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DELTA 2015




THEME:

AGRICULTURE: THE NIGERIAN ECONOMY BEYOND OI

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DELTA STATE UNIVERSITY, ASABA CAMPUS,
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EDITED BY

S. I. OMEJE, S. O. EMOSAIRUE, C. C. CHUKWUJI, L. BRATTE, J. O. ISIKWENU R. A. ISIORHOVOJA & M. O. AGBOGIDI

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EFFECTS OF DICHLORVOS (DDVP) ON THE HAEMATOLOGICAL PROPERTIES OF WISTAR RATS

S. O. OLAOYE[1], O. O FADINA[2], O. O. FAYINMINNU[2], O. M. ADEDIRE[1], W. F. OGUNDIPE[1], A. K FAJOBI[1] & A. O FARINU[1]
[1]federal College of Agriculture, P.M.B. 5029, Moor Plantation, Ibadan
[2]department of Crop Protection and Environmental Biology, University of Ibadan
phemosay@yahoo.com

ABSTRACT
The potential sub-chronic toxicological effects of oral administration of Dichlorvos (DDVP) on wistar rats was investigated for a period of 6 weeks. Thirty-two (32) wistar rats (equal number of both sexes) were uniformly divided into two groups while each group comprised of 4 divisions with four rats each. At the end of experiment, animals were sacrificed and haematological test was carried out to investigate the possible toxicological effects of the oral administration of the pesticide on the rats. Results generally showed a dose-dependent response with PCV, Hb, RBC and Plat values that are significantly different from each other ($P \leq 0.05$) among the male rats while PCV and RBC values are significantly different from each other ($P \leq 0.05$) while all other haematological parameters in both sexes showed no significant difference from each other at $P \leq 0.05$. This result suggests that ingestion of the pesticide (Dichlorvos) may not be toxic at the doses *investigated.*

Keywords: pesticide, Dichlorvos, DDVP, toxicity, haematology.

INTRODUCTION
Dichlorvos is a synthetic organic chemical used as an insecticide. It does not occur naturally in the environment, but manufactured by an industrial process and generally belongs to the class of Organophosphates. It is also referred to as DDVP which is the abbreviation for its full chemical name O, O-Dimethyl-O-(2,2-DichloroVinyl) Phosphate according to USEPA (2007). In Nigeria, Dichlorvos is used as a household and agricultural pesticide traded under names such as *Nuvan, Sniper, No pest, Festoff, Sedge etc* and used to control insects on crops, household and stored products, and also to treat external parasitic infections in farmed fish, livestock, and domestic animals (Erdogan *et al.*, 2007). Musa *et. al.*, (2010) reported that Dichlorvos, a volatile organophosphate was the active ingredient in the local formulation of *Ota-piapia* and its popularity, acceptance, and widespread proliferation in Nigeria has been due solely to its cheap production, efficacy, accessibility, and affordability.

Dichlorvos (DDVP) has been in use since the early 1960s and has been the subject of many toxicity studies and review articles (Durkin and Follansbee, 2004). It is rapidly absorbed through the gastrointestinal and respiratory tracts and skin, it enters human system via inhalation, dermal, and oral routes, and it is metabolized by the liver and excreted by the kidney (CERI, 2007).

Studies have shown that not all applied pesticides may actually reach targeted pests and the remaining have potential to get into the soil, water, and the atmosphere (Goodman *et. al.*, 2006). Pesticides are designed to alter specific body processes (Darren and Cynthia, 2007) and to this end, human and animals are occasionally and unintentionally exposed to lethal and sub-lethal doses of pesticides (Martin *et al.*, 2003). Apart from the direct exposure, indirect exposure occurs in animals by consuming food that contains high residues of the pesticides (Caroline, 1991) hence, the need to determine the toxicity clinical signs associated with exposure to sub-chronic doses of Dichlorvos and investigate its effects on the haematological parameters using male and female wistar (albino) rats as test organism.

