PRELIMINARY TOXICOLOGICAL STUDIES ON 0, 0-DIETHYL, 0(2-ISOPROPYL - 6 - METHYL - 4, PYRIMIDINYL) PHOSPHOROTHIOATE (DIAZINON) IN BALAMI SHEEP.

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ABSTRACT

Toxicity studies of 0,0-diethyl-0-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate (Diazinon[®]) was carried out in the Balami breed of sheep. The agent was administered orally daily for one week at the rate of 20, 25 and 30mg/kg body weight to groups of sheep. The 30mg/kg dose produced transient clinical signs in the sheep. Diazinon inhibited plasma and red blood cell cholinesterase activity. The intensity of the inhibition was more with 30mg/kg dose and occurred as from day 3 and lasted till the end of the investigation 7 days post treatment. Leucocytosis was also observed with the 30mg/kg dose. There was no observable effect of the chemical on the RBC, Hb, and PCV values in sheep at the dosages used.

Key words: Sheep, Diazinon, Toxicity.

INTRODUCTION

0,0-diethyl,0-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate (Diazinon[®]), Geigy company, Switzerland) is an organophosphorus compound with low mammalian toxicity, higher biodegradability and low persistence in the tissues of the body (WHO, 1974). The agent also has high insecticidal properties (Johnson *et al.*, 1952); and has been used to control ectoparasites and DDT resistant pests (Holland, Hazleton, and Hanzal, 1952; Cuthrie and Backer, 1954). Diazinon is used extensively for the control of cotton pests and other insects of agricultural importance in Borno State of Nigeria. As a result of increasing use of pesticides and other agrochemicals, there is an acknowledged concern over the possible toxicological hazards

posed by a spectrum of agrochemicals (Paul, 1991).

In Nigeria, the incidence of poisoning by insecticides is not fully documented, although reports of poisoning are not uncommon. In Borno State, three incidents of accidental poisoning of animal due to the ingestion of crops sprayed with Fenitrothion[®] were reported between July 1979 and August, 1981 (Paul, 1991). Accidental poisoning in man due to inhalation of Queleatox[®] organophosphate has also been reported in Yobe and Borno States (Paul, 1991). In 1986, 100 cattle and 70 sheep were reported dead after grazing in an area sprayed with Dichlorvos[®] in Gamboru, Borno State, while in Okpiko, Benue State 203 cattle were reported dead after grazing in an Endrin[®] sprayed area (Ameh, 1988). The information on poisoning with diazinon[®] is lacking in Nigeria.

Animals usually vary in susceptibility to organophosphorus toxicity. The susceptibility of animals to these chemicals may be influenced by genetic factors (Smith, 1970), which may be related to the rate of hydrolysis of the phosphate in the plasma. The clinical signs of organophosphorous compounds in animals include salivation, frequent urination, increased lacrimation, restlessness, incoordination, hyspnea, rigidity of limbs, convulsions and death (Radeleff and Woodard, 1957; O'Brien, 1957; Jackson *et al.*, 1960; Radeleff, 1970) and the severity depends upon the degree of inhibition of cholinesterase enzyme.

Although, toxicity of diazinon in sheep has been reported over 20 years ago in other countries (Smith, 1970), the toxicity of this

chemical on our indigenous breeds of sheep has not been investigated. Therefore, the purpose of this study is to determine the toxicity of diazinon in Balami breed of sheep following oral administration.

MATERIALS AND METHODS

Experimental Animals:

Twelve clinically healthy Balami rams belonging to the University of Maiduguri, Department of Animal Science farm were used for the study. The sheep were dewormed with Fenbendazole (Panacur[®], Hoechst AG, Frankfurt Main, Germany) at a dose of 100mg/kg body weight *per os*. The animals weighed between 19 to 23kg at the start of the experiment and were 6 to 12 months old. They were kept in the experimental animal house and fed with hay, groundnut cake and water *ad libitum*.

Insecticide

Diazinon sixty percent emulsifiable concentration (Geigy Company, Switzerland) used in this study was obtained from the Federal Pest Control Unit, Maiduguri, Nigeria.

Experimental groups and sample collection

The sheep were divided into four groups with three animals in each group. Group 1 was the untreated control group. Diazinon was administered orally to groups II, III, and IV at doses of 20, 25 and 30mg/kg body weight respectively. The agent was administered daily for one week. Five millilitres of blood samples were collected from the jugular vein into test tube containing an anti-coagulant disodium-ethylene-diamine tetra acetate (EDTA), at 24 hours after each day of treatment and at 3 and 7 days after the last diazinon administration.

The parameters used for assessing the toxicity of diazinon in the sheep were the clinical signs, plasma and red blood cell cholinesterase levels and haematological parameters such as haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cell (RBC) counts,

and total white blood cell (WBC) counts. The cholinesterase activity was assayed using the method of Michel (1949). This electrometric method for measurement of the erythrocyte or plasma cholinesterase activity is principally based on the hydrolysis of the choline ester substrate (acetylcholine) in a standard buffer solution (pH 8) by the cholinesterase of the biological sample used, producing an acid which lowers the pH of the mixture.

