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STUDIES ON GOLDFLEECE® AND DIAZOLE 60EC® BRANDS OF DIAZINON PESTICIDE AS A FOLLOW-UP TO INTOXICATION IN CATTLE DIPPED WITH GOLDFLEECE®

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SUMMARY

Acute oral and dermal toxicity studies in rats and thin layer chromatographic analysis of two brands of diazinon: Goldfleece® and Diazole 60EC® were carried out. The Goldfleece® diazinon has LD_{50} of 2.83 mg/kg and a TLC of two compounds with R_f values of 0.91 and 0.48, respectively, while Diazole 60EC® has LD_{50} of 141.42 mg/kg and a single compound with R_f value of 0.91 *per os*. The dermal LD_{50} and LC_{50} for Goldfleece® were 1,732 mg/kg and 0.866 mg/ml, respectively, while the corresponding values for Diazole 60EC® were 2236 mg/kg and 1.0006mg/ml, respectivey. The higher toxicity of Goldfleece® may be due to its second (metabolic) compound content. The clinical signs (muscarinic, nicotinic and CNS disturbances) observed in the treated rats with both brands of diazinon are classical for organophosphate poisoning.

KEY WORDS: Goldfleece®, Diazole 60EC®, Toxicity, Rats, TLC analysis

INTRODUCTION

Diazinon is the common name for the organophosphate insecticide with the chemical name 0,0-diethyl-0- (6-methyl-2-[1-methylethyl]-4-pyrimidinyl) phosphorothioate. It is widely used to control various insect pests in the soil, ornamental plants, fruits and vegetable crops (Worthing and Walker, 1983) as well as those of household (Curie *et al.,* 1990) and livestock (Ester *et al.*, 1993) including lice, ticks, ked fleas and tse-tse flies. In veterinary practice, diazinon preparations are applied as sprays or as a dip, which the animals swim through, using a concentration that ranges from 0.04 to 0.1 percent aqueous solution.

This compound is marketed under the common trade names of Alfatox®, Dazzel®, Knoxout®, Gardentox®, AG500®, Gold Fleece®, Diazole®, Basudin, Diazajet, Diazide, Sarorex, Spectrocide, *et cetera* by various companies.

Diazinon, is moderately hazardous, with acute oral LD_{50} of 300 400 mg/kg and dermal LD_{50} of 2,150 mg/kg in rats (Bartsch, 1974). The clinical signs of diazinon intoxication *per os* in treated rats are characteristic of organophosphate poisoning, categorized as muscarinic, nicotinic and central nervous system (CNS) effects.

The muscarinic signs may include hypersalivation, lacrimation, nasal discharges, diarrhea, frequent defecation and urinary incontinence. These toxic manifestations are the result of the stimulation of the secretory glands while the frequent defecation is due to increased smooth muscle contraction leading to increased gastrointestinal tone and motility.

The nicotinic effects may include fasciculation of the muscles and the weakness, respiratory distress, cheyne-stroke respiration and generalized paralysis and are due to the accumulation of acetylcholine (Ach) at the skeletal muscle junction and the sympathetic preganglionic nerve endings.

The central nervous system signs of O-P intoxication may include nervousness, apprehension, ataxia, respiratory distress, convulsion and coma, and are ascribable to accumulation of Ach in the cerebral cortex, hippocampus, extrapyramidal motor system with depression of respiratory centres in the CNS. The

stimulation of bronchial glands which result in secretions to clog the airways, also contribute to the respiratory distress.

In the year 2002, at a farm in Kyaudan Bako village in the outskirt of Zaria, an incidence of Goldfleece® poisoning was reported (Personal Communication). A dip, with a capacity of 1,500 gallons (6,000 litres) was used to treat cattle for ectoparasites. The dip contained 6 litres of Goldfleece® of 60% w/w diazinon in water and was made up to a final concentration of 0.6 mg/ml.

Seventy of the 85 cattle, 5-7 years of age herded through the prepared clinical dip died within an hour after the dipping, an additional 6 died within the next 48 hours. Only 9 of the cattle survived field observation. Three cattle egrets that apparently fed on the ticks from the treated cattle also died. However, there were neither clinical signs of poisoning nor deaths among the 20 local breeds of sheep (Yankasa) that were simultaneously dipped along with the cattle. Clinical signs of intoxication started ten minutes after the dipping, with the cattle showing signs of dyspnoea, lacrimation, salivation, convulsive jerky movements and bellowing; the first death was observed 20 minutes after the exposure to the toxicant.