MATERIALS AND METHODS
The experiment was carried out at the Rabbitary unit of Federal College of Agriculture, Moor plantation, Ibadan using 32 eight week-old Wistar rats of both sexes (65.50 $\pm$6.25g for females and 72.00$\pm$8.5g for males) procured from the Animal House of the Physiology Department, University of Ibadan, Nigeria comprised of equal number of both sexes and then transported to the rabbitary unit of Federal College of Agriculture where the animals were made to acclimatize to room conditions for 2 weeks before being randomized into 4 groups of 4 rats per group for each sex. The pesticide (Dichlorvos) and other chemicals required for haematological estimation of tested high quality were purchased locally. The rats were fed on growers mash throughout the duration of the study and this was purchased locally while water and feed were supplied *ad libitum*.

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Serial Dilution

Appropriate volume of the pesticide (Dichlorvos) (determined by its oral LD_{50} of 56mg/kg in rats) was mixed with water since Dichlorvos is readily soluble in water. The concentration of the active ingredient in *Pestoff* is 1000g/L, this is also equivalent to 1,000,000mg/L. 0.5mls of the pesticide solution was taken and distilled water added to make up to 50ml this gives a solution of the active ingredient in 10,000ppm. Also, 5mls was taken from this 1st intermediate solution and then distilled water added to make up to 50ml forming the second intermediate solution (1,000ppm). These concentrations were determined using the formula

$C_1V_1 = C_2V_2$ Where;

C_1 = initial concentration of stock; V_1 = volume of stock used

C_2 = desired concentration; V_2 = desired volume

From this solution, the followings were taken and distilled water added to make up to 50mls.

Treatment 1: 7mls equivalent to 140mg/L of the active ingredient (a.i)

Treatment 2: 3.5mls equivalent to 70mg/L of the a.i

Treatment 3: 1.8mls equivalent to 35mg/L of the a.i

Treatment 4: 0mls of the a.i (control)

Experimental Layout

The experiment was laid out in a completely randomized design (CRD) with 4 treatments replicated 4 times.

Data collected

The animals were weighed weekly in order to determine weight gain/loss; the feed given was weighed in order to ascertain the daily feed intake and mortality.

Physical observations such as shyness, behavioural pattern of the rats, signs of pesticide toxicity such as tremors, loss of appetite, muscle jerking, constricted pupils, vomiting etc were recorded per animal in each treatment. On termination of the experiment, blood samples (5mls) was collected for haematological analysis.

Haematological Analysis

The PCV and haemoglobin (Hb) values were determined using the micro haematocrit method and cyanmethemoglobin method respectively as described by Mitruka and Rawnsley (1977) while RBC and WBC were determined using the improved Neubauer haemocytometer after the appropriate dilution (Schalm *et. al.*, 1975).

The differential leucocyte counts were determined by scanning Giemsa's stained slides in the classic manner (Schalm et al., 1975)

Statistical Analysis

Data obtained was subjected to one-way analysis of variance ANOVA (SAS Version 9.2, 2002-2003) while group means was compared for significant differences at 95% confidence level ($P<0.05$) (Duncan, 1959) and results were presented as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Physical/Behavioural Observations

Fig. 1 shows the effects of the various doses of the pesticide (Dichlorvos) on the average weekly weight (g) of male rats while fig.2 shows that of the female rats. Both sexes showed a dose-dependent response with a sharp decline in weight one week after the commencement of the doses (week 4) as all the rats exhibited loss of appetite after the treatment administration leading to a loss of weight. However, male rats which received 70mg/kg of body weight of the dosage still had an increased weight on week 4 but subsequently, their weight reduced gradually. All the controls (both sexes) did not experience any loss in weight.

A total of 4 mortalities (preceded by loss of coordination, muscle jerking, nervous breakdown and convulsion) was recorded from the experiment. This consists of 2 males and 1 female rats from the 140mg/kg group and 1 male rat from the 70mg/kg group. Prior to the administration of the doses, the rats were observed to cuddle together in a corner of the cage while after the administration of the doses, they were restless and moved randomly within the cage. However, this uncoordinated movement ceases within 20-40mins after the dosage administration and the rats movements returned to normal.

