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Acute Oral Toxicity of Warfarin to Poultry, *Gallus domesticus:* A Non-target Species

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Anticoagulant rodenticides like warfarin, pindone, diphacinone, dicoumarol etc. have been used for the control of rodent pests besides their use in clinical medicines as therapeutic agents for treating thrombophlebitis disease (Gleason et al. 1957; Harrison 1974; Platt 1979). They have also been used extensively to combat rodent menace both outdoors and indoors due to their properties, such as low single and high multiple dose toxicity, besides being less hazardous to non-target species. However, warfarin-(3-(alpha-phenyl-beta-acetyl-ethyl)-4 hydroxycoumarin), enjoyed the status of 'most safe' and 'innocuous rodenticide' and dominated the field of rodent control till the reports on its primary and secondary poisoning including human fatalities were reported (Lange and Terveer 1954; Evans and Ward 1967; Carson 1977).

As a chronic rodenticide the recommended period of baiting with warfarin (0.025%) to control the rodent pests ranges from 9-15 or 30 days, and at times it has been extended even upto 20 weeks (Rowe and Chudley 1963). Due to its long period of availability as baits, consequential accidental poisoning to various non-target species are not unlikely.

Consideration of toxicities of single doses of anticoagulants is necessary to assess the risk of accidental poisoning of non-target species. While reports on massive single ingestion of warfarin to other animals, viz. dogs, cats, swine and ruminants are available (William et al. 1973), information on toxicity to poultry is meagre. Hence, an attempt is made here to establish acute oral toxicity of warfarin to poultry.

MATERIALS AND METHODS

Cockerels (White leghorn) were obtained from a local poultry farm and conditioned in the laboratory for two weeks prior to the experimentation. They were weighed, statistically grouped, numbered, maintained in individual cages and fed on grower mash and water ad libitum. Six birds of 9-10 weeks old, weighing between 580-680 g were used for each dose.

Each bird was weighed prior to intubation and the required amount of warfarin (99.8% pure, Pest Control India Pvt. Ltd., Bombay), calculated on mg/kg body weight basis was weighed into empty gelatin capsules. The dosages tested were 500, 750, 1000 and 2000 mg/kg b.w. The capsules were administered to birds by placing them into their gullet with forceps. Birds administered empty gelatin capsules served as controls. These were observed daily for four weeks for toxic signs, mortality, etc. At the end of 4th week, birds were autopsied and the weights of liver, kidney, heart, spleen, lungs, testis, brain, adrenals and thyroid were recorded. These tissues were fixed in formalin, paraffin embedded, sectioned, stained with haematoxylin and eosin and examined for histopathological changes. The organ weights and weekly body weights were subjected to statistical analysis. The LD_{50} and LD_{90} values were calculated by probit analysis (Finney 1977).

RESULTS AND DISCUSSION

Out of four dosages tested (500, 750, 1000 and 2000 mg/kg b.w.) the first two dosages killed only 16.6% of the test birds, while 66.6% and 100% of the birds succumbed to the two higher doses (Table 1). The signs of poisoning generally appeared at one to two hours of feeding the capsules. The common signs observed were frequent

Table 1. Acute oral toxicity of warfarin to poultry

Dosage (mg/kg b.w.)	Av.b.w. (g)	Mortality (%)	Death time (h)	mg/kg b.w.	
				LD_{50} (95% confidence limits)	LD_{90}
500	643	16.6	14	942 (730-1215)	1985
750	649	16.6	29		
1000	634	66.6	6-68		
2000	667	100	3-30		
0.0	651	0.0	0		

fluttering of the wings, gasping and paralysis (commencing from the legs and extending towards neck region). A few showed haemorrhages in the eyes and mouth. A dose-dependent blanching of comb was observed. Severe blanching was recorded in birds which died from warfarin intoxication. Recovery was indicated in survivors by the gradual reappearance of red colour in the combs. Most of the birds died within 3 days (14-68 h) of administration, and those not dying within that period gradually recovered. Of the twelve birds that died, nine took more than 20 h, while the remaining three died within 6 h of administering warfarin capsules. The LD_{50} and LD_{90} values obtained were 942.0 and 1985 mg/kg b.w.

respectively (Table 1). All survivors showed reduced gain in body weight compared to controls. The reduction in weight gain was dose-dependent (Table 2).

The liver, heart, spleen and brain of the survivors administered different doses of warfarin were increased in weight ($P<0.05$) as compared to controls (Table 3). However, no significant differences were noticed in the weights of other organs. Mild cellular hypertrophy and sinusoid dilatations were noticed in the liver of all treated survivors; kidney and lungs showed slight to moderate haemorrhages. The hearts from some birds showed areas of mild cellular infiltration. No significant changes were observed in the remaining organs.