The above mentioned incident and other observed cases of poisoning in herds of cattle, and canine species dipped with the same batch of Goldfleece® in the Large and Small Animal Clinics at Faculty of Veterinary Medicine, Ahmadu Bello University Zaria necessitated the present toxicologic studies to elucidate the possible causes of toxicity associated with this pesticide.

Therefore the study was carried out to determine the oral and dermal acute toxicity and thin layer chromatographic (TLC) analysis of two brands of diazinon: Goldfleece® and Diazole®.

MATERIALS AND METHODS

Experimental Animals

Adult Wistar rats, weighing between 70 170 g, 8 weeks old obtained from the National Veterinary Research Institute, Vom, near Jos, Plateau State of Nigeria were used for the studies. The experimental rats were observed for a period of five weeks prior to the commencement of the experiments. The animals were fed with chick mash (Sander's Feeds, Kaduna) mixed with ground cassava flour as binder and ground roasted soya beans in a ratio of 4: 1 : 0.5, respectively. The feed was mixed, moulded and dried in a laboratory oven.

Test Chemicals

Goldfleece® is manufactured by the Bimedia Chemicals Export, Dublin Republic of Ireland but repackaged apparently at Lagos, Nigeria for the Petroleum (Special) Trust Fund (P.T.F.) with date of manufacture, expiry date and batch number, indicated as Mar 2000, Mar 2003 and FMA063A, respectively. The Diazol® was manufactured by the Makhtesin Chemical Works Ltd., P. O. Box 60 Beer-Sheva, Israel with dates of manufacture, expiry date and batch number as March, 2003, March, 2006 and 84 100 respectively. The Diazole 60EC® is distributed in Nigeria by Dizengoff W.A. (Nig) Ltd. Others are Ferric Chloride Solution. Dragendorff's (potassium bismuth iodide) solution, Shikimic acid, Acetic acid, Chloroform, and Methanol.

Test Solution Preparation

The Goldfleece® test chemical containing 60% w/w diazinon was diluted with distilled water to produce 10 ml volumes in concentrations of 15 mg/ml and 150 mg/ml. These stock solutions were variously diluted with distilled water to obtain the required test solutions for treatment of the rats.

Experimental Protocol

Goldfleece® Acute Oral Toxicity Studies

The determination of the median lethal dose was carried out using the method of Lorke (1983). In the first phase, three groups of 3 Wistar rats per group were weighed individually and placed in cages and approximately marked. The rats were treated with Goldfleece® orally at dose levels of 10, 100 and 1,000 mg/kg body weight. The animals were then monitored for a period of 72 hours for clinical signs of intoxication and mortality. In the second phase, 3 groups of 1 rat per group were given 2, 4 and 8 mg Goldfleece® per kg body weight based on the results of the first phase study. They were also monitored for clinical signs of intoxication and mortality for 72 hours.

The final LD_{50} value was calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

Goldfleece® Thin Layer Chromatographic (TLC) Studies

A silica TLC plate was prepared and then impregnated with Goldfleece® and placed in a tank containing a methanol ethanol mixture in a ratio of 4 : 1 to attain saturation. This preparation was then left for 24 hours to allow the components of the Goldfleece® to separate out (Stahl, 1969).

Goldfleece® Dermal Acute Toxicity studies

Twenty rats were separated into four groups designated as groups I, II, III and IV respectively. Each group consisting of five male rats, were treated as follows. The rats in group I, which served as the control, were individually immersed up to the neck for 1 min in 400 ml liquid containing diluted water only. The rats in groups II, III and IV were each immersed up to the neck for 1 min in 400 m l solution containing 0.06, 0.3 or 1.5 mg Goldfleece® per ml of water, respectively. The rats were thereafter placed per cage by groups (5 rats/cage) and observed for 3 hours for clinical signs of toxicity and death. The above procedure was repeated for two more days, selecting the same time periods after 24 hours interval. The animals were then observed for 14 days without further exposure to diazinon.

Diazole® Acute Oral Toxicity Studies

The method of Lorke (1983) as previously described for Goldfleece® studies was used. Same doses were used in the first phase of the study. In the 2nd phase however, the rats\ in each of the 3 groups were given 50, 100 and 200mg Diazole® per kg body weight respectively.

Diazole® Acute Dermal Toxicity Studies

Using Diazole 60EC®, the acute dermal toxicity studies were repeated exactly as for Goldfleece® using different sets of rats.

