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REPRODUCTIVE INDICES AND HAEMATOLOGICAL RESPONSE OF MALE ALBINO RATS (*Rattus norvegicus*) EXPOSED TO DICHLORVOS

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Abstract: **The effects of exposure to Dichlorvos was evaluated through different exposure routes in male albino rats using haematological and sperm quality parameters such as; PCV, HB, RBC, McV, McH and McHc, Motility quantitative (M1), Debris quantitative (D1), sperm count (C), and morphology primordial (Mp). The results revealed the following mean value of PCV for group 1, 2, 3, 4 and 5 as 40.75, 21.5, 34.25, 27.5 and 18.66 respectively with significant difference (P<0.05) in group 2 and 4, for HB 13.95, 8.76, 20.43, 8.68 and 6.93 for group 1, 2, 3, 4 and 5 respectively with significant difference (P<0.05) only in group 3. RBC and McV had a mean value of 3.83 and 12.37, 2.44 and 98.69, 2.89 and 13.62, 2.06 and 14.04 then 3.0 and 8.89 for group 1, 2, 3, 4 and 5 respectively with significant difference (P<0.05) recorded only in group 5 for MCV group. While McH and McHc had a mean value of 4.28 and 0.32, 3.58 and 0.41, 6.80 and 0.58, 4.89 and 0.31 then 3.41 and 0.38 for group 1, 2, 3, 4 and 5 respectively with significant difference (P<0.05) across the group. For semen analysis, M1 was 77.25, 26.25, 72.5, 21.25 and 10 for group 1, 2, 3, 4 and 5 respectively, Debris D1 was 6.25, 49.50, 6.25, 46.25 and 20.0 for group 1, 2, 3, 4 and 5 respectively, C and Mp were 69.5 and 5.0, 61.75 and 6.25, 74.75 and 10.0, 54.0 and 7.50 then 15.0 and 15.0 for group 1, 2, 3, 4 and 5 respectively also. The results indicate that dichlorvos affects sperm quality in males and generally had negative effect on the haematology irrespective of the route of exposure, hence care should be taken in the use and handling of Dichlorvos.**

Keywords: **Reproductive Indices, Haematological Response, male albino rats, Dichlorvos.**

1. INTRODUCTION

Dichlorvos is an organophosphate pesticide used to control household pest and ectoparasite in animals, it has been classified by WHO (1997) as highly hazardous and toxic compound. Most human poisoning usually results from the splashing of the concentrated formulations unto the skin (Blair, *et al*., 1975). The mechanism by which Dichlorvos exerts its toxicity as a pesticide is mainly by blocking acetylcholinestrase- an enzyme that decomposes acetylcholine, immobilization of this enzyme results in the accumulation of excessive amounts of acetylcholine in the nerve tissues and muscle motor plates, as well as, symptoms of endogenic poisoning by this neuro hormone (Bisby and Simpson, 1995). The use of pesticide has become a regular phenomenon in both agricultural and general household needs, and one widely used pesticide is organophosphate pesticides, this pesticide has been reported to have serious toxic effects on humans and animals because of its regular use (Bedford *et al*., 1996). There has been an increased concern that the exposure to dichlorvos may have adverse effects on the reproductive systems of humans and other non-targeted organisms,and may damage sperm, alter DNA structure which might lead to birth defects or inability to conceive and may also cause epigenetic effects (Aquilina et al., 1999). In humans and other experimental animals, dichlorvos is rapidly absorbed into

