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POTENTIATION OF ANTICOAGULANT TOXICITY TO *Rattus rattus* BY TWO NON-STEROID ANTI-INFLAMMATORY DRUGS

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ABSTRACT: In view of resistance reported to have developed towards second generation anticoagulants and the problem of bait shyness and neophobia when acute rodenticides are used it becomes imperative that methods be evolved to overcome these problems. Attempts to potentiate anticoagulants for effective rodent control is a new concept with very few studies. Experiments using two non-steroid anti-inflammatory, drugs namely ibuprofen and phenylbutazone at 80 mg/kg and 50 mg/kg body weight respectively to potentiate the action of two second generation anticoagulants, brodifacoum and bromadiolone yielded positive results for *Rattus rattus.* The drugs reduced the lethal dose required for 100% mortality as well as days to death. Field trials confirmed laboratory findings.

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INTRODUCTION

The concept of total rodent eradication still remains a dream with acute rodenticides like zinc phosphide leaving bait shy populations and anticoagulants resulting in resistant strains. Even the second generation anticoagulants are reported to have led to development of resistance (Rowe et al. 1981). This new trend makes it invitable to potentiate second generation anticoagulants to ensure complete mortalities quickly. Certain drugs are known to interact with oral anticoagulants by a variety of mechanisms such as altered absorption, binding and/or metabolism of protein. The action of phenylbutazone in potentiating anticoagulant effect is well known (Lewis et al. 1974). Hence experiments were designed to study the possibility of potentiation of two second generation anticoagulants viz bromadiolone (C_{30} H_{23} Br O_4), 3-(4 hydroxy-3- coumarinyl), 3 phenyl-l-(4 bromo-p-biphenyl) propanol) also known as 'Supercaid' and 'Maki' and brodifacoum (C_{31} H_{23} O_3 Br), (-(3-(4 bromobiphenyl-4-yl) - 1,2,3,4-tetra hydronaphthyl-I-yl)-4-hydroxy coumarin) by two non-steroidal, anti-inflammatory drugs (NSAIDs) namely phenylbutazone and ibuprofen.

METHODS

The experimental procedure consisted of five steps: First to establish toxicity of wax cakes and technical grade concentration of bromadiolone and brodifacoum to *Rattus rattus.* Secondly to establish the tolerance limits of*R. rattus* to ibuprofen and phenylbutazone. Third to evaluate the potentiation of wax cakes of brodifacoum and bromadiolone by the two drugs. Fourth to test the potentiation of technical grade concentrations of the two rodenticides. Finally field evaluation of wax cakes mixed with the two drugs was carried out. All experiments were conducted on wild, field caught *Rattus rattus.*

Bromadiolone Toxicity

No choice test—Rats (N = 12) after being acclimated to laboratory conditions were weighed and caged individually. Each rat was exposed to a single wax cake (20g) containing 0.005% rodenticide for 24 hrs and its intake recorded. Thereafter the subjects were fed standard rat feed and observed for poison ingested, days to death and mortality for 3 weeks. A similar no choice test on 12 subjects was conducted but by exposing them to the cake for 48 hrs. Data on poison ingested, mortality and days to death were recorded over 3 weeks.

Choice test—Two batches of rats (N=12) were exposed to wax cakes and non-poisonous ragi bait (*Eleusine coracana*) + 10% groundnut oil (*Arachis hypogea*) for 24 and 48 hrs respectively. At the end of exposure period, data on the amount of ragi bait and poison cake consumed, days to death and mortality were documented.

Oral toxicity studies—After acclimation to laboratory conditions *R. rattus* with equal representation of the two sexes were weighed and caged individually and starved overnight. Calculated amount of technical grade (99.99%) bromadiolone, were dissolved in known quantities of polyethylene glycol to get the required percent concentration. Depending on the weight of the animal, calculated amounts of bromadiolone was injected directly into the stomach of 20 animals by oral intubation in order to get 1, 2, 3, 4 and 5 mg poison/kg body weight. Six hours later the rats were given standard rat feed and were maintained for 3 weeks on the same feed during which time the days to death and mortality were recorded. Using probit analysis (Litchfield and Wilcoxin 1949), the LD_{50} values were calculated.

