Scientific Research Publishing

Journal of Biosciences and Medicines, 2023, 11, 37-49
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Genomic Instability Is a Mechanism for Diminished Male Fertility Following Chronic Dichlorvos Exposure

Raymond A. Vhriterhire[1], Samuel O. Odeh[2], Gideon U. Egesie[2]

[1]Department of Anatomical Pathology, College of Health Sciences, Benue State University, Makurdi, Nigeria
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Jos, Jos, Nigeria
Email: akp4ray@yahoo.com

How to cite this paper: Vhriterhire, R.A., Odeh, S.O. and Egesie, G.U. (2023) Genomic Instability Is a Mechanism for Diminished Male Fertility Following Chronic Dichlorvos Exposure. *Journal of Biosciences and Medicines*, **11**, 37-49.
https://doi.org/10.4236/jbm.2023.117005

Received: June 4, 2023
Accepted: July 8, 2023
Published: July 11, 2023

Abstract

Background and Objectives: Chronic low-dose exposure to dichlorvos occurs in communities in Africa where the substance is used indiscriminately for a variety of purposes. This experiment used an animal model to evaluate genomic instability induced by this pattern of chronic exposure and its relationship with some measures of fertility in males. **Methods:** Seventy-five male *Rattus norvegicus* rats obtained for this experiment, were randomly allotted into five groups. Dichlorvos was given by oral gavage at doses of 0.28 mg/kg, 0.56 mg/kg and, 1.68 mg/kg, respectively, to three of the groups, on alternate days for 50 weeks. The remaining two groups received plain drinking water and cyclophosphamide as negative and positive controls, respectively. Samples were collected at 17, 34, and 50 weeks. Sperm count, sperm morphology and serum levels of follicle-stimulating hormone, luteinizing hormone, dihydrotestosterone, oestrogen and progesterone were determined. Furthermore, the frequency of micronucleated polychromatic erythrocytes was determined in bone marrow cells obtained from the femur. **Results:** The mean ranks of micronuclei frequency had an increasing trend. The frequency of micronucleated polychromatic erythrocytes (MnPCE) had a significant negative correlation with oestrogen ($r_s = -0.47$, $p = 0.00$, $n = 50$), follicle-stimulating hormone ($r_s = -0.41$, $p = 0.00$, $n = 50$) and progesterone ($r_s = -0.37$, $p = 0.01$, $n = 50$) serum levels. A positive monotonic relationship also existed between micronuclei frequency and those of tubular necrosis, tubular vacuolation, and residual bodies. A positive significant moderate correlation was found between MnPCE and the proportion of immotile sperms ($r_s = 0.41$, $p = 0.00$, $n = 50$). **Conclusion:** The nature of the correlations between micronuclei frequency and the proportion of immotile sperms, adverse histological changes and serum hormone levels found in this study suggest genomic instability as the possible mechanism for diminished fertility in males chronically exposed to dichlorvos.

Keywords

Genomic Instability, Micronuclei, Male Infertility, Dichlorvos, Chronic Organophosphate Exposure

1. Introduction

Diminished male reproductive capacity constitutes a substantial component of infertility in global reports and local studies in the sub-Saharan African region [1] [2] [3]. This "male factor" may be the consequence of causes which range from failure of the sexual act or fertilisation to intrinsic genetic anomalies and exposure to adverse environmental agents. These environmental factors appear to have a significant impact on male fertility through various mechanisms [4]. These include hormone-disruption chemicals [5], which mimic or interfere with the production, release, transport, metabolism, action, and elimination of hormones [6] and consequently, interfere with the regulation of spermatogenesis [7]. One such agent is dichlorvos (2,2-Dichlorovinyl-O, O-dimethyl phosphate, DDVP, "Sniper®"). This is a volatile colourless liquid which is indiscriminately used by individuals in communities in sub-Sahara Africa as an insecticide and pesticide in their homes or farms, contaminating foods and water [8] [9]. It may be absorbed into the body via the skin, respiratory tract or by oral ingestion of contaminated foods, water, and drinks [10]. Dichlorvos and other similar chemicals have genotoxic potentials described in the literature, in addition to the hormone-disrupting properties [11] [12]. However, in most of these experiments, dichlorvos was administered over short durations and thus, there exists a relative gap in the literature on studies which model the pattern of persistent low-dose chronic exposure to dichlorvos which takes place in our communities [13]. This experiment aimed to explore the impact of this pattern of exposure on male reproductive function and particularly the hypothesis that reduced reproductive capacity might be the result of genetic damage caused by this substance. Genetic damage may manifest as reduced testosterone serum levels and decreased volume of the testes, ineffective spermatogenesis, azoospermia, oligospermia and infertility [14] [15] [16].

In this research, an animal model was used to evaluate genomic instability and its relationship with other fertility measures such as serum hormone levels, sperm count, sperm morphology, motility and structural changes in the testes, in a pattern which simulate the low dose dichlorvos community exposure.

