

Research Article

Immuno-splenic Toxicity in Rats Fed with Dichlorvos-Treated Bean Diets

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ABSTRACT

Dichlorvos an organophosphate compound is used in the agricultural sector for both pre-harvest and postharvest protection of crops. Large quantities are used globally every year with the developing nations having the highest patronage. The hazardous consequences of this quantity on human and animal health are enormous and a matter of urgent public health concern. This work is aimed at determining the effect of dichlorvos-treated bean diets on immune profiles and the structural integrity of the spleen in rats. A total of twenty-eight (28) kilograms of bean (*Phaseolus vulgaris*) was acquired and divided into seven groups of 4kg each. Group I is the control group and did not receive any dichlorvos treatment. Groups IIa, IIb, and IIc only received high, medium, and low doses respectively while groups IIIa, IIIb and IIIc received high, medium, and low doses of dichlorvos respectively thereafter parboiled and dried. All samples were stored for six months, separately ground into powder form using a blender then mixed with rat feed and fed to the rats for thirty days. At the end of the treatment, the rats were anaesthetized with chloroform, and blood samples were collected through cardiac puncture for determination of white blood cell profile. The spleen was harvested and preserved in 10% formaldehyde for histological analysis. The result showed that in groups IIa, IIb, and IIc all the immuno-protective components of the cell increased significantly when compared to the control. Cross-examination of the spleen showed various degrees of structural abnormalities caused by dichlorvos.

Keywords: Dichlorvos Treated-bean diets, Immuno-splenic toxicity, Immuno-protective components, Integrity of the spleen, Structural abnormalities

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INTRODUCTION

Dichlorvos known as 2, 2-dichlorovinyl dimethyl phosphate or DDVP and locally called Sniper, Nuvan, *Ota pia-pia* etc. is an organophosphate compound used in agriculture for the protection of crops against insects, as disinfectants in hospitals, industries, and homes. It was first introduced into the market in 1948¹ and has since gained widespread acceptance, especially in developing countries. In some more developed countries, its use has either been

restricted or completely banned. Plant pests and diseases destroy about 45% of world crops yearly making the use of pesticides in improving the existing state of harvest and postharvest production of crops an inevitable project in meeting the global food demand (Islam *et al.*, 2014).

In Nigeria, the use of dichlorvos in preserving dry bean seeds has become a common practice with several reported cases of the presence of dichlorvos

in bean samples across many markets in the country (Ogah *et al.*, 2012, Yusuf *et al.*, 2018, Adoga *et al.*, 2019). Nigeria imports over 130,000 metric tons of dichlorvos yearly with about 76% of it being consumed in the agro-allied sector (Oshatunberu *et al.*, 2023).

The consequences of this large volume on human health and its' unregulated and unrestricted use is likened to a "time bomb" and a matter of urgent public health concern (Okoroiwu and Iwara, 2018). United Nations reports published in 2018, showed that approximately 200,000 people worldwide die each year from toxic exposure to pesticides (Rifai, 2017 and Karigidi, 2018). Several studies have been conducted to assess the toxic effects of dichlorvos on organisms in the environment using indices including mortality, immobilization, and growth inhibition (Yusuf and Buhari, 2017). Evidence of dichlorvos toxicity on animal and human health shows that dichlorvos toxicity can cause neurotoxicity, haematotoxicity, genotoxicity, carcinogenicity, irritation and corrosion, and reproductive and developmental toxicity (CERI, 2007), and other associated abnormalities such as leukemia, brain, and prostate cancer (Mourad, 2005).

Haematological Alterations

The human blood is an important tool in clinical assessment of the physiological or general health condition of the body. Alterations in blood constituents which may be of genetic and non-genetic origin, exposure to toxicants, age-related origin, sex, breed, or management systems, give crucial information on the nutritional, physiological, and pathological status of the animal (Etim *et al.*, 2014).

The haematotoxic effect of dichlorvos can be assessed by frequently monitoring the various components of blood and comparing them with recommended normal levels. This serves as a good check on an individual's health status, like immune status, therapeutic status, monitoring disease progression, and treatment outcomes (Amilo, 2020). Haematological components include leucocytes (white blood cell), red blood cells (erythrocytes), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC).

