		Plain food				
		Mean consump-tion/day (Mean±SE)	Mean total conumption (Mean±SE)	Mean conump-tion/day (Mean±SE)	Mean total consumption (Mean±SE)			
Set-I	4.17±0.11	3.53±0.35	16.88±2.35	2.97±0.44	29.28±11.83	83.34 (5/6)	8.20 (4-11)	16.67 (1/6)
Set-II	3.73±0.39	3.06±0.25	44.03±5.12	3.33±0.68	29.07±11.93	66.67 (4/6)	17.50 (11-22)	33.33 (2/6)
Set-III	4.25±0.35	4.52±0.16	22.61±0.82	3.57±0.49	50.80±20.38	66.67 (4/6)	11.75 (9-17)	33.33 (2/6)
Set-IV	4.44±0.35	4.42±0.40	66.30±6.07	3.88±0.49	52.31±8.58	16.67 (1/6)	24 (-)	83.33 (5/6)
Set-V (Control)	4.25±0.14	–	–	3.34±0.47	18.25±4.87	100 (6/6)	5.84 (3-9)	Nil (0/6)

Set I: Intake of vitamin K_1 supplemented diet during post-poisoning period ranged from 1.0 to 4.9 g by the individual test rats. Out of six animals 5 could survive the entire vitamin K_1 supplementation period (5 days) because their consumption of the supplemented diet was 3.0 g, whereas in case of sixth animal (No.4) this consumption dropped on 2nd day resulting in its death on 4th day itself. The fatal anticoagulation effect was also noticed in another rat (No.3) immediately after withdrawal of vitamin supplement. Similarly three rats (Nos. 2, 5 and 6) too died subsequently on day 10, 7 and 11 even after vitamin K_1 supplementation for 5 days. However, out of 6 animals, one (No.1) could recover from fatal effect of anticoagulant, because its daily intake of vitamin K_1 diet on all the five days of exposure was maximum (4.0-4.9 g/100 b. wt.). Total intake of vitamin K_1 supplemented bait was 16.88 g/100 g b. wt. in five days with a daily mean of 3.53 g/100 g b. wt. However, mean daily intake of plain food after vitamin K_1 supplementation was further reduced to 2.97 g/100 g b. wt. (Table 1). Thus reduced intake and recovery of only one out of six test rats (16.7%) indicated poor antidoting effect of vitamin K_1 supplementation @ 1 mg kg^{-1} for 5 days on the difethialone poisoned rats.

Set II: Increasing the period of vitamin K_1 supplementation (1 mg kg^{-1} dose) from 5 to 15 days recorded some effect of antidoting the toxicity of difethialone in house rats as 33.33% poisoned rats survived. The mean daily consumption of difethialone bait, vitamin K_1 supplemented diet and plain food was almost at par i.e. 3.73, 3.06 and 3.33 g/100 g b. wt., respectively (Table 1). Mortality and consumption pattern of test rodents indicated that one animal (No.2) recording drastic reduction in vitamin feed intake (1.35-2.7 g) died within the period of vitamin K_1 supplementation, whereas other 3 rats (Nos. 1, 3 and 5) could survive up to period of vitamin K_1 feeding, but died within 2-3 days after cessation of antidote supplementation. However, the two surviving rodents (Nos. 4 and 6) maintained a steady pattern of feed intake and became normal within three weeks of poisoning. Besides the complete recovery of one-third test animals, the dead rodents too survived for longer duration (11-22 days) as compared to Set I (4-11 days). This revealed that steady supply of vitamin K_1 for 2 weeks played an important role in reversal of anticoagulation process to some extent.

Set III: When dosage of antidote supplementation was doubled to 2 mg kg^{-1} for a five-day exposure period, 33.3% of test rodents recovered from anticoagulant poisoning even after consuming 3.42-4.9 g/100 g b. wt. (av. 4.25 g) of difethialone (0.0025%) bait. However, increased dosage of vitamin K_1 supplementation could help other rodents to survive the entire period of dosing with antidote (5 days) and died 4-6 days after withdrawal of vitamin K_1 treated food. The completely recovered animals (Nos. 5 and 6) registered 4.0-5.10 g/100 g b. wt. consumption of the vitamin K_1 supplemented food. Analysis of overall consumption during five day period of vitamin K_1 supplementation revealed a total intake of 22.61 g/100 g b. wt. Daily intake of vitamin K_1 treated bait (4.52 g/100 g b. wt.) was at par with that of difethialone bait (4.25 g/100 g b. wt.), however, post treatment plain food intake after 5 days

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