2. Materials and Methods

2.1. Animal Husbandry

Seventy-five male Wistar albino rats, *Rattus norvegicus*, were obtained and used for this experiment. They were managed in spacious wire cages, under similar conditions of temperature and humidity, and adequately fed with pellet feed and

water until maturity before the commencement of the experiment. The animals were maintained in optimal environmental conditions to minimize stress.

2.2. Experimental Design

There were five experimental groups and each group had fifteen animals. The negative control (Group 1), was given only plain drinking water, while Group 5, the positive control, received cyclophosphamide, a chemotherapeutic agent known for inducing genotoxicity, at the dose of 5 mg/kg at intervals of 28 days [17] [18]. The Groups 2, 3, and 4 animals were given commercial-grade dichlorvos of 98% purity at the dose of 0.28 mg/kg, 0.56 mg/kg, and 1.68 mg/kg, respectively. This corresponded to 0.5%, 1%, and 3%, respectively, of dichlorvos LD50 using 56 mg/kg used as the reference value (California Environmental Protection Agency, 1996). These treatments were administered orally using an oral-gastric gavage tube on alternate days for 50 weeks. Samples were collected at intervals of 17 (T_1), 34 (T_2), and 50 (T_3) weeks for analysis. Each experimental animal was sedated with chloroform and euthanized by cervical dislocation and other procedures following standard ethical recommendations.

2.3. Testes Histology, Sperm Count and Morphology

The animal's testes were dissected out, preserved in 10% buffered formalin, processed routinely tissue, and embedded in paraffin wax, and sections were stained with hematoxylin and eosin. The histological findings in the seminiferous tubules were semi-quantitatively graded using a system modified from Lanning *et al.* (2002), as 0 (absent), 1 (minimal or <5% area of tubule affected), 2 (slight or 5% - 25% tubule area affected), 3 (moderate or 25% - 50% tubule area affected), 4 (marked or 50% - 75% tubule area), and 5 (severe or >75% tubule area affected) [19]. Sperms obtained from the epididymis were used for sperm count determination and the method described by Wyrobek and Bruce was employed to evaluate changes in sperm morphology [20].

2.4. Blood Hormone Assay

Blood levels of dihydrotestosterone (T), oestrogen (E_2), follicle-stimulating hormone (FSH), luteinising hormone (LH) and progesterone (P_4), were determined by ELISA method (Accu-Bind Elisa Microwells kit, manufactured by Monobind Inc., 100 North Pointe Drive, Lake Forest California 92630, USA).

2.5. Micronuclei Assay

The protocol for micronuclei assay previously described by Schmid (1975) as cited by Abrevaya *et al.* (2007) and Nazam *et al.* (2013) was followed in the course of this research with some minor modifications [18] [21]. About 2 ml of foetal calf serum (Sigma Aldrich, Germany) was drawn into a 5 ml syringe and maintained at 2˚C - 4˚C. Both femurs were rapidly dissected after the animal was sacrificed. Foetal calf serum was then used to flush out the bone marrow in-

to an Eppendorf tube and incubated at 37˚C for 30 minutes in a water bath. The cells were then aggregated by centrifuging at 1000 rotations per minute for five minutes. The resulting cellular sediment was further suspended in 50 µml of fresh foetal serum. A smear was made from this suspension on a microscope glass slide and fixed in 70% methanol for 15 minutes, and then stained with Giemsa stain [22]. The stained slides were examined with a light microscope for micronuclei at 1000 times magnification with immersion oil. One thousand randomly selected polychromatic erythrocytes and normochromatic erythrocytes were scored for micronuclei per animal. A micronucleus was defined as a round small nucleus, less than a third of the main nucleus in size but with a similar staining consistency [23].

2.6. Research Ethics Clearance

Research ethics clearance was obtained from the research ethics committee of the University of Jos (Protocol date: 4th September 2018, Reference Number: F17-00379). The recommendations of the Declaration of Helsinki and the guiding principles in the care and use of animals for experimentation were followed in the course of this research.

2.7. Statistical Analysis

The data collected was managed and analyzed using SPSS® Statistics (IBM Corporation, Armonk, New York, USA). The non-parametric Kruskal-Wallis H test was used for hypothesis testing following Shapiro-Wilk and Levene's tests for normality and homogeneity, respectively. Mann Whitney U test with Bonferroni correction was used for *post hoc* pairwise comparison. The relationships between variables were determined by the non-parametric Spearman's correlation coefficient ($-1 \leq rs \leq 1$), and stratified as very weak (0.00 - 0.19), weak (0.20 - 0.39), moderate (0.40 - 0.59), strong (0.60 - 0.79) and very strong (0.80 - 1.0).

3. Results

Samples from sixty-two out of the 75 animals were analyzed because there was an attrition of 17.3% (13 deaths) spread across the groups during the 50 weeks of experimentation.