The acute oral LD_{50} value of 942 mg/kg b.w. determined in our experiment indicates that poultry (Gallus domesticus) are more tolerant to warfarin than rats (LD_{50} being 5.6 mg/kg b.w. Muktha Bai et al., 1986). The value (1000 mg/kg b.w.) is very similar to that reported by Spector (1955). The rapid appearance of poisoning signs within an hour or two of administering warfarin suggests that the toxic action of warfarin is different in birds compared to rats. In rats, signs always appear after 2-3 days of feeding and never earlier. This early onset of toxic signs in poultry is advantageous to treat them in cases of accidental poisoning. The highest dose tested (2000 mg/kg b.w.) although resulted in 100% mortality, could not bring instantaneous death or reduction in the death time as compared to lower doses (Table 1). This delayed action in birds fed higher doses could be related to a slow (but complete) absorption of warfarin from the intestine at enteric pH (William et al. 1973). The fact that 83.3% of the birds survived at 500 and 750 mg/kg b.w. of warfarin (Table 1), loss in body weight gain, increase in relative organ weights (viz., liver, brain, heart and spleen), coupled with histopathological observations of liver (cellular infiltration), kidney and lungs (haemorrhage), indicates the persistence of toxicity at single massive doses.

The high LD_{50} value (942.0 mg/kg b.w.) obtained for poultry compared to rats (5.6 mg/kg b.w.) could be due to the differences in the coagulation pattern between birds and mammals (Grimminger 1965; Stopforth 1970; Archer 1971) and the presence of lower quantities of Factor-VII in chicken (which is required during the process of coagulation and which gets depressed in warfarin poisoned animals; Bell and Freeman 1971). This difference in the toxicity of warfarin to birds and rats is adequate for developing "selective baits" for practical purposes, besides considering warfarin as one of the "safe" and "innocuous" rodenticides to combat rodent pests where poultry is a major non-target species. To cause toxicity, a bird weighing 1-1.5 kg would have to consume at least 4-6 kg of 0.025% warfarin bait (recommended concentration) at a time, which is unlikely. Assuming that, by chance they consume that quantity of bait or even less having higher concentration of warfarin, due to quick manifestation of poisoning

Table 2. Body weights of cockerels administered single doses of Warfarin

Dosage (mg/kg b.w.)	Body weights (g/bird) at week				
	Initial (g)	I	II	III	IV
0	651	858.0^{b} ±19.7	970.7^{b} ±15.9	1056.3^{b} ±18.2	1202.0^{b} ±16.6
500	643	796.0^{ba} ±29.2	900.4^{ab} ±35.3	1031.0^{ab} ±52.6	1172.0^{ab} ±46.7
750	649	812.4^{b} ±25.1	916.0^{ab} ±28.6	1034.8^{b} ±26.2	1146.8^{ab} ±28.1
1000	634	684.5^{a} ±24.5	801.0^{a} ±13.0	905.0^{a} ±32.0	1024.0^{a} ±19.0

Means of the same column followed by different letters differ significantly at 5% level (Student's 't' test).

Table 3. Relative organ weights of cockerels administered single doses of Warfarin at 4th week

Dosage (mg/kg b.w.)	Weight (g/1000 g. body weight)								
	Liver	Heart	Kidney	Spleen	Lungs	Testis	Brain	Adrenals	Thyroid
0	19.8^{d} ±0.7	4.7^{ab} ±0.3	7.9^{a} ±0.5	1.6^{b} ±0.1	5.5^{a} ±0.3	1.8^{a} ±0.8	2.4^{c} ±0.06	0.10^{a} ±0.01	0.07^{a} ±0.004
500	24.4^{ab} ±1.5	4.5^{b} ±0.2	8.9^{a} ±0.5	1.7^{b} ±0.1	6.3^{a} ±0.4	1.9^{a} ±0.7	2.6^{b} ±0.04	0.12^{a} ±0.02	0.09^{a} ±0.02
750	22.5^{ac} ±0.6	4.5^{b} ±0.07	8.7^{a} ±0.2	1.9^{ab} ±0.2	8.0^{a} ±1.4	1.2^{a} ±0.9	2.5^{bc} ±0.09	0.10^{a} ±0.01	0.08^{a} ±0.01
1000	26.4^{a} ±3.9	5.6^{a} ±0.3	10.5^{a} ±1.4	2.3^{a} ±0.07	5.6^{a} ±0.02	1.1^{a} ±0.4	3.4^{a} ±0.004	0.06^{a} ±0.0	0.08^{a} ±0.001

Means of the same column followed by different letters differ significantly ($P<0.05$) (Student's 't' test).

symptoms the birds could be treated on time before they succumb to warfarin poisoning.

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