the body through the gastrointestinal, respiratory tract and the skin and is easily metabolized by esterase that exist in most tissue, and it was distributed in the kidney and adipose tissue in a relatively high concentration when exposed to rats (Cheng, 1991; Dean et al., 1991). There are major concerns that focus on the acute and chronic toxicity of this pesticide considering the fact that this pesticide is prevalent in urban waterways (USEPA, 1991), people can be exposed to dichlorvos in their work places by breathing it in, skin absorption etc and in line with this, there have been set limits or permissible exposure limit for dichlorvos exposure in the workplace as $1mg/m^3$ over an 8h work day and at levels of $100ml/m^3$ dichlorvos is immediately dangerous to life and health (USEPA, 1991; USEPA, 1997; Barolo, 1993). Animal studies suggest that exposure to dichlorvos may be associated with immunosuppression and effect on diurnal rhythm/normal daily cycles, while another study found alterations in the diurnal rhythm of the pituitary-adrenal axis in rats exposed to dichlorvos and changes in hormonal levels (Civen et al., 1980; Desi et al., 1998). In vivo studies, there was no effect on antibody production but it was reported by another author that dichlorvos interfered with circadian rhythm of red blood cell acetyl cholinesterase in mice and humans (Jian and Zhiying, 1990; Dunier et al., 1991). According to some studies, exposure to dichlorvos caused birth defects and reproductive effects in laboratory animals (Dambska et al., 1999; Kimbrough and Gaines, 1998), and adverse testicular effects in the treated male mice (ACC, 1987) on the other hand, in several breeding studies no adverse effect or birth defect were observed in rabbits, swine or experimental mammals after exposure to dichlorvos (Stanton et al., 1989; Vogin et al., 1991). Some authors have reported skin irritations and allergic reactions when exposed to experimental animals, while human data regarding the dermal effects of the pesticide is relatively sparse but generally support the animal studies (Fujitu, 1985; Arimatsu et al., 1987; Bisby and Simpson, 1995). Man is exposed to insecticides due to occupational, residential, dietary or combined exposure especially in third world countries where adequate personal protective equipments is not used when working in farms etc and the pesticide can be absorbed by the skin, or inhaled, or contaminated food and water. This study is therefore aimed at evaluating the effect of Dichlorvos on sperm quality and haematological parameters of albino rats through different routes of exposure (inhalatory, oral, feed incorporated and dermal routes).

2. MATERIALS AND METHODS

Experimental design: A total of twenty (20) healthy albino adult males weighing 160-180g were used in this study, and were allowed to acclimatize to laboratory condition (24-26^{o}C) for 14 days before the commencement of the study. They were housed in a plastic cage during the duration of study. Complete Randomized design was used and the animals were divided into 5 groups; Group 1(control, no chemical), Group 2 (1ml dichlorvos – inhalatory), Group 3 (0.5ml dichlorvos - Dermal), Group 4 (2ml/300g dichlorvos – incorporated in feed) and Group 5 (0.1ml dichlorvos - oral). In the inhalatory method, the chemical was applied on a piece of cotton wool and the rats were made to sniff it for one minute while the oral administration was done using a gavage tube. The period of study was 10 days

Biochemical Analysis: Standard procedures were ensured during the collection of the blood, and sperm samples prior to biochemical analysis. Sperm fluid/semen was collected from the sperm duct by maceration on the glass slide and the analysis procedure was done according to Ochei and Kolhatkar (2000), Sperm motility, viability and abnormalities were determined using one step eosin method Björndahl, *et al*., (2003) and the epididymal sperm count was done with Neubauer haemocytometer (Deep 1/10 mm, LABART, Munich, Germany) and light microscope at 40× magnifications.

Method of Data Analysis: Analysis of variance was used to assess the data by using SPSS program version 10 and Duncan multiple test method and results were expressed as mean ± standard deviation.

3. RESULTS

Effects of Dichlorvos on Haematological Parameters of Albino Rats

The results for the effect of Dichlorvos on Haematological parameters of Albino rats are shown in Table 1. Group 1, 2 and 3 had a mean value of 40.75, 21.5, and 34.25 respectively for Pack cell volume (PCV) and group 4 and group 5 had a mean value of 27.5 and 18.66 respectively for PCV also with significant difference ($P<0.05$) in group 1 and 5 when compared to the control group. Haemoglobin (Hb) had a mean value of 13.95, 8.76, 20.43, 8.68 and 6.93 for group 1, group 2, group 3, group 4 and group 5 respectively with a significant difference ($P<0.05$) recorded groups 2, 4 and 5 when compared to the control. Group 1, 2, 3, 4 and 5 had a mean value of 3.83, 2.44, 2.89, 2.06 and 3.0 respectively for red blood cell (RBC) with no significant difference ($P>0.05$) in groups. Mean corpuscular Volume (McV) had mean value of 12.3, 8.69, 13.62, 14.04 and 8.89 for group 1, group 2, group 3, group 4 and group 5 respectively with significant