3.1. Micronuclei Frequency

Micronucleated polychromatic erythrocytes (MnPCE) had a mean of 2.96 ± 2.01 s.d. at T_1, 3.24 ± 1.90 s.d. at T_2 and 8.44 ± 1.11 s.d. at T_3. The micronuclei score values at the three-time intervals are shown in **Tables 1-3** below. The data had a skewness and kurtosis of 0.58 ± 0.48 and −0.80 ± 0.94 s.e. at T_1, 0.45 ± 0.50 s.e and −1.33 ± 97 s.e. at T_2, and 0.85 ± 0.54 s.e. and -262 ± 1.04 s.e. Shapiro-Wilk test showed a non-normal distribution at T_1 ($W(23) = 0.91$, $p = 0.04$) and T_2 ($W(21) = 0.85$, $p = 0.01$) and a normal distribution at T_3 ($W(18) = 0.90$, $p = 0.05$). Levene's test of homogeneity failed to reject the hypothesis that the group variances

Table 1. Effects of dichlorvos on mean micronuclei counts per 1000 bone marrow cells of Wistar rats at 17 weeks (T_1).

Group	PCE	NCE	PCE/NCE Ratio	MnPCE	MnNCE	Other Marrow Cells
1	168.80 ± 94.91	239.00 ± 92.38	0.71 ± 0.27	2.20 ± 2.39	0.80 ± 1.30	589.40 ± 179.02
2	279.20 ± 178.69	198.40 ± 112.97	1.86 ± 1.63	2.80 ± 2.05	2.60 ± 1.14	517.00 ± 193.92
3	174.33 ± 74.57	174.67 ± 109.41	1.14 ± 0.63	1.33 ± 0.58	3.33 ± 0.58	646.33 ± 165.15
4	120.20 ± 53.18	170.40 ± 79.61	0.93 ± 0.83	4.60 ± 2.07	4.00 ± 2.55	700.80 ± 88.63
5	115.40 ± 60.80	93.00 ± 26.33	1.24 ± 0.50	3.20 ± 1.30	4.40 ± 1.34	784.00 ± 75.77

PCE: Polychromatic erythrocytes; NCE: Normochromatic erythrocytes; MnPCE: Micronucleated polychromatic erythrocytes; MnNCE: Micronucleated normochromatic erythrocytes.

Table 2. Effect of dichlorvos on mean micronuclei counts per 1000 bone marrow cells of Wistar rats at 34 weeks (T_2).

Group	PCE	NCE	PCE/NCE ratio	MnPCE	MnNCE	Other marrow cells
1	163.75 ± 59.87	183.75 ± 109.90	1.17 ± 0.85	2.00 ± 0.82	1.75 ± 0.96	648.75 ± 137.64
2	119.80 ± 39.10	170.00 ± 58.15	0.77 ± 0.30	3.60 ± 1.52	0.60 ± 0.55	706.00 ± 75.44
3	116.33 ± 83.72	95.67 ± 5.77	1.20 ± 0.82	4.00 ± 2.00	3.00 ± 1.00	781.00 ± 85.98
4	87.40 ± 9.02	102.40 ± 12.36	0.86 ± 0.13	4.00 ± 2.35	2.20 ± 1.30	804.00 ± 17.31
5	148.00 ± 77.91	110.00 ± 31.70	1.30 ± 0.49	2.50 ± 2.38	1.00 ± 0.00	738.50 ± 105.28

PCE: Polychromatic erythrocytes; NCE: Normochromatic erythrocytes; MnPCE: Micronucleated polychromatic erythrocytes; MnNCE: Micronucleated normochromatic erythrocytes.

Table 3. Effect of dichlorvos on mean micronuclei counts per 1000 bone marrow cells of Wistar rats at 50 weeks (T_3).

Group	PCE	NCE	PCE/NCE Ratio	MnPCE	MnNCE	Other Marrow Cells
1	148.67 ± 50.52	192.67 ± 83.34	0.83 ± 0.22	5.67 ± 1.53	1.33 ± 0.58	651.67 ± 127.16
2	123.67 ± 21.08	87.00 ± 27.22	1.48 ± 0.30	5.00 ± 1.00	1.67 ± 0.58	782.67 ± 47.54
3	87.80 ± 13.29	173.00 ± 43.06	0.55 ± 0.24	5.80 ± 3.56	0.60 ± 0.55	732.80 ± 35.66
4	145.25 ± 81.14	118.25 ± 57.15	1.34 ± 0.94	13.75 ± 4.57	4.00 ± 2.71	718.75 ± 114.50
5	139.33 ± 67.11	148.00 ± 65.85	1.10 ± 0.79	12.00 ± 2.65	4.00 ± 2.65	696.67 ± 76.97

PCE: Polychromatic erythrocytes; NCE: Normochromatic erythrocytes; MnPCE: Micronucleated polychromatic erythrocytes; MnNCE: Micronucleated normochromatic erythrocytes.

at the three-time intervals were not equal, $F(2, 59) = 14.01$, $p = 0.00$.