Diazole® Thin Layer Chromatography Studies

A thin layer plate was coated with silica gel and after activation, spotted with Diazol®. Fifty milliliters (50ml) of chloroform-methanol (in a ratio of 6:1) mixture was poured into the tank and covered so as to allow uniform saturation. The thin plate, after spotting, was placed in the saturated tank for 24 hours to allow the chemical constituent(s) of Diazol® to separate (Stahl, 1969). After separation, the plate was removed from the tank and allowed to dry. It was then developed with Dragendoff spray. The R_f value(s) of the separate component(s) were then recorded.

Acute Dermal LD_{50} and LC_{50} of Goldfleece® Toxicity Studies

The method described by Lorke (1983) with modification, was employed in the determination of dermal LD_{50} and LC_{50}. The experiment, performed by different trained individuals, employed the dermal route of exposure to administer the compound and distilled water as the vehicle. The experiment was performed in two stages.

In stage I, 18 rats were separated into three groups of six animals, each consisting of 3 males and 3 females (n = 6). They were fasted overnight and then the rats in groups I, II and III were immersed in the Goldfleece® at the following doses: 10, 100 or 1000 mg/kg (mass/volume), respectively by precutaneous route.

In stage II, eight rats were separated into four groups of two rats, each consisting of one male and one female (n = 2). They were the fasted overnight and the rats in groups I, II, III and IV were exposed to the Goldfleece® agent at concentrations of 1500, 2000, 2500 and 3000 mg per kg (mass/volume) respectively, by percutaneous route.

The rats were observed for 72 hours after treatment and the final LD_{50} values was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose (Lorke, 1983).

Similarly, the LC_{50} was calculated as the product of the lowest lethal concentration and the highest non-lethal concentration. That is, the geometric mean of the consecutive doses for which 0 and 100 % survival rats were recorded.

Studies for Determination of the Acute Dermal

LD_{50} and LC_{50} for Diazole 60EC®

Using Diazole 60EC® and the method described by Lorke (1983) dermally, the above procedure for Goldfleece® was carried out with a different set of rats.

Statistical Analysis

The data deduced from the dermal acute toxicity studies were statistically analyzed using analysis of variance (ANOVA) and expressed as mean ± S.E.M. The significance of differences between mean was tested by the student's t-test. Differences in means were considered to be significant at P = 0.05.

RESULTS

Goldfleece®

In stage I of acute toxicity studies (Lorke, 1983), there were no survival in all the three stages of rats dosed *per os* at 10, 100 and 1000 mg/kg body weight for groups I, II and III, respectively. The clinical signs and symptoms of intoxication observed were: increased salivation, rhinorrhoea, incoordination, buckling, staggering, diarrhea, polymia, tremors, respiratory distress and convulsions (group I); muscle twitching, tremors, ocular haemorrhoage, respiratory distress and tonic/clinic convulsions (group II); severe tremors, ocular haemorrhage, respiratory distress and tonic/clinic convulsions in group III rats. The stage II experimental rats had 100% survival in the group III rats treated at 2 mg/kg body weight, while there were no survival in groups I and II, treated at 8 and 4 mg/kg body weight respectively. The clinical signs in all the three groups include incoordination, knuckling, stagerring, rhinorrhoea, increased salivation, polymia, tremors, respiratory distress and convulsions with or without death terminally.

The oral LD_{50} was calculated to be 2.83mg/kg in the rats. The result of the thin layer chromatographic analysis indicated the presence of two compounds with R_f values of 0.48 and 0.91, respectively.

Diazole 60EC®

In the stage I of acute toxicity studies for Diazole® (Lorke, 1983), all the rats in group III dosed at 1000 mg/kg body weight showed symptoms of wet furs (= sweating), salivation, dyspnoea, lacrimation, prostration, abdominal discomfort, respiratory distress, sneezing, muscle twitching and fasciculation, convulsion, coma and death within 10 minutes after treatment.

The rats in group II showed mild symptoms of intoxication (with abdominal and respiratory distress) while those of group I did not show signs of intoxication. There were no deaths in groups I and II rats within 72 hours after treatment and 14 days thereafter.

In stage II experiment, only the rat in group III died. The LD_{50} was calculated to be 141.42 mg/kg *per os*. The thin layer chromatographic result of Diazole® revealed the presence of only one compound with an R_f value of 0.91.

Acute Dermal Toxicity Studies of Goldfleece® and Diazole 60EC®

Both Goldfleece® and Diazole® administered dermally produced similar clinical signs of toxicity as earlier observed during oral toxicity studies. A dose-dependent decrease in the mean onset of toxic effect manifestations after repeated daily exposure for 1 minute at the concentrations of 0.06, 0.3 or 1.5 mg/ml of diazinon was observed throughout the period of exposure (that is 24, 48 and 72 hours).