difference (P<0.05) in all groups 2 and 5 when compared to the control group. Group 1, 2, 3, 4 and 5 had a mean value of 4.28, 3.58, 6.80, 4.89 and 3.41 respectively for McH (Mean Corpuscular Haemoglobin). Statistically, there was no significant difference (P>0.05) in the treated groups when compared to the control group. For McHc (Mean Corpuscular Haemoglobin Concentration), group 1, 2, 3, 4 and 5 had a mean value of 0.32, 0.41, 0.58, 0.31 and 0.38 with significance difference (P<0.05) recorded when all the treated groups were compared to the control group.

Effects of Dichlorvos on Sperm/Semen quality of Albino Rats

The results for sperm/semen analysis is outlined in Table 2, The control group (group 1) had a mean value of 77.25 for M1 (Motility quantitative), while the group 2 had a mean value of 26.25, group 3 had a mean of 72.5, group 4 had a mean value of 21.25 and group 5 had a mean value of 10. Statistically, there were significant differences among the groups when comparing group 2, 3, 4 and 5 with the control group (Group 1). Group 1, Group 2 , Group 3, Group 4 and Group 5 had a mean value of 6.25, 49.50, 6.25, 46.25 and 20.0 respectively for D1 (Debris quantitative) with a significant difference (P<0.05) recorded when comparing the treated groups 2 and 4 with the control. The sperm count carried out (C) had a mean value of 69.50, 61.75, 74.75, 54.0 and 15.0 in group 1, 2, 3, 4 and 5 respectively, with a significant difference (P<0.05) only in group 5 when compared to the control. The Morphological premodial (Mp) had mean values of 5.0, 6.25, 10.0, 7.50 and 15.0 respectively in group 1, 2, 3, 4 and 5. There was no significant difference (P>0.05) for group 2, 3, 4 and 5 when compared to the control.

Table 1: Effect of nitrocellulose Dichlorvos on Hematological parameters.

Group	PCV	Hb	RBC	MCV	MCH	MCHC
1	40.75 ± 7.63^{b}	$13.95\pm1.7^{a,b}$	3.83 ± 1.49^{a}	12.37 ± 5.95^{b}	4.28 ± 2.18^{a}	$0.32\pm0..83^{b}$
2	21.5 ± 7.23^{a}	8.76 ± 3.08^{a}	2.44 ± 0.67^{a}	8.69 ± 0.57^{a}	3.58 ± 0.61^{a}	0.41 ± 0.08^{a}
3	34.25 ± 3.10^{b}	20.43 ± 15.92^{b}	2.89 ± 1.13^{a}	13.62 ± 6.55^{b}	6.80 ± 2.94^{a}	0.58 ± 0.42^{a}
4	27.5 ± 0.58^{b}	8.68 ± 0.62^{a}	2.06 ± 0.62^{a}	14.04 ± 3.16^{b}	4.89 ± 1.25^{a}	0.31 ± 0.03^{a}
5	18.66 ± 5.51^{a}	6.93 ± 1.0^{a}	3.0 ± 0.61^{a}	8.89 ± 0.03^{a}	3.41 ± 0.67^{a}	0.38 ± 0.08^{a}

PCV: Packed Cell Volume, Hb: Haemoglobin, RBC: Red Blood Cell, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration

Table 2: Effect of hardener and Dichlorvos on the sperm parameter.