Brodifacoum Toxicity

No choice and choice tests were carried out on brodifacoum wax cakes (0.005%). The procedure followed, being similar to bromadiolone evaluation.

Acute oral toxicity studies—Five concentrations of technical grade brodifacoum (99.99%) namely 0.5, 1.0, 1.5, 2.0 and 2.5 mg/kg were employed for determining LD_{50} values. The experimental details were similar to bromadiolone oral toxicity tests.

Tolerance limit for ibuprofen and phenylbutazone

Ibuprofen—Four batches of *R. rattus* comprising of 10 rats each (5 males and 5 females) were acclimated and starved overnight prior to the test. To one batch of rats 5ml ibuprofen (Fenlong, Sol Pharmaceuticals Ltd, Hyderabad) caliberated to contain 20 mg/kg body weight was directly fed into the stomach by oral intubation. Similarly the remaining three groups of rats were given dosages of 40 mg/kg, 80 mg/kg and 100 mg/kg. Twelve hours later experimental rats were fed normally and were kept under observation for mortality for three days.

Phenylbutazone—Forty rats divided into four batches of 10 each were subcutaneously injected with calibrated doses

of 50 mg/kg, 100 mg/kg, 150 mg/kg and 200 mg/kg after starving them overnight. Twelve hrs after treatment the rats were put on regular diet for 3 days and mortalities recorded.

Potentiation of wax cakes of bromadiolone and brodifacoum by ibuprofen and phenylbutazone

Bromadiolone—No choice test—Twenty-four *R. rattus* were individually caged and starved overnight. One batch of 12 rats was fed ibuprofen at 80 mg/kg body weight by oral intubation and the second batch subcutaneously injected with 50 mg/kg phenylbutazone. In the afternoon all the rats were given one bromadiolone wax cake (20 mg) for 24 hours after which the rats were fed normally and kept under observation for mortality.

Choice test — The acclimated rats, numbering 24, were starved overnight. Next morning they were divided into two batches: one batch was injected with 50 mg/kg phenylbutazone and the other intubated with 80 mg/kg body weight with ibuprofen. Both groups were exposed to a choice test of 30g each of mixed cereals and standard rat feed and 20g bromadiolone wax cake containing 0.005% rodenticide for 24 hrs. Later they were maintained on standard rat feed and observed for mortality.

Brodifacoum—Evaluation of potentiation of brodifacoum wax cakes (0.005%) was done by carrying out no choice and choice tests as described for bromadiolone. In all the tests quantity of wax cake consumed was weighed and converted to g consumed/lOOg body weight for comparisons.

Potentiation of technical grade anticoagulants.

Bromadiolone—A total of 20 *R. rattus* were acclimated to laboratory, starved overnight and weighed in the morning. To one batch, ibuprofen at 80 mg/kg was administered by stomach intubation and to the second batch of 10 rats phenylbutazone at 50 mg/kg was injected subcutaneously. In the afternoon all the 20 animals were administered bromodiolone dissolved in polyethylene glycol at 2 mg/kg. After four hours they were fed standard rat feed and observed for percent mortality and duration to death.

Brodifacoum—To ibuprofen and phenylbutazone administrated rats as in the previous experiments, 1 mg brodifacoum/kg was stomach fed by intubation followed by observations on mortality and days to death.