The percentage survival and mean time of onset of signs of dermal toxicity for Goldfleece® and Diazole 60EC® in Wistar rats are presented in Tables I and II, while clinical signs observed for dermal toxicity studies are contained in Table III.

The dermal LD_{50} values for Goldfleece® and Diazole 60EC® in Wistar rats were calculated to be 1,732 mg/kg and 2,236 mg/kg (Tables IV and V); while the LC_{50} values were calculated to be 0.866 mg/ml and 1.006 mg/ml, respectively (Tables VI and VII).

TABLE I: Percentage survival and mean onset of toxicity of Goldfleece® in Wistar rats

Number of exposure	Group	Number of animals	Concentration of Diazinon (mg/kg)	% Survival	Mean onset of toxicity ± SEM (min)
1	I	5	0	100	-
	II	5	0.06	100	*62.35 ± 3.25
	III	5	0.3	100	*19.47 ± 1.21
	IV	5	1.5	0	*3.26 ± 0.53
2	I	5	0	100	-
	II	5	0.06	100	*60.14 ± 2.13
	III	5	0.03	100	*18.01 ± 1.57
3	I	5	0	100	-
	II	5	0.06	100	*50.01 ± 3.01
	III	5	0.3	100	*14.46 ± 2.33

* Significantly different from Diazole 60EC® and represents P < 0.05, student's t-test. n = 5.

TABLE II: Percentage survival and mean onset of toxicity of Diazole 60EC® in Wistar rats

Number of exposure	Group	Number of animals	Concentration of Diazinon (mg/kg)	% Survival	Mean onset of toxicity ± SEM (min)
1	I	5	0	100	-
	II	5	0.06	100	*77.45 ± 1.15
	III	5	0.3	100	*31.30 ± 2.36
	IV	5	1.5	0	*19.25 ± 0.58
2	I	5	0	100	-
	II	5	0.06	100	*73.25 ± 2.43
	III	5	0.03	100	*28.20 ± 1.55
	IV	5	1.5	100	17.15 ± 1.28
3	I	5	0	100	-
	II	5	0.06	100	*70.30 ± 2.25
	III	5	0.3	100	*25.20 ± 1.47
	IV	5	1.5	100	15.20 ± 1.12

* Significantly different from Goldfleece® and represents P < 0.05, student's t-test. n = 5

TABLE III: Clinical Signs observed from acute dermal toxicity study

Clinical signs	Gold Fleece®			Diazol 60 EC®		
(Conc. mg/ml)	1.5	0.3	0.06	1.5	0.3	0.06
Salivation	+++	++	+	++	+	+
Lacrimation	+++	-	-	++	-	-
Rhinorrhea	+++	++	-	++	+	_
Frequent defecation	+++	++	+	++	+	+
Urinary incontinence	+++	++	+	++	+	+
Abdominal distension	+++	-	-	++	-	-
Diarrhoea	+++	+	-	++	+	-
Respiratory distress	+++	+	-	++	+	-
Chene-stokes respiration	+++	-	-	-	-	-
Weakness	+++	++	+	++	+	+
Trenor	+++	++	+	++	+	+
Spasms	+++	++	+	++	-	-
Convulsion	+++	-	-	-	-	-
Paralysis	+++	-	-	-	-	-
Coma	+++	-	-	-	-	-

+++Severe, ++Moderately severe, +Slightly severe, -Absent

TABLE IV: Calculation of dermal LD_{50} of Goldfleece® brand of Diazinon in Wistar rats

Stage 1 (n = 6)		Stage 2 (n = 2)		LD_{50}
Dose (mg/kg)	% Survival	Dose (mg/kg)	% Survival	
10	100	1500	100	
100	100	2000	0	√1500 x 2000
1000	100	2500	0	*1732 mg/kg
		3000	0	

*** This value is the median lethal dose that will kill 50% of the population**

TABLE V: Calculation of dermal LC_{50} of Goldfleece® brand of Diazinon

Stage 1 (n = 6)		Stage 2 (n = 2)		LC_{50}
Conc. (mg/kg)	% Survival	Conc. (mg/kg)	% Survival	
0.005	100	0.75	100	
0.05	100	1.0	0	√0.75 x 1
0.5	100	1.25	0	*0.866 mg/ml
		1.5	0	

* This value is the median lethal concentration that will kill 50% of the population.