Dichlorvos is documented to have toxic effects on the immune components of the cell (Makarizadeh *et al.*, 2015). Increased white blood cell (WBC) values and

platelet counts have been documented in rats treated with dichlorvos (Holy *et al.*, 2015, Nwauzobilom *et al.*, 2020). Inhalation of dichlorvos resulted in increased values of WBC and platelet counts in rats (Kanu *et al.*, 2016). These works cited here validate the immunotoxicity potential of dichlorvos.

In an age-related study, dichlorvos caused immunotoxicity of dichlorvos in young and old rats while there was no toxic effect in the middle age group of rats studied. The study proved that the effect of dichlorvos on haematological parameters is age-dependent (Ige, 2021). A previous work (Mfaume *et al.*, 2023) showed significant increases in the values of white blood cell components and increased intensity of dark brown deposits of diaminobenzidine (DAB) in the spleen in both the red and white pulp surrounding the central artery of rats exposed to dichlorvos indicating the toxic effect of DDVP on the spleen.

MATERIALS AND METHODS

Collection of Beans

A total of twenty-eight (28) kilograms of freshly harvested bean sample (*Phaseolus vulgaris*) was obtained from farmers for this research. The beans were properly processed by removing all the farm debris and thereafter divided into three different groups: I, II, and III. Group I is the control group while the treated groups, II and III were divided into three sub-groups each, labelled as IIa, IIb, IIc, IIId, IIIE, and IIIf, and packaged into air-tight containers of four kilograms of beans each.

Procurement and Application of Dichlorvos to Bean Sample

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) in a 100mL bottle labelled as 'sniper 100EC' was procured from an agrochemical store and mixed with the beans sample according to the manufacturer specifications:

The beans sample in group I (control) was not treated with dichlorvos while groups IIa and IIId were treated with high doses of dichlorvos (8mL of dichlorvos/4kg of beans), groups IIb and IIIE were treated with medium doses (4mL of dichlorvos/4kg of beans) and groups IIc and IIIf received low dosage (2mL of beans/4kg of beans). Group III samples were parboiled after the application of dichlorvos and dried while groups I and II were left un-parboiled. All the samples were packaged into seven separate airtight

containers and stored for six months. At the end of the storage, the samples were separately ground to powdered form, mixed with normal rat feed, and used for feeding the rats.

Procurement and Feeding of albino Wistar Rats

Thirty-five (35) albino Wistar rats were procured and acclimatized for 14 days in the Animal House Laboratory, College of Health Sciences, Benue State University, Makurdi, Nigeria. Water was administered *ad libitum*.

The rats were divided into seven groups of five rats each and fed with normal rat feed mixed with different preparations of beans diets per group for thirty days. Group I (control) was fed with normal rat feed mixed with untreated bean powder while feed for groups IIa, IIb, and IIc contained normal rat feed mixed with un-parboiled dichlorvos-treated bean diets of high, medium, and low doses respectively. Groups IIId, IIle, and IIIf were fed with normal rat feed mixed with parboiled dichlorvos-treated bean diets of high, medium, and low doses respectively.

At the end of the exposure period (30 days), the rats were anaesthetized, a blood sample (1mL) was collected by cardiac puncture using a sample needle and immediately transferred into sterile EDTA-K3 Vacuum tubes and used for haematological analysis. White blood cell components were analyzed by

complete blood count (CBC). The spleen was harvested, placed in formaldehyde (10%), and stored in a refrigerator for histological studies. Slides of liver sections were prepared using the paraffin technique as outlined by Oyeyemi *et al.* (2020) and photomicrographs were obtained at x400 magnification (haematoxylin and eosin stain).

Data Analysis

Multiple factor analysis was carried out by using analysis of variance while differences between means that were of statistical significance was analyzed by using Duncan’s multiple range test at P<0.05. Values were presented as mean value ± standard deviation.

RESULTS

Haematological Parameters

The results of immuno-toxic effects of dichlorvos treated beans diets on albino Wistar rats is represented by the white blood cell profiles shown in Table 1.

Histological Analysis

Results of examinations of cross-sections of spleen tissues for histological changes in rats induced by dichlorvos-treated bean diets are displayed in plates 1-7.