Group	M1	D1	C	Mp
1	77.25 ± 2.89^{b}	6.25 ± 2.50^{a}	69.50 ± 5.45^{b}	5.00 ± 0.00^{a}
2	26.25 ± 4.79^{a}	49.50 ± 9.54^{b}	61.75 ± 8.50^{b}	6.25 ± 2.50^{a}
3	72.5 ± 6.45^{b}	6.25 ± 2.50^{a}	74.75 ± 6.85^{b}	$10.00\pm7.07^{a,b}$
4	21.25 ± 8.54^{a}	46.25 ± 4.79^{b}	54.00 ± 5.35^{b}	7.50 ± 2.89^{a}
5	10.0 ± 0.0^{a}	20.0 ± 4.08^{a}	15.00 ± 7.07^{a}	15.00 ± 4.08^{a}

M1: Motility quantitative, D1: Debris quantitative, C: Count in million per litre, and Mp: Morphology premodial.

4. DISCUSSION

It was observed from the results that the toxicity of dichlorvos was route dependent with the oral route (group 5) having the most severe toxic effect generally. The PCV and Hb was observed to be significantly very low in the treated groups when compared to the control group with the lowest mean value recorded in the group that was exposed to the treatment via oral route. This indicates that dichlorvos exerts more adverse effect on PCV and Hb when exposed via oral route. But that notwithstanding, dichlorvos had a general negative effect on the PCV and Hb of the test subjects indicating the ability of dichlorvos to induce an anaemic condition because low PCV and Hb is known to be an indicator for anaemia and kidney damages (Dunn *et. al.*, 2007). The RBC was observed also to be lower in the treated group when compared to the control group, although statistically it wasn't significant. The lowest value of RBC was recorded on group 2 which was through the inhalatory route, this might be because when inhaled, the treatment had direct contact with the blood through the thin walls of the lungs, this must have made more of the chemical to reach the kidney and caused some damages that

interfered with red blood cell production through interrupting the secretion and release of the hormone erythropoietin from the peritubulary capillary lining cells of the kidney (Adamson, 1996; NIDDK, 2014; AKF, 2018), hence the reason for the very low RBC recorded when compared to the groups with other routes of exposure.

The MCV was observed to be significantly lower in the group 2 and 5 but higher in group 3 and 4 when compared to the control group. High MCV indicates macrocytic anaemic conditions, while low MCV indicates macrocytic anaemic conditions (Curry, 2015; Jenny, 2018), High MCV according to Jenny (2018) is a sign of liver diseases or damage which decreases liver function. High MCH was recorded in group 3 and 4 and according to Johnson, (2017) a high value of McH is a sign of macrocytic anaemia, it is also known to arise due to liver damage. This means that there is increased chance of liver damage when dichlorvos is exposed through dermal and when incorporated in feed. Group 2 and 5 recorded a low value showing that the concentration of haemoglobin in the red blood cells reduced due to the presence of dichlorvos in the blood stream or due to liver damage etc (Mayo Clinic, 2018).

The level of MCHC was also observed to be high in the treated group when compared to the control group and this is also known to be a sign of anaemia resulting due to liver damages. In the sperm analysis, it was observed that for motility quantitative M1, that the treated group had a very poor result showing that the motility of the sperm cells reduces significantly due to the treatment. Group 5 which was the oral route followed by group 4 which is the feed incorporated group had the lowest mean value due to the fact that dichlorvos is also rapidly absorbed through the gastrointestinal route (Cheng, 1991; Dean et al., 1991). The debris was also seen to increase significantly in the treated group alongside the sperm count which had a considerably low value in groups 5 and 4. The sperm morphology was also adversely affected in the treated groups; this cumulatively will reduce the fertility of the individual by decreasing the number of viable sperm cells that can successfully fertilize the egg on account of any mating. The mechanism by which dichlorvos exerts this negative effects on the sperm parameters might be through its mechanism of interrupting neural communication by inhibiting the enzyme acetyl cholinesterase thereby adversely affecting the central nervous system (Bisby and Simpson, 1995), and interfering with the normal functioning of the Hypothalamus and Pituitary glands that play a vital role in sperm production. This result is in agreement with (Aquilina et al., 1999) who reported possible adverse effect of Dichlorvos on the reproductive system.

5. CONCLUSION

An exposure to Dichlorvos through the various routes in this study had negative effects on male albino rats hence care should be taken while handling and applying Dichlorvos, also the use of personal protective equipments should be made mandatory during the application of Dichlorvos.

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