Field Evaluation of potentiated anticoagulants

Nine poultry sheds measuring 100' x 40' and housing 2,000 chicks each at the Central Poultry Breeding and Research Institute, at Hesaraghatta, 25 km from Bangalore were selected for evaluating the potentiated anticoagulant activity of bromadiolone and brodifacoum. The sheds were infested with only *Rattus rattus.* The population in each shed was estimated by surplus baiting method (Barnett et al.1951) using poultry feed. The rates of bait consumption per shed are presented in Table 1. Based on earlier observations that a single *R. rattus* weighs an average of 11Og and consumes a mean of 14g poultry feed (Krishnamurthy 1990) the number of rats present in each shed and their total weights were extrapolated (Table. 1). The tolerable and effective dosages of ibuprofen and phenylbutazone for the rat population of each shed was calculated as in Table 1. The evaluation of their potentiation was carried out in two sets of experiments. In the first set of tests, wax cakes of brodifacoum and bromadiolone were powdered and mixed with calculated amounts of ibuprofen and phenylbutazone separately. Thus shed 1 was exposed to brodifacoum + ibuprofen, shed 2 to brodifacoum + phenylbutazone, shed 3 to bromadiolone + ibuprofen, shed 4 to bromadiolone + phenylbutazone at the dosages presented in Table 1. Each shed had 10 bait points. Similarly sheds 5 to 8 were exposed to 10 bait stations of poultry containing the two drugs as calibrated in Table 1. In sheds 5 and 6 brodifacoum cake was placed beside each bait container and in sheds 7 and 8 bromadiolone cakes were placed similarly. The 9th shed served as control. After 48 hrs of exposure the baits were removed and population estimates carried out by surplus baiting. The difference in the bait consumed prior to and after treatment were converted into percent reduction in bait consumed and tabulated (Table 2). The significance of potentiation of bromadiolone and brodifacoum by ibuprofen

Table 1. Details of rodenticide and calibrated dosages of anti-inflammatory drugs evaluated in poultry sheds.

Shed No.	Kg bait consumed	Equivalent rats calculated at 14g/rat	Total wt. of rat (kg/shed) (@110g/rat)	Rodenticide plus calibrated dosage of NSAIDs
1	1.331	95	10.45	25g brodifacoum[a]+38.36 ml IP [b]
2	1.148	82	9.05	25g brodifacoum[a]+2.25 ml PB [c]
3	1.512	108	11.88	25g bromadiolone[a]+9.5 ml IP
4	1.064	76	8.36	25g bromadiolone + 2.9 ml PB
5	0.742	53	5.83	0.742 kg poultry feed + 4.66 ml IP
6	1.092	78	8.58	1.092 kg poultry feed + 2.15 ml PB
7	1.204	86	9.46	1.204 kg poultry feed +7.57 ml IP
8	1.61	115	12.65	1.61 kg poultry feed + 3.16 ml PB
9	1.274	91	10.01	1.274 kg poultry feed

[a]Powdered brodifacoum and bromadiolone
[b]IP = Ibuprofen at 80 mg/kg.
[c]PB = Phenylbutazone at 50 mg/kg.

Table 2. Field Trials of anticoagulants potentiated by ibuprofen and phenylbutazone.

Treatment	% Reduction in bait consumed
Brodifacoum + Ibuprofen	72.5
Brodifacoum + Phenylbutazone	85.5
Bromadiolone + Ibuprofen	55.6
Bromadiolone + Phenylbutazone	62.7
Brodifacoum + Ibuprofen in poultry feed	87.5
Brodifacoum + Phenylbutazone in poultry feed	91.7
Bromadiolone + Ibuprofen in poultry feed	75.6
Bromadiolone + Phenylbutazone in poultry feed	83.3
Control	9.4

ANOVA table for above data.

Source of variation	Df	Mean square	F. ratio
Replicates	4	55.09	2.33[a]
Rodenticides (A)	1	2250.00	95.16[b]
Potentiating agent (B) mixed in feed	1	640.03	27.07[b]
Potentiating agent mixed with wax powder (C)	1	2387.05	100.95[b]
A x B	1	3.59	0.15[a]
A x C	1	235.23	9.95[b]
B x C	1	42.00	1.78[a]
A x B x C	1	55.22	2.34[a]
ERROR	28	23.64	—

[a]Not significant
[b]Very highly significant ($p<0.001$)

and phenylbutazone was arrived at by applying Duncan's Multiple Range test (Fedrer 1963).