Table 1. White blood cell profile

Parameters	Control	Group II			Group III		
		a	B	c	D	E	F
WBC (10³/μL)	9.05±0.14	10.58±0.17*	10.51±0.03*	10.45±0.03*	9.55±0.14 ^a	9.38±0.06 ^b	9.24±0.09 ^c
LYM (10³/mL)	2.24±0.17	3.78±0.14*	3.36±0.05*	3.13±0.04 ^a	2.38±0.05 ^a	2.28±0.07 ^b	2.26±0.06 ^c
MID (10³μL)	1.06±0.06	1.43±0.04*	1.37±0.04*	1.20±0.03 ^a	1.17±0.04 ^a	1.14±0.02 ^b	1.10±0.04
GRA (10³μ/L)	3.60±0.09	4.06±0.04*	3.77±0.11	3.71±0.05	3.68±0.08 ^a	3.64±0.06	3.68±0.06
LYM%	33.90±1.65	50.74±2.33*	46.98±1.74*	43.20±0.99 ^a	41.10±0.36 ^a	37.00±0.36 ^b	34.70±0.62 ^c
MID%	14.70±0.37	19.48±0.53*	16.72±0.40 ^a	15.80±0.44 ^a	15.70±0.17 ^a	15.20±0.16	14.49±0.14 ^b
GRA%	51.40±1.62	54.11±0.89	53.55±0.75	52.93±0.77	52.94±0.30	51.72±0.26	51.50±0.23

N – 5, * = significant relative to control at P < 0.05, a = significant relative to a P < 0.05, b = significant relative to b at P < 0.05, c = significant relative to C at P < 0.05, d = significant relative to d at P < 0.05, e = significant relative to e P < 0.05, f = significant relative to f at P < 0.05. WBC = white blood cell, LYM = lymphocyte, MID= Any Other types of WBC not classified as lymphocytes or granulocytes (e.g. eosinophil, basophil, and monocyte), GRA= Granulocytes

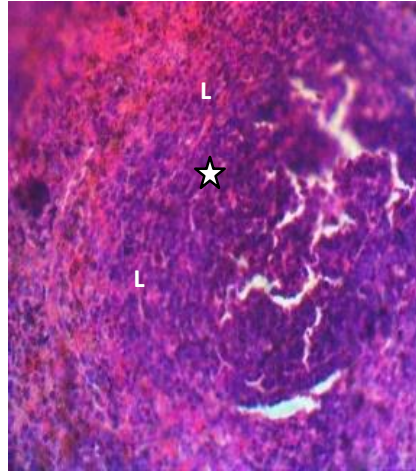


Plate 1 (Group 1 – Control): Spleen photomicrograph shows a distribution of lymphocytes (L) consisting of small mature lymphocytes (white star). L = Lymphocytes. No observable lesion. *Hematoxylin & eosin stain, x 400*

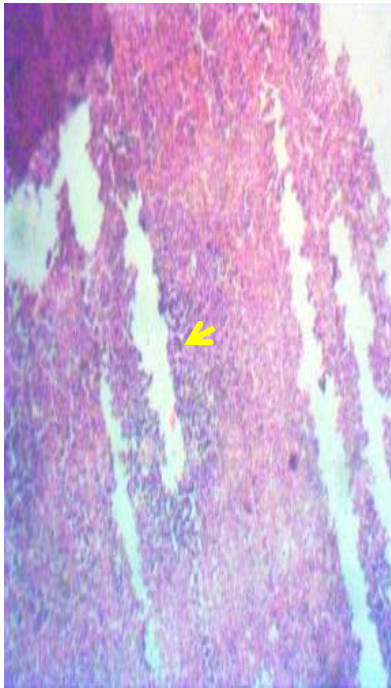


Plate 2 (Group IIa: High dose of dichlorvos): Spleen- There is severe atrophy of follicular lymphoid cells. *Hematoxylin & eosin stain x 400*

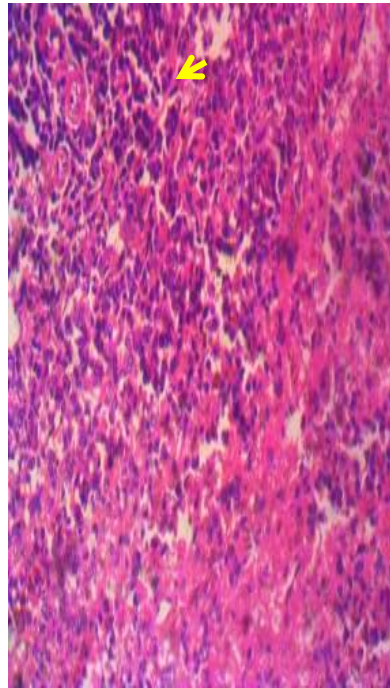


Plate 3 (Group IIb: Medium dose of dichlorvos): There is severe atrophy of follicular lymphoid cells. *Hematoxylin & eosin stain x400*