Loss of appetite was observed on all the rats except the control immediately after the administration of the various dose. This might be the cause of a rapid loss in weight of both sexes (male and female) of rats 1 week after the commencement of the treatments as shown in Fig.1 and 2 respectively.

Effects of Dichlorvos on Haematological Parameters of wistar rats

From table1 (a and b), male rats that received 140mg/kg weight of the pesticide showed an abnormally high value of PCV (52%). PCV is the volume percentage (%) of red blood cells in blood. An elevated PCV is most often associated with dehydration, which is a decreased amount of water in the tissues, diarrhea etc. Chineke *et.*

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al., (2006) documented that high PCV haematocrit reading indicated either an increase in the number of circulating RBC or reduction in circulating plasma volume.

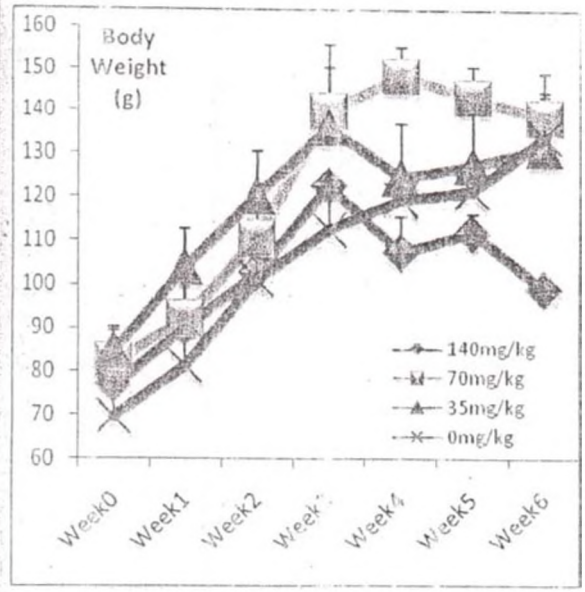

Fig 1: Effects of varying doses of Dichlorvos on the average weekly weight (g) of male wistar rats rats

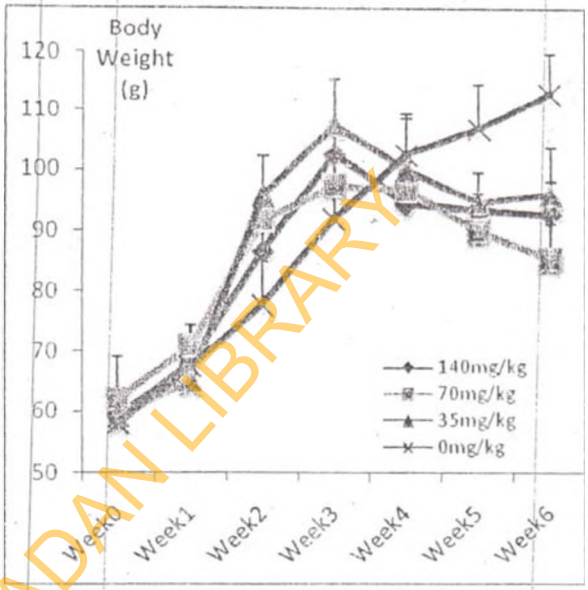

Fig 2: Effects of varying doses of Dichlorvos on the average weekly weight (g) of female wistar

Table 1: Effects of varying levels of Dichlorvos on haematological parameters of Male wistar rats