The null hypothesis that the distribution of micronucleated polychromatic erythrocytes (MnPCE) was the same across the groups was retained at T_1 (Kruskal-Wallis, χ^2 (4) = 6.70, p = 0.15, n = 23). Thus, at seventeen weeks, treatment with dichlorvos failed to induce observable changes in the number of micronucleated polychromatic erythrocytes in the bone marrow. Similarly, the null hypothesis was also retained at T_2 (Kruskal-Wallis, χ^2 (4) = 4.06, p = 0.40, n = 21), but was rejected at T_3 as significant differences existed between the groups (Kruskal-Wallis, χ^2 (4) = 10.59, p = 0.03, n = 18). The results of multiple comparisons at T_3 are presented in the table below. The absence of differences be-

tween group 1 (the control group) invalidates the differences between the other experimental groups. The treatment, therefore, failed to induce a statistically significant dose-related genomic instability.

Furthermore, to assess the effect of duration of exposure, the null hypothesis that the distribution of micronucleated polychromatic erythrocytes was the same across the three categories of time (T_1, T_2 and T_3) was rejected (Kruskal-Wallis, χ^2 (2) = 21.42, p = 0.00, n = 62). The mean ranks of MnPCE frequency at 17, 34 - and 50-week intervals had increasing trends, 23.67, 26.05 and 47.86, respectively. On pairwise comparison, there was no difference between T_1 - T_2 MnPCE frequencies but differences existed between the T_1 - T_3 ($p < 0.05$) and T_2 - T_3 ($p < 0.05$) pairs. Figure 1 presents an image of a micronucleus in the cytoplasm of a polychromatic erythrocyte stained with Giemsa and viewed at 1000 times magnification.

3.2. Correlation between Micronuclei Frequency and Serum Hormone Levels

3.2.1. Micronuclei Frequency and Dihydrotestosterone Correlation

There was a weak monotonic relationship between the frequency of micronucleated polychromatic erythrocytes and serum levels of dihydrotestosterone at T_1 ($r_s = 0.29$, p = 0.25, n = 18). At T_2, the correlation between the two variables was weak ($r_s = 0.36$, p = 0.15, n = 17) and was very weak ($r_s = 0.07$, p = 0.82, n = 15) at T_3. When considered together, a very weak non-statistically significant negative correlation was obtained ($r_s = -0.04$, p = 0.81, n = 50). The correlation between micronuclei and dihydrotestosterone is shown in Table 4.

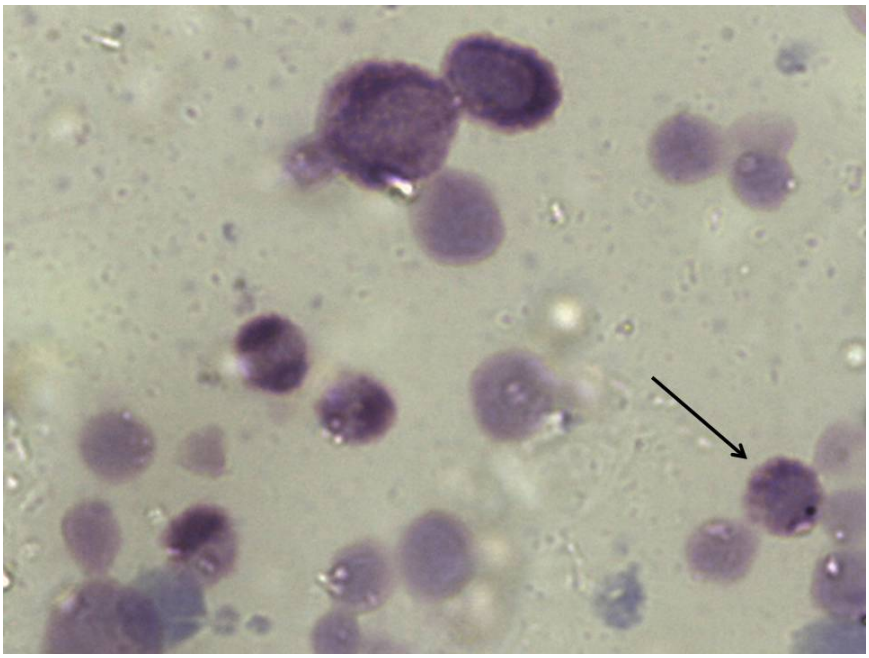

Figure 1. Photomicrograph of a smear of bone marrow aspirate showing a micronucleated polychromatic erythroblast (arrow). Giemsa stain; ×100 objective magnification; immersion oil.

3.2.2. Micronuclei Frequency and Follicle-Stimulating Hormone (FSH) Correlation

There was an overall, significant moderate negative correlation ($r_s = -0.41$, p = 0.00, n = 50) obtained between micronuclei frequency and blood follicle-stimulating hormone levels (alpha = 0.01). At T_1, there was a moderate negative correlation between micronuclei and FSH ($r_s = -0.42$, p = 0.09, n = 18). It was a very weak negative correlation between micronuclei and FSH at T_2 ($r_s = -0.03$, p = 0.92, n = 17) and T_3 ($r_s = -0.17$, p = 0.56, n = 15). This is shown in Table 4.