Electrometric determination of the change in pH produced over a definite period of time (1hr) (ph/hr) at a specific temperature of 25°C represents the enzyme activity. The blood parameters were determined by the method of Brown (1976).

Statistical analysis of the results were done using analysis of variance (Steel and Torrie, 1980).

RESULTS

Clinical Signs

There was no observable clinical sign in the sheep given diazinon at the rate of 20, and 25mg/kg. Those treated with 30mg/kg of the chemical showed clinical signs within one to six hours after each day's diazinon administration beginning from the 3rd day of treatment. The earliest signs observed were dullness, restlessness, and fatigue. These were later followed by profused salivation, lachrymation, frequent urination, incoordination, rigidity of limbs and convulsions. The clinical signs were observed in all the animals in this group, and disappeared within 7 hours of onset.

Cholinesterase activity

The effect of diazinon on the cholinesterase activities of the plasma and the red blood cells are shown in Table 1. The oral administration of 20mg/kg dose had no effect on both the plasma cholinesterase (PChE) and red blood cell cholinesterase (RChE) activities. With 25mg/kg dose, there was inhibition of PChE and RChE activities. The inhibition of RChE occurred on the 7th day of diazinon

TABLE 1: THE EFFECT OF DIAZINON ON RED BLOOD CELL AND PLASMA CHOLINESTERASE ACTIVITY IN SHEEP FOLLOWING ORAL ADMINISTRATION.

Parameter	DOSE OF DIAZINON (mg/kg/Day)	DAYS				
		MEDICATION PERIOD			POST MEDICATION	
		1	3	7*	10	14
	0.0	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
PchE	20.0	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Percent Activity**	25.0	100.0±0.00	100.0 ±0.00	100.0 ±0.00	89.20±2.41	89.20±1.98
	30.0	100.0±0.00	83.33±3.20	83.33±3.92	50.10±6.36	60.0 ±4.77
RchE	0.0	100.0±0.00	100.0±0.00	100.0 ±0.00	100.0 ±0.00	100.0 ±0.00
Percent Activity**	20.0	100.0±0.00	100.0±0.00	100.0 ±0.00	100.0 ±0.00	100.0 ±0.00
	25.0	100.0±0.00	100.0±0.00	93.20±0.92	93.20±1.02	93.20±1.00
	30.0	100.0±0.00	93.20±0.84	93.20±0.68	53.30±7.24	55.0 ±5.33

* Date of termination of diazinon treatment

**Mean o/o activity.

activity occurred on the 3rd day post diazinon administration.

The administration of 30mg/kg of diazinon daily to sheep, resulted in the inhibition of RChE and PChE which occurred from the 3rd day and persisted throughout the experimental period.

Haematological Parameters

Tables 2 and 3 show the effect of various doses of diazinon on the haemoglobin, packed cell volume, red blood cell counts and white blood cell counts of sheep. There was no change in the values of Hb and PCV either during or post administration of diazinon. The RBC and WBC counts were not affected when diazinon was administered at 20 and 25mg/kg (Table 3). At the dose of 30mg/kg there was a slight but insignificant ($P>0.05$) increase in RBC counts, while the animals showed a significant ($P<0.05$) increase in

WBC counts from the 7th day of administration. This increase in WBC counts persisted throughout the experimentation period.

DISCUSSION

The administration of diazinon at the dose of 30mg/kg orally to sheep in the present study resulted in noticeable clinical signs. Similar signs have been reported in Dorset Down breed of sheep and other animal species such as cattle and chickens (Radeleff and Woodard, 1957; O'Brien, 1957; Smith, 1970). The clinical signs observed in this study such as excessive salivation, hyperlacrimation and frequent urination were due to muscarinic stimulation while the other signs such as rigidity of limbs, incoordination and convulsions are due to nicotinic and central nervous system effects of diazinon.

TABLE 2: THE EFFECT OF ORAL ADMINISTRATION ON DIAZINON ON THE HAEMOGLOBIN AND PACKED CELL VOLUME IN SHEEP

PARAMETER	DOSE OF DIAZINON mg/kg/DAY	DAYS				
		MEDICATION PERIOD		POST MEDICATION		
		1	3	7*	10	14
Hb (gm Percent) (Mean $\pm$ S.E)	0.0	11.20	11.20	11.25	11.25	11.35
	(Group I)	± 0.14	± 0.21	± 0.21	± 0.21	± 0.42
	20.0	11.25	11.45	11.30	11.45	11.50
	(Group II)	± 0.21	± 0.07	± 0.14	± 0.07	± 0.42
	25.0	11.40	11.20	11.35	11.65	11.35
	(Group III)	± 0.29	± 0.14	± 0.07	± 0.07	± 0.07
PCV (Percent) (Mean $\pm$ S.E)	0.0	30.5	30.50	31.0	31.0	31.0
	(Group I)	± 0.70	± 0.70	± 0.70	± 1.41	± 1.41
	20.0	31.0	31.50	31.50	31.0	31.0
	(Group II)	± 1.41	± 0.50	± 0.70	± 1.47	± 1.46
	25.0	32.50	31.75	31.50	32.00	32.62
	(Group III)	± 0.02	± 0.35	± 0.70	± 1.41	± 0.09
	30.0	32.0	32.50	32.0	32.0	40.0
	(Group IV)	± 0.04	± 0.35	± 0.35	± 0.06	± 0.70