RESULTS

Toxicity of bromadiolone and brodifacoum

Bromadiolone—In no choice test, 24 hrs exposure of bromadiolone cake (0.005%) resulted in mean 70% mortality of *R. rattus* with females significantly ingesting more of wax cake than males and succumbing 80% as compared to 60% mortality of males. Forty eight hrs exposure to the poison resulted in 90% mortality in males. Females ingested more poison and succumbed 100%. Days to death was an average of 7.9 days for 24 hrs poisoned rats and 5.78 days for 48 hrs fed rats.

In choice tests only 60% of both sexes died in 7.8 days when bromadiolone cake was fed for 24 hrs. Forty eight hours exposure to the same resulted in 100 percent mortality in 6.7 days.

Oral toxicity experiments demonstrated LD_{50} value of bromadiolone as 2.2 mg/kg with a confidence limit of 1.1-4.4 mg/kg for males and 1.75 mg/kg for females with confidence limits of 0.76-4.02 mg/kg.

Brodifacoum—Both the sexes succumbed 100% to brodifacoum wax cakes in both choice and no choice tests as well as 24 and 48 hours exposure. Death occurred faster in no choice test i.e., 4.6 days and 3.5 days in 24 and 48 hrs exposure respectively, whereas in choice tests death occurred in 6.7 and 6.1 days for 24 and 48 hrs exposed rats respectively. Comparatively lower amount of poison was ingested during choice test: i.e., 3.31 mg/kg (24 hrs) and 4.39 mg/kg (48 hrs) than during no choice test i.e., 6.5 mg/kg and 7.98 mg/kg for 24 and 48 hrs poisoned rats respectively

Experiments on oral toxicity established LD_{50} value of brodifacoum as 0.9 mg/kg for male rats with confidence limits of 0.276-2.923 mg/kg and for females 0.7 mg/kg with confidence limits of 0.165-2.975 mg/kg.

Tolerance limits of *R.rattus* to ibuprofen and phenylbutazone

Ibuprofen—Rats treated with ibuprofen at 20 mg/kg, 40 mg/kg, 60 mg/kg and 80 mg/kg did not die even after 72 hrs but those which ingested 100 mg/kg died within 24 hrs suggesting the tolerance limit to be 80 mg/kg.

Phenylbutazone at 100 mg/kg, 150 mg/kg and 200 mg/kg induced 100 percent mortality after 24, 2 and half an hour respectively. Therefore 50 mg/kg of phenylbutazone seems to be the tolerance limit for *R.rattus.*

Potentiation of anticoagulant activity of wax cakes by the two drugs

Bromadiolone—Rats fed with 80 mg/kg ibuprofen followed by exposure to bromadiolone wax cake for 24 hours succumb within 24 hours compared to 100% mortality and 6.7 days to death when only bromadiolone cake was given that too for 48 hrs. Even the amount of poison ingested, 2.52 mg/kg in choice test and 2.49 mg/kg in no choice test, was less when compared to rats fed only on bromadiolone (6.13 mg/kg),

Subcutaneous injection of phenylbutazone at 50 mg/kg to bromadiolone poisoned rats also resulted in 100% overnight mortality in both no choice and choice tests compared to 6.7 days when rats were exposed for 48 hrs to bromadiolone cakes. Even the poison ingested was only 1.25 mg/kg in choice test and 1.73 mg/kg in no choice test (Table 3).

Brodifacoum — The potentiation of brodifacoum wax cakes by the two NSAIDs was similar to that of bromadiolone. Ibuprofen + brodifacoum wax cake fed rats succumbed 100% overnight (Table 4) compared to 4.6 and 3.5 days taken by rats fed only on brodifacoum cakes for 24 and 48 hrs respectively in no choice tests and 6.7 and 6.1 days taken by rats tested in choice tests after 24 and 48 hrs exposure. The poison ingested was similarly far less in ibuprofen treated rats in choice test and no choice test compared to rats poisoned with only brodifacum cake.