3.2.3. Micronuclei Frequency and Oestrogen Correlation

A moderate negative correlation ($r_s = -0.47$, p = 0.00, n = 50, alpha = 0.01) occurred between micronuclei frequency and oestrogen as presented in Table 4. It was very weak positive at T_1 ($r_s = 0.03$, p = 0.91, n = 18) but reversed to become moderately negative correlation at T_2 ($r_s = -0.55$, p = 0.02, n = 17) and weakly negative at T_3 ($r_s = -0.28$, p = 0.31, n = 15).

3.2.4. Micronuclei Frequency and Luteinizing Hormone (LH) Correlation

The correlation of micronuclei with luteinizing hormone was not statistically significant ($r_s = -0.22$, p = 0.12, n = 50). A weak negative correlation was observed between micronuclei frequency and luteinizing hormone at T_1 ($r_s = -0.26$, p = 0.30, n = 18) and T_2 ($r_s = -0.39$, p = 0.12, n = 17) and a very weak positive correlation at T_3 ($r_s = 0.07$, n = 0.80. n = 15) as shown on Table 4.

3.2.5. Micronuclei Frequency and Progesterone Correlation

Overall, a significant moderate negative relationship was found between serum progesterone levels and micronuclei frequency ($r_s = -0.37$, p = 0.01, n = 50) as shown in Table 4. A very weak positive correlation was found at T_1 between micronuclei frequency and serum levels of progesterone ($r_s = 0.03$, p = 0.91, n = 18) and at T_2 ($r_s = 0.02$, p = 0.94, n = 17) but became weakly negative at T_3 ($r_s = -0.31$, p = 0.27, n = 15).

Table 4. Micronuclei frequency and serum hormone levels correlation.

Hormone	T_1 (n = 18)		T_2 (n = 17)		T_3 (n = 15)		Summary (n = 50)	
	rs	p	rs	p	rs	p	rs	p
DHT	0.29	0.25	0.36	0.15	0.07	0.82	−0.04	0.81
FSH	−0.42	0.09	−0.03	0.92	−0.17	0.56	−0.41	0.00
E2	0.03	0.91	−0.55	0.02	−0.28	0.31	−0.47	0.00
LH	−0.26	0.30	−0.39	0.12	0.07	0.80	−0.22	0.12
P4	0.03	0.91	0.02	0.94	−0.31	0.27	−0.37	0.01

DHT = Dihydrotestosterone; FSH = Follicle stimulating hormone; E2 = Oestrogen; LH = Luteinizing hormone; P4 = Progesterone; rs: Spearman's rho correlation coefficient; p = p-value.

3.3. Micronuclei and Histological Changes Correlation

There was a positive weak correlation between micronuclei frequency and the grades of fourteen abnormal histological findings (see Table 5). These included tubular vacuolation (r_s = 0.30, p = 0.03, n = 50), residual bodies (r_s = 0.31, p = 0.03, n = 50) and tubular necrosis (r_s = 0.38, p = 0.01, n = 50). Table 5 below presents the details of the relationship.

3.4. Micronuclei Frequency Correlation with Sperm Count and Motility

MnPCE frequency did not have a statistically significant correlation with sperm count (r_s = −0.13, p = 0.37, n = 50). There was, however, a positive significant moderate correlation with the proportion of immotile sperms (r_s = 0.41, p = 0.00, n = 50) at an alpha level of 0.01 (two-tailed). The correlation coefficients of micronuclei frequency versus measured sperm parameters are shown in Table 6 below.

Table 5. Correlation between micronuclei frequency and histological findings (n = 50).

Histological findings	rs	p-value
1. Tubular atrophy/Degeneration	0.26	0.06
2. Germ cell depletion	0.17	0.24 h
3. Germ cell exfoliation	0.02	0.90
4. Tubular vacuolation	0.30*	0.03
5. Tubular contraction	0.20	0.17
6. Spermatid retention	0.25	0.08
7. Residual bodies	0.31*	0.03
8. Tubular necrosis	0.38**	0.01
9. Tubular neutrophilic inflammation	0.19	0.18
10. Interstitial oedema	0.24	0.10
11. Multinucleated giant cells	0.24	0.10
12. Sperm stasis/spermatocoele	0.07	0.63
13. Leydig cell hyperplasia	0.23	0.11

rs: Spearman's rho correlation coefficient; *: Correlation is significant at the 0.05 level; **: Correlation is significant at the 0.01 level.

Table 6. Correlation between micronuclei frequency and sperm parameters (n = 50).

Sperm parameter		rs	p-value
Sperm count		−0.13	0.37
Sperm Motility	1. Rapidly progressive	−0.13	0.38
	2. Slowly progressive	−0.23	0.11
	3. Immotile	0.41	0.00

rs: Spearman's rho correlation coefficient, α = 0.01, two-tailed.