Dosage (mg/kg bw)	PCV(%)	Hb	RBC	WBC	Lymp	Neut	Mono	Eos	Plat
140	52±1.75a	14.8±0.22a	6.98±1.65ab	5800	70	24	3.5	2.5	74000±23.68ab
70	30±1.65b	14.3±1.25ab	7.44±0.89a	1875	68.5	25.5	3.5	2.5	46500±35.85b
35	34±1.50b	14.2±1.05ab	8.04±0.85a	6900	72	23	3.5	1.5	115000±22.20a
0	40±0.95ab	13.75±0.85b	6.85±0.35ab	6500	68	25	4.5	2.5	112500±20.56a
LSD (P<0.05)	6.95	3.65	4.65	34.68 ns	6.3 ns	2.56 ns	0.55 ns	0.02 ns	36.9

Means followed by the same alphabets are not significantly different from each other (P≤0.05)

PCV = Packed cell volume, Hb = Haemoglobin count, RBC = Red blood cell count, WBC = White blood cell count, Lymp = Lymphocytes, Neut = Neutrophiles, Mono = Monocytes, Eos = Eosonophile, Plat = Platelets count

An elevated haematocrit may also be caused by an absolute increase in blood cells, called *polycythemia*. However, other treatments (including the control) had various PCV levels within the reference (normal) values for wistar rat. Also, the highest Hb value of 14.8 was recorded from male rats administered 140mg/kg body weight of the pesticide however this value is within the normal (reference) value (Table 2) though all the treatments showed a significant difference (P<0.05). Both RBC and Plat were significantly different from each other at P<0.05 though the values were also within the normal values while other parameters measured such as WBC, Lymp, Neut, Mono and Eos showed no significant difference among the treatments at P<0.05. Among the female rats (table2), only the PCV (%) showed a significant difference among the treatments at P<0.05 however, all values obtained were within the reference value.

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Furthermore, both RBC and Plat were significantly different from each other at P<0.05 though the values were also within the normal values while other parameters measured such as WBC, Lymp, Neut, Mono and Eos showed no significant difference among the treatments at P<0.05. Among the female rats (table2), only the PCV (%) showed a significant difference among the treatments at P<0.05 however, all values obtained were within the reference value.

Table 2: Effects of varying levels of Dichlorvos on haematological parameters of Female wistar rats

Dosage (mg/kg bw)	PCV	Hb	RBC	WBC	Lymp	Neut	Mono	Eos	Plat
140	40+1.85ab	13.95	6.91	5700	75.5	27	3.5	4	88500
70	34+1.35ab	15.1	7.88	6025	65.5	30.5	3	1	107000
35	32+1.70ab	14.75	7.33	9150	66.5	29	2.5	2	154000
0	46+0.90a	14.95	7.52	6800	73.5	19	3.5	4	134500
LSD(P<0.05)	10.48	1.88	0.29	45.9	13.6	3.65	0.07	0.14	62.5
		ns		ns	ns	ns	ns	ns	ns

Means followed by the same alphabets are not significantly different from each other (P≤0.05)
PCV = Packed cell volume, Hb = Haemoglobin count, RBC = Red blood cell count, WBC = White blood cell count, Lymp = Lymphocytes, Neut = Neutrophiles, Mono = Monocytes, Eos = Eosonophile, Plat = Platelets count

CONCLUSION AND RECOMMENDATION

Clinical signs of pesticide poisoning was observed at various levels of pesticide treatment. Also, the haematological investigation revealed a negative effect of the pesticide on the PCV and Hb of both male and female rats however, majority of the haematological were not significantly different from each other in the female rats, this is probably due to hormonal interaction by the female rat. Dichlorvos is readily absorbed into the body of mammals via all routes of exposure, where it is rapidly metabolized and eliminated (Durkin and Follansbee, 2004). The nature and magnitude of the toxic effects produced by a given exposure to Dichlorvos by any route are directly related to the dose and rate at which the exposure occurs.

Previously published report (Robert and Richard, 2001) specified the need to pay a much more global attention to the possible effects of environmentally persistent pesticide which are produced in the developed countries and exported to the underdeveloped where a gross abuse and misuse is observed because of poor enforcement on its transport, handling, storage and use hence, there might be the need to critically review some pesticides earlier thought to be safe.

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