Phenylbutazone too potentiated the action of brodifacoum wax cakes by inducing death overnight and at concentrations far below that of rats exposed only to cakes both in no choice and choice tests (Table 4).

Duncan's Multiple Range test (Fedrer 1963) revealed following significant differences between bromodiolone cake and brodifacoum cake poisoned rats and those rats whose poison action was potentiated by ibuprofen and phenylbutazone: (1) Between bromadiolone and brodifacoum, the later was more potent as seen by lesser amounts

Table 3. Evaluation of potentiation of bromadiolone cake by ibuprofen and phenylbutazone.

Treatment	Sex	Body weight (g)	Qty of bait consumed (mg/kg)	% Mortality	Days to death
Choice Test					
Bromadiolone cake + Ibuprofen @ 80 mg/kg (Oral intubation)	M	190.0±3.54	2.97±0.76	100	4.0±0.28
	F	187.5±4.15	2.66±0.38	100	4.0±0.28
	—	180.75±2.76	2.92±0.6	100	4.0±0.2
Bromadiolone cake + Phenylbutazone @ 50 mg/kg (Subcutaneous)	M	110.0±13.11	1.51±0.27	100	
	F	71.25±9.42	1.34±0.19	100	Overnight
	—	90.63±10.59	1.25±0.23	100	
No Choice Test					
Bromadiolone cake + Ibuprofen @ 80 mg/kg (Oral intubation)	M	132.5±18.58	2.16±0.03	100	4.3±0.21
	F	142.5±18.58	2.82±0.21	100	4.0±0.35
	—	137.5±13.26	2.49±0.12	100	4.3±0.28
Bromadiolone cake + Phenylbutazone @ 50 mg/kg (Subcutaneous)	M	122.5±15.26	1.61±0.21	100	
	F	120.0±15.41	1.85±0.19	100	Overnight
	—	121.25±10.85	1.73±0.15	100	

Table 4. Evaluation of potentiation of brodifacoum cakes by ibuprofen and phenylbutazone.

Treatment	Sex	Body weight (g)	Qty of bait consumed (mg/kg)	% Mortality	Days to death
Choice Test					
Brodifacoum cake + Ibuprofen @ 80 mg/kg (Oral intubation)	M	138.0±13.89	1.92±0.24	100	4.2±0.33
	F	131.0±12.52	2.32±0.67	100	3.6±0.36
	—	134.5±9.42	2.12±0.14	100	3.9±0.26
Brodifacoum cake + Phenylbutazone @ 50 mg/kg (Subcutaneous)	M	110.0±17.74	0.28±0.03	100	
	F	119.0±13.52	0.26±0.05	100	Overnight
	—	115.0±35.49	0.27±0.03	100	
No Choice Test					
Brodifacoum cake + Ibuprofen @ 80 mg/kg (Oral intubation)	M	94.0±9.53	0.71±0.13	100	4.4±0.22
	F	109.0±23.81	0.72±0.16	100	4.4±0.22
	—	101.5±13.04	0.71±0.09	100	4.4±0.15
Brodifacoum cake + Phenylbutazone @ 50 mg/kg (Subcutaneous)	M	142.5±9.88	0.13±0.02	100	
	F	123.75±9.05	0.12±0.02	100	Overnight
	—	133.13±10.04	0.13±0.01	100	

of bait requirement and shorter duration to death in both 24 and 48 hrs exposure to poison. (2) Both ibuprofen and phenylbutazone potentiated the action of bromadiolone and brodifacoum cakes. (3) The potentiation by phenylbutazone was higher than that of ibuprofen. (4) The potentiation of brodifacoum cakes by phenylbutazone was higher than that of bromadiolone cakes (Table 5).