4. Discussion

This experiment determined the impact of chronic dichlorvos exposure on male infertility and examined genomic instability as a possible mechanism for diminished reproductive capacity in males using a *Rattus norvegicus* rat model. Dichlorvos and other similar substances are used indiscriminately in many local African communities for domestic and agricultural purposes either to control insects in homes, prevent the destruction of stored grains or as pesticides [24].

The scientific literature has reported a wide spectrum of direct and indirect effects of this substance on both animal and human reproductive functions. These include hormone disruption [25], alteration in complex metabolic and energy pathways [26], induction of oxidative stress [27] [28] [29] and genetic pathways [18]. Disruptions in redox homeostasis have long been recognised as a cause of cell injury, and cell death and the underlying basis for many disease states [30]. This occurs through several mechanisms including lipid peroxidation of membranes, oxidative modification of cellular enzymes and structural proteins and breaks in DNA [31]. Disruption in redox homeostasis is a significant harbinger of genomic instability and mutagenesis [30].

The frequency of MnPCE significantly increased with the duration of exposure to dichlorvos in this study. This is consistent with previously published reports of similar studies in which genomic instability induced by dichlorvos and other organophosphate pesticides was assessed by quantification of micronuclei frequency [18] [32].

The correlation of MnPCE with sperm count was weak and statistically insignificant contrary to the direct inverse relationship expected. Since any form of genetic injury, besides hormone disruption, can potentially perturb the process of spermatogenesis with changes to enzyme functions, structural defects, etc, it is difficult, in this situation, to infer a direct link of causality. However, the animals with higher MnPCE also had increased proportions of immotile sperms. Hence, it appeared that dichlorvos-induced genomic instability had minimal effect on sperm maturation and sperm count but had a more profound impact on the motility of the sperms. This inference is consistent with the findings of Moghbeli-Nejad *et al.* in a study in which the researchers induced chromosomal instability in lymphocytes collected from both infertile and fertile men using 2 and 4 Gy gamma rays from a cobalt-60 source. They found a statistically significant difference in micronuclei formation between normal and infertile men and concluded that genomic instability could contribute to diminished reproductive capacity [33]. It appeared that the chronic exposure to dichlorvos induced genetic damage which adversely affected the motility of the spermatozoa. This is because MnPCE frequency increased in tandem with the proportion of immotile spermatozoa but not with germ cell depletion. While the genetic damage spared the germ cells' survival, neither inducing apoptosis nor anoikis, it significantly impaired the motile capacity of the formed spermatozoa. This inference is consistent with the findings of Zhang *et al.* in which almost null sperm motility occurred rats with a

loss-of-function mutation in CEP78 [34].

Micronuclei are small membrane-bound nuclear bodies found in the cytoplasm. They occur following damage to chromosomes during cell division or broken anaphase bridges leading to chromosomal fragments which are left behind in the cytoplasm (anaphase lag) [35]. The clastogenic effects of substances which induce genomic instability are mirrored by the frequency of micronuclei in the cytoplasm particularly of rapidly dividing cells [36]. Thus, the simplicity of this procedure makes it a useful tool for the assessment of genomic instability or genetic damage. Genetic damage or genomic instability has been evaluated, using micronuclei frequency determination in polychromatic erythrocytes as a surrogate measure [11] [12], and the authors advocate that it should be incorporated into routine infertility work-up since genetic causes have been reported to be responsible for up to 10% - 15% of severe male infertility cases [14].

5. Conclusion

This study shows that exposure to chronic low-dose dichlorvos in animal models resulted in genomic instability. The degree of genomic instability induced was found to increase with the duration rather than the dose of exposure. The nature of the correlations between micronuclei frequency with the proportion of immotile sperms, adverse histological changes and serum hormone levels found in this study suggest genomic instability as the possible mechanism for diminished fertility in males chronically exposed to dichlorvos.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Kumar, N. and Singh, A. (2015) Trends of Male Factor Infertility, an Important Cause of Infertility: A Review of Literature. *Journal of Human Reproductive Sciences*, **8**, 191-196.

[2] Ekwere, P.D., Archibong, E.I., Bassey, E.E., Ekabua, J.E., Ekanem, E.I. and Feyi-Waboso, P. (2007) Infertility among Nigerian Couples as Seen in Calabar. *Port Harcourt Medical Journal*, **2**, 35-40. https://doi.org/10.4314/phmedj.v2i1.38890

[3] Agarwal, A., Mulgund, A., Hamada, A. and Chyatte, M.R. (2015) A Unique View on Male Infertility around the Globe. *Reproductive Biology and Endocrinology*, **13**, Article No. 37. https://doi.org/10.1186/s12958-015-0032-1

[4] Kumar, N. and Singh, A.K. (2022) Impact of Environmental Factors on Human Semen Quality and Male Fertility: A Narrative Review. *Environmental Sciences Europe*, **34**, Article No. 6. https://doi.org/10.1186/s12302-021-00585-w