TABLE VI: Calculation of dermal LD_{50} of Diazole 60EC® brand of Diazinon in Wistar rats

Stage 1 (n = 6)		Stage 2 (n = 2)		LD_{50}
Dose (mg/kg)	% Survival	Dose (mg/kg)	% Survival	
10	100	1500	100	
100	100	2000	100	√2000 x 2500
1000	100	2500	0	*2236 mg/kg
		3000	0	

* This value is the median lethal dose that will kill 50% of the population.

TABLE VI: Calculation of dermal LC_{50} of Diazole 60EC® brand of Diazinon in Wistar rats

Stage 1 (n = 6)		Stage 2 (n = 2)		LD_{50}
Conc. (mg/ml)	% Survival	Conc. (mg/ml)	% Survival	
0.0045	100	0.675	100	
0.045	100	0.9	100	√0.9 x 1.125
0.45	100	1.125	0	*1.006 mg/ml
		1.35	0	

* This value is the median lethal concentration that will kill 50% of the population

DISCUSSION

The *per os* LD_{50} 72 hours for Goldfleece® (60% w/w diazinon) and Diazole 60EC® are 2.83 mg/kg and 141.42 mg/kg body weight, respectively. The acute dermal LD_{50} of Goldfleece® and Diazole 60EC® are 1,732 mg/kg and 2,236 mg/kg body weight, respectively. The acute dermal LD_{50} in rats for diazinon is greater than 2,150 mg/kg (Bartsch, 1974).

According to the WHO (1996) classification of pesticides, LD_{50} of 2.83 mg/kg body weight in rats puts this brand and batch of diazinon in the category of the highly toxic chemical for Goldfleece® while 141.42 mg/kg body weight for Diazole 60EC® brand is in the category of the moderately toxic chemicals.

The R_f values of 0.91 and 0.48 for the two compounds observed for Goldfleece® and an R_f value of 0.91 observed for Diazole 60EC® indicate that there is the presence of an additional chemical compound in the Goldfleece® brand of diazinon which is apparently the agent producing toxicity that was observed at a level well below the 100-200 mg/kg body weight documented LD_{50} range of diazinon (EPA, 1990).

The results of the present study can be extrapolated to summarise that the incidence of poisoning of cattle and egrets that were exposed to Goldfleece® was due to the presence of a degradation product in this brand of diazinon. Also, the differentially high toxicity for Goldfleece® as compared to Diazole 60EC® treated rats in the laboratory was also probably due to the presence of this metabolite.

The toxicity of diazinon is very variable in the presence of water as such formulations hydrolyse to the highly poisonous tetraethyl-monothionopyrophosphate. The presence of these metabolites has at times led to serious accidental poisoning (Mello *et al.*, 1972).

This assertion was further corroborated by Vittorazzi (1976), by reporting that some formulations of diazinon were unstable and contained a number of potent impurities such as tetraethyl-pyrophosphate (TEPP) or sulfo-TEPP and monthiono-TEPP, which increase the health and environmental risk associated with diazinon use.

The R_f values of 0.91 and 0.48 that were obtained for the chromatographic studies were compared to the reference R_f value of diazinon and those of its metabolites; the R_f value of 0.91 correlates closest to that of diazinon (R_f value 0.86), while the lower R_f value of 0.48 could be that of tetraethyl-monothionopyrophosphate (R_f value 0.69) or sulfo-TEPP or monothioto-TEPP. The lower values for R_f values obtained could be due to difference in the solvent selection for TLC analysis.

The toxicity observation points to an apparent aqueous dilution or introduction of impurities during repackaging of the PTF Goldfleece® brand of the diazinon or a metabolic breakdown during storage to produce the highly poisonous tetraethylmonothionopyrophosphate. This compound is formed if insufficient water is added to diazinon and left unused for a long time. The apparent non-susceptibility to poisoning of the exposed sheep can be explained on the basis that diazinon poisoning to sheep is influenced by genetic factors; some breeds are more sensitive than others to the toxicant (Smith, 1970). Thus the non-toxicity to sheep may be due to genetic resistant of the exposed Yankasa breed of sheep.

The death of the egrets that fed on ticks and fleas from the exposed animals may be explained on the basis that birds are significantly susceptible to diazinon toxicity (Kendal *et al.*, 1992). The LD_{50} for birds, range from 2.75 to 40.8 mg/kg per day for normal diazinon (Bartsch, 1974).

CONCLUSION

This work indicates a need to test for effects of diazinon group of pesticides in lower laboratory animals from time to time to confirm their safety before usage in veterinary practice, especially if the stock has been in storage for long (Mello *et al.*, 1972).

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