Potentiation of technical grade anticoagulants by the two drugs

When rats were fed on sub-lethal doses of bromadiolone (2 mg/kg) and brodifacoum (1 mg/kg) by stomach intubation and potentiated with ibuprofen and phenylbutazone, death occured in comparatively shorter duration in ibuprofen treated rats but overnight in phenylbutazone injected subjects (Table 6). Duncan's Multiple Range test of experiments on potentiation of technical grade bromadiolone and brodifacoum by ibuprofen and phenylbutazone indicated three significant points: (1) That the two drugs significantly potentiated the action of the two anticoagulants. (2) Between the two drugs, phenylbutazone was more potent as seen by overnight mortality. (3) Brodifacoum was potentiated more than

Table 5. Duncan's analysis of the efficacy and potentiation of bromadiolone and brodifacoum cakes by NSAIDs in *Rattus rattus.*

Sl. No.	Rodenticide treatment	Sex	Bromadiolone 0.005% wax a	Bromadiolone 0.005% wax b	Brodifacoum 0.005% wax a	Brodifacoum 0.005% wax b
1	24 hrs exposure to wax cake	M	8.0 A	3.58 A	6.6 A	3.33 A
		F	7.7 A	3.75 A	6.8 A	3.28 A
2	48 hrs exposure to wax cake	M	7.2 AB	5.73 D	6.4 A	4.26 B
		F	6.2 B	6.55 D	5.8 A	4.51 B
3	Potentiation with	M	4.0 C	2.97 AB	4.2 B	0.71 C
	ibuprofen @ 80 mg/kg	F	4.0 C	2.66 ABC	3.6 B	0.72 C
4	Potentiation with	M	—	1.15 C	—	0.13 D
	phenylbutazone @ 50 mg/kg	F	—	1.34 BC	—	0.12 D
	SD & (df)		±0.8 (20 df)	±0.52 (32 df)	±0.34 (24 df)	±0.17 (32 df)

Legend: a - Days to death
b - Quantity of bait consumed in mg/kg

Note: The significance of the treatment is calculated through Duncan's Multiple Range test between means of the columns. The difference between the two means is significant if they are followed by a different alphabet ($p<0.005$).

bromadiolone as seen by shorter duration to death (Table 7).

Field evaluation of potentiated anticoagulants

Brodifacoum cake potentiated with ibuprofen and phenylbutazone gave higher reduction of poultry shed rats than similarly potentiated bromadiolone (Table 2) and between the two NSAIDs, phenylbutazone was more effective. The same trend was seen when NSAIDs were added to poultry feed and exposed to rats along with the rodenticides (Table 2). Thus the field trials when subjected to Duncan's Multiple Range test proved laboratory findings (Table 2).

DISCUSSION

With reports of development of resistance to brodifacoum and difenacoum (Rowe et al. 1981) it becomes imperative to evolve methods to overcome this problem. One such approach could be to potentiate the second generation anticoagulants by employing additives which can act as absorbers of vitamin K, capable of damaging capillaries, inducing injuries and interfering with the mechanism of clotting. Activated clay, charcoal and carbon when combined with warfarin resulted in 88%, 75% and 63% mortality which was attributed to vitamin K absorption (Muktha Bai and Rao 1979). Similarly L-hystidine combined with low dosages of warfarin caused 100% mortality of *R.rattus* due to haemorrhage caused by warfarin coupled with vasoconstrictor action and release of natural heparin from liver by L-hystidine (Muktha Bai and Rao 1979). The two non-steroidal anti-inflammatory drugs, phenylbutazone and ibuprofen potentiated the action of bromadiolone and brodifacoum in the present study by reducing time to death to overnight and reducing the lethal doses. The NSAIDs are known to interact with oral anticoagulants by a variety of mechanisms. Evidence is available to show that anticoagulants are affected by drugs which alter their absorption, protein binding or metabolism (Breckenbridge et al. 1978). Phenylbutazone displaces warfarin from albumin and invariably leads to increased hypothrombinemia of anticoagulated patients (Koch-Weser

Table 6. Potentiation of technical grade bromadiolone and brodifacoum by ibuprofen and phenylbutazone.