[5] Kaur, R.P., Gupta, V., Christopher, A.F. and Bansal, P. (2015) Potential Pathways of Pesticide Action on Erectile Function—A Contributory Factor in Male Infertility. *Asian Pacific Journal of Reproduction*, **4**, 322-330. https://doi.org/10.1016/j.apjr.2015.07.012

[6] Latini, G., Knipp, G., Mantovani, A., Marcovecchio, M.L., Chiarelli, F. and Soder,

O. (2010) Endocrine Disruptors and Human Health. *Mini-Reviews in Medicinal Chemistry*, **10**, 846-855. https://doi.org/10.2174/138955710791608271

[7] Wong, E.W.P. and Cheng, C.Y. (2011) Impacts of Environmental Toxicants on Male Reproductive Dysfunction. *Trends in Pharmacological Science*s, **32**, 290-299. https://doi.org/10.1016/j.tips.2011.01.001

[8] Agina, O., Omoja, V., Ekere, S., Odo, S. and Agina, B. (2017) A Study on Dichlorvos Induced Hematology, Clinical Biochemistry and Reproductive Abnormalities in Male Albino Rats. *Journal of Molecular Pathophysiology*, **6**, 5-11.

[9] Ogwuche, J.A. and Aginde, M.T. (2014) Use Practices and Health Impact of Nuvan Insecticide in Makurdi Urban Area, Benue State, Nigeria. *Research Journal of Agriculture and Environmental Management*, **3**, 326-333.

[10] Okoroiwu, H.U. and Iwara, I.A. (2018) Dichlorvos Toxicity: A Public Health Perspective. *Interdisciplinary Toxicology*, **11**, 129-137. https://doi.org/10.2478/intox-2018-0009

[11] Costa, C., García-Lestón, J., Costa, S., Coelho, P., Silva, S., Pingarilho, M., *et al.* (2014) Is Organic Farming Safer to Farmers' Health? A Comparison between Organic and Traditional Farming. *Toxicology Letters*, **230**, 166-176. https://doi.org/10.1016/j.toxlet.2014.02.011

[12] Nersesyan, A.K. (2007) Assessment of Cytogenetic Effect of Antiblastic Therapy by Means of Micronucleus Assay in Exfoliated Epithelial Cells. *Gene Therapy and Molecular Biology*, **11**, 177-184.

[13] Mansour, S.A., Mossa, A.H. and Heikal, T.M. (2008) Cytogenetic and Hormonal Alteration in Rats Exposed to Recommended "Safe Doses" of Spinosad and Malathion Insecticides. *International Journal of Agriculture and Biology*, **10**, 9-14.

[14] Ferlin, A., Arredi, B. and Foresta, C. (2006) Genetic Causes of Male Infertility. *Reproductive Toxicology*, **22**, 133-141. https://doi.org/10.1016/j.reprotox.2006.04.016

[15] Dong, Y., Du, R.C., Jiang, Y.T., Wu, J., Li, L.L. and Liu, R.Z. (2012) Impact of Chromosomal Translocations on Male Infertility, Semen Quality, Testicular Volume and Reproductive Hormone Levels. *Journal of International Medical Research*, **40**, 2274-2283. https://doi.org/10.1177/030006051204000625

[16] Ayesha, B., Ansari, A. and Lohiya, N. (2018) An Overview on the Genetic Determinants of Infertility. *Biomedical Journal of Scientific & Technical Research*, **10**, 7960-7964. https://doi.org/10.26717/BJSTR.2018.10.001984

[17] Doherty, A.T., Hayes, J., Holme, P. and O'Donovan, M. (2012) Chromosome Aberration Frequency in Rat Peripheral Lymphocytes Increases with Repeated Dosing with Hexamethylphosphoramide or Cyclophosphamide. *Mutagenesis*, **27**, 533-539. https://doi.org/10.1093/mutage/ges016

[18] Nazam, N., Iqbal Lone, M., Shaikh, S. and Ahmad, W. (2013) Assessment of Genotoxic Potential of the Insecticide Dichlorvos Using Cytogenetic Assay. *Interdisciplinary Toxicology*, **6**, 77-82. https://doi.org/10.2478/intox-2013-0014

[19] Lanning, L.L., Creasy, D.M., Chapin, R.E., Mann, P.C., Barlow, N.J., Regan, K.S., *et al.* (2002) Recommended Approaches for the Evaluation of Testicular and Epididymal Toxicity. *Toxicologic Pathology*, **30**, 507-520. https://doi.org/10.1080/01926230290105695

[20] Wyrobek, A.J. and Bruce, W.R. (1975) Chemical Induction of Sperm Abnormalities in Mice. *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 4425-4429. https://doi.org/10.1073/pnas.72.11.4425