*Date for the termination of diazinon treatment

The result of this study shows that the administration of diazinon inhibited plasma and red blood cell cholinesterase in a dose-dependent manner. The intensity of inhibition was more with 30mg/kg dose and was more in the red blood cell (See Table 1). In previous studies of the toxicity of diazinon in calves, steers and sheep, similar inhibitions were observed. Inhibition of cholinesterase enzyme activity was also noticed with other organophosphorus compounds French and MacFarlane, 1970; Vadlamudi, 1974). The inability by 20mg/kg and 25mg/kg doses of diazinon to inhibit plasma and RBC cholinesterase activities may be due to rapid biodegradability of this compound (WHO, 1974) which results in non phosphorylation of the enzymes, since reduction in activity is

linked with enzyme phosphorylation (Buck and Osweiler, 1981). It should be noted that the various doses used in this study fall between the oral maximum non-toxic dose (20mg/kg) and the minimum toxic dose (30mg/kg) (Buck and Osweiler, 1981). Furthermore, earlier studies have shown that animals and man may develop tolerance to repeated sublethal doses of organophosphates (Murphy, 1975). Cholinesterase enzyme is responsible for the hydrolysis of acetylcholine liberated at the neuromuscular junction. The specific function of the cholinesterase found in the blood is not well established (Cuthrie and Backer, 1954; Ellin *et al.* 1973), but the levels can be used as a diagnostic tool for exposures to organophosphates and other anti-cholinesterase compounds, since gross lesions

TABLE 3: THE EFFECT OF ORAL ADMINISTRATION OF DIAZINON ON THE RBC AND WBC VALUES IN SHEEP

PARAMETER	DOSE OF DIAZINON mg/kg/DAY	MEDICATION PERIOD			POST MEDICATION	
		DAYS				
RBC						
Million/cumm (Means $\pm$ S.E)	0.0	1	3	7*	10	14
	(Group I)	8.56 ± 0.70	8.45 ± 0.21	8.57 ± 0.42	8.59 ± 0.63	8.59 ± 0.77
	20.0 (Group II)	8.66 ± 0.70	9.65 ± 0.21	8.65 ± 0.42	8.67 ± 0.63	8.69 ± 0.70
	25.0 (Group III)	8.72 ± 1.00	8.76 ± 0.70	8.92 ± 0.70	9.02 ± 0.10	8.86 ± 1.96
	30.0 (Group IV)	9.22 ± 1.40	9.96 ± 0.45	10.35 ± 0.70	9.92 ± 0.16	8.86 ± 1.40
t WBC						
Thousand/cumm (Mean $\pm$ S.E)	0.0	11.27	11.26	11.32	11.25	11.37
	(Group I)	± 0.70	± 0.07	± 0.42	± 0.07	± 0.07
	20.0 (Group II)	11.54 ± 0.16	11.58 ± 0.29	11.52 ± 1.04	11.80 ± 0.31	11.37 ± 0.70
	25.0 (Group III)	11.63 ± 0.14	11.77 ± 1.49	11.63 ± 0.77	11.73 ± 0.77	11.88 ± 0.56
	30.0 (Group IV)	11.92 ± 0.35	12.85 ± 0.56	13.37 ± 0.50	13.33 ± 1.00	13.22 ± 0.14

*Date for the termination of diazinon treatment

may not be evident on post mortem (Paul, 1991). According to Maber and Creaby (1960), acute exposure to organophosphates results in a decreased RBC, plasma and brain cholinesterase activity.

Leucocytosis was also observed following the administration of diazinon at 30mg/kg in the present study. Leucocytosis was also observed in other species of animals treated with organophosphorus compounds (French and MacFarlane, 1970; Gupta and Paul, 1972). Intoxications by chemicals especially those that stimulate stress reactions are known to produce leucocytosis (Coles, 1980). The high leucocyte count obtained may be indicative of the bone marrow response to the toxic

condition caused by diazinon at 30mg/kg dose, which is a reflection of the response of the sheep to the chemical.

In the present study diazinon had no effect on the RBC, Hb and PCV values in sheep, although Vadlamudi (1974) in his study observed hypohaemoglobinaemia and erythrocytopaenia after feeding of fodder sprayed with 100mg/kg of malathion and 250mg/kg of sumithion for 28 days to buffalo calves. The hypohaemoglobinaemia and erythrocytopaenia observed by Vadlamudi (1974), which was absent in the present study is due to the toxic effect of malathion and sumithion resulting in bone marrow depression.

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