Treatment	Sex	Body weight	Days of death
A. BROMADIOLONE @ 2 mg/kg			
Bromadiolone only	M	177.5±11.29	6.0±1.41
(control)	F	175.7±14.89	6.6±0.83
	—	176.5±9.57	6.38±0.75
Bromadiolone +	M	149.0±16.64	4.4±0.83
Ibuprofen	F	115.0±6.16	4.4±0.46
	—	132.0±10.37	4.4±0.47
Bromadiolone +	M	138.0±15.79	
Phenylbutazone	F	139.0±19.41	Overnight
	—	138.5±12.51	
B. BRODIFACOUM @ 1 mg/kg			
Brodifacoum only	M	112.0±7.67	4.8±0.49
(control)	F	123.0±18.75	4.4±0.25
	—	117.5±11.39	4.6±0.37
Brodifacoum +	M	111.0±9.84	2.8±0.18
Ibuprofen	F	101.0±11.52	2.2±0.33
	—	106.0±7.74	2.5±0.21
Brodifacoum +	M	103.0±10.03	
Phenylbutazone	F	99.0±9.21	Overnight
	—	107.0±17.64	

Table 7. Duncan's analysis of potentiation of orally intubated bromadiolone and brodifacoum by anti-inflammatory drugs—ibuprofen and phenylbutazone.

S1 No.	Rodenticide	Sex	Bromadiolone @ 2 mg/kg		Brodifacoum @ 1 mg/kg	
			Weight (g)	Days to death	Weight (g)	Days to death
Potentiation as agents						
1	Phenylbutazone	M	138.00 A	—	107.00 A	—
	@ 50 mg/kg	F	139.00 A	—	99.00 A	—
2	Ibuprofen	M	143.00 A	4.4 A	111.00 A	2.8 A
	@ 80 mg/kg	F	115.00 A	4.4 A	101.00 A	2.2 A
3	Control	M	177.5 A	6.0 A	112.00 A	4.8 B
	(only anticoagulants)	F	175.7 A	6.6 A	123.00 A	4.4 B
	SEm		±17.27 (24 df)	±0.82 (16 df)	±13.77 (24 df)	±0.38 (16 df)

Remarks: Duncan's Multiple Range test conducted to check the significance of potentiation revealed that there is no dependence on the weight of the rat and phenylbutazone has a very high potentiating effect on both the rodenticides as it brings down the days to death to overnight.

and Sellers 1971). It is also known to displace both warfarin isomers from plasma albumin in vivo and in vitro. In humans it is known to inhibit the biotransformation of the S isomer while stimulating the elimination of R isomer. Since the S isomer is 5 times more potent than R isomer, potentiation of warfarin induced hypothrombinemia is reported to occur (Sellers 1978).

A second way of potentiation of anticoagulants by NSAIDs seems to be due to injuries caused. Many NSAIDs including ibuprofen and phenylbutazone cause gastrointestinal damage by way of ulceration and bleeding (Van kolfschoten et al. 1983). Of the two drugs phenylbutazone was more potent ulcerogenic than ibuprofen (Diamentis et al. 1980). The present observations of shorter duration to death at comparitively lower doses of anticoagulants when rats are fed/injected with ibuprofen and phenylbutazone supplements the findings of Diamentis et al. (1980).

The third way in which NSAIDs potentiate anticoagulants may be by affecting wound healing. Healing of wounds has many phases such as granulation, collagenation, contraction, epithelization and scar remodelling. All these phases excepting the last are dependent on each other and occur simultaneously. Many NSAIDs are known to interfere with one event or other. Diwan and Kulkarni (1986) observed ibuprofen and phenylbutazone depressing granulation, collagenation and initial phase of contraction of wounds.

It may be concluded that both ibuprofen and phenylbutazone enhance the efficacy of bromadiolone and brodifacoum by affecting their binding, inducing gastric ulceration, bleeding and finally by interfering with natural healing of wounds.

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