[21] Abrevaya, X.C., Carballo, M.A. and Mudry, M.D. (2007) The Bone Marrow Micro-

nucleus Test and Metronidazole Genotoxicity in Different Strains of Mice (*Mus musculus*). *Genetics and Molecular Biology*, **30**, 1139-1143. https://doi.org/10.1590/S1415-47572007000600018

[22] Nersesyan, A., Kundi, M., Atefie, K., Schulte-Hermann, R. and Knasmuller, S. (2006) Effect of Staining Procedures on the Results of Micronucleus Assays with Exfoliated Oral Mucosa Cells. *Cancer Epidemiology Biomarkers & Prevention*, **15**, 1835-1840. https://doi.org/10.1158/1055-9965.EPI-06-0248

[23] Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S. and Zeiger, E. (2003) HUMN Project: Detailed Description of the Scoring Criteria for the Cytokinesis-Block Micronucleus Assay Using Isolated Human Lymphocyte Cultures. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **534**, 65-75. https://doi.org/10.1016/S1383-5718(02)00249-8

[24] Musa, U., Hati, S.S., Mustapha, A. and Magaji, G. (2010) Dichlorvos Concentrations in Locally Formulated Pesticide (Ota-Piapia) Utilized in Northeastern Nigeria. *Scientific Research and Essays*, **5**, 49-54.

[25] Sharma, B., Sharma, P. and Joshi, S.C. (2017) Mitigating Effects of *Zingiber officinale* against Reproductive Toxicity Induced by Dichlorvos in Male Rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, **9**, 107-113. https://doi.org/10.22376/ijpbs.2018.9.2.b166-172

[26] Bui-Nguyen, T.M., Baer, C.E., Lewis, J.A., Yang, D., Lein, P.J. and Jackson, D.A. (2015) Dichlorvos Exposure Results in Large Scale Disruption of Energy Metabolism in the Liver of the Zebrafish, *Danio rerio*. *BMC Genomics*, **16**, Article No. 853. https://doi.org/10.1186/s12864-015-1941-2

[27] Dirican, E.K. and Kalender, Y. (2012) Dichlorvos-Induced Testicular Toxicity in Male Rats and the Protective Role of Vitamins C and E. *Experimental and Toxicologic Pathology*, **64**, 821-830. https://doi.org/10.1016/j.etp.2011.03.002

[28] DSouza, U.J.A. (2017) Pesticide Toxicity and Oxidative Stress : A Review. *Borneo Journal of Medical Sciences*, **11**, 9-19. https://doi.org/10.51200/bjms.v11i2.466

[29] El Ayari, T., Mhadhbi, L., Debara, N., Znati, M. and Dab, H. (2020) The Detoxifying Effect of *Polygonum equisetiforme* Extracts against Dichlorvos (DDVP)-Induced Oxidative Stress and Neurotoxicity in the Commercial Clam *Ruditapes decussatus*. *Environmental Science and Pollution Research International*, **27**, 10449-10458. https://doi.org/10.1007/s11356-019-07554-0

[30] Poetsch, A.R. (2020) The Genomics of Oxidative DNA Damage, Repair, and Resulting Mutagenesis. *Computational and Structural Biotechnology Journal*, **18**, 207-219. https://doi.org/10.1016/j.csbj.2019.12.013

[31] Gaschler, M.M. and Stockwell, B.R. (2017) Lipid Peroxidation in Cell Death. *Biochemical and Biophysical Research Communications*, **482**, 419-425. https://doi.org/10.1016/j.bbrc.2016.10.086

[32] Torres-Bugarín, O., Garcia-Arellano, E., Onel Salas-Cordero, K. and Molina-Noyola, L. (2019) Micronuclei and Nuclear Abnormalities as Bioindicators of Gene Instability Vulnerability. *Austin Journal of Pathology & Laboratory Medicine*, **6**, Article ID: 1023.

[33] Moghbeli-Nejad, S., Mozdarani, H. and Aleyasin, A. (2012) Increased Frequency of Micronuclei in Lymphocytes of Infertile Males after Exposure to γ Irradiation: A Possible Sign of Genomic Instability. *Journal of Assisted Reproduction and Genetics*, **29**, 89-94. https://doi.org/10.1007/s10815-011-9550-8

[34] Zhang, X., Zheng, R., Liang, C., Liu, H., Zhang, X., Ma, Y., *et al.* (2022) Loss-of-Function Mutations in CEP78 Cause Male Infertility in Humans and Mice. *Science*

Advances, **8**, eabn0968. https://doi.org/10.1126/sciadv.abn0968

[35] Lindberg, H.K., Wang, X., Järventaus, H., Falck, G.C.M., Norppa, H. and Fenech, M. (2007) Origin of Nuclear Buds and Micronuclei in Normal and Folate-Deprived Human Lymphocytes. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **617**, 33-45. https://doi.org/10.1016/j.mrfmmm.2006.12.002

[36] Norppa, H. and Falck, G.C.M. (2003) What Do Human Micronuclei Contain? *Mutagenesis*, **18**, 221-233. https://doi.org/10.1093/mutage/18.3.221