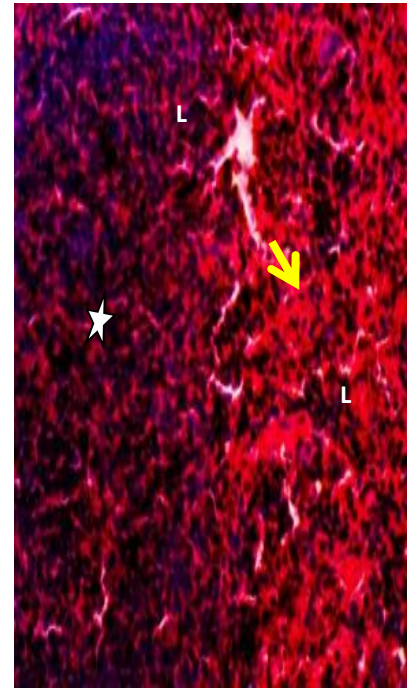


Plate 4 (Group IIc: Low dose of dichlorvos): Spleen photomicrograph shows moderate congestion of red pulp (arrow) and lymphoid hyperplasia with aggregates of small lymphocytes with dark nuclei (white star). L = Lymphocytes. *Hematoxylin & eosin stain x400*

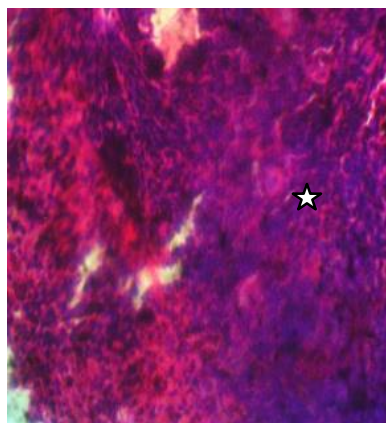


Plate 5 (Group III d): High dose of dichlorvos): Spleen photomicrograph shows follicular lymphoid hyperplasia consisting of small mature lymphocytes (white star). *Hematoxylin & eosin stain, x 400*

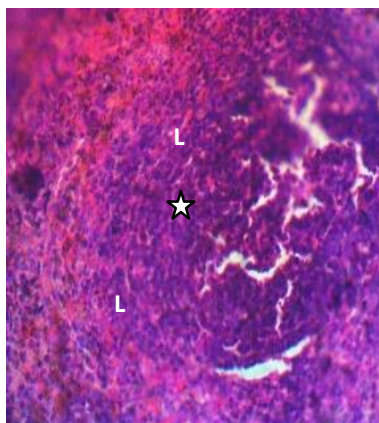


Plate 6 (Group III e): Medium dose of dichlorvos): Spleen photomicrograph shows follicular lymphoid hyperplasia consisting of small mature lymphocytes (white star). L = Lymphocytes. *Hematoxylin & eosin stain, x 400*

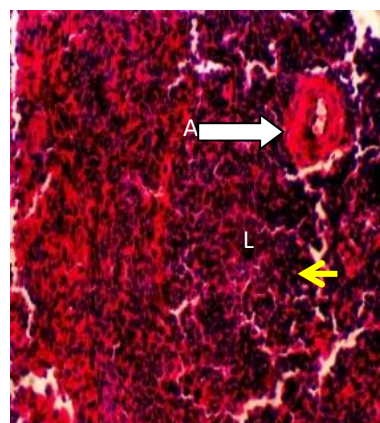


Plate 7 (Group III f): Low dose of dichlorvos): Spleen photomicrograph shows an arteriole (upper right) surrounded by a broad rim of small lymphocytes, depicting moderate lymphoid hyperplasia (arrow). L = lymphocytes, A = Arteriole. *Hematoxylin & eosin stain x400*

DISCUSSION

Dichlorvos is a potent toxic compound with deleterious effects on several human organs and tissues such as the liver, kidney, spleen, blood, and brain (Holy *et al.*, 2015; Mfaume *et al.*, 2023; Amali *et al.*, 2023). The result showed a general increase in levels of white blood cell (WBC), lymphocytes (LYM), MID (i.e. other types of WBC not classified as lymphocytes or granulocytes e.g. eosinophil, basophil, and monocyte) and granulocytes (GRA) when compared to the control group. The increase is dose-dependent with the highest elevation being recorded in group II which contained the highest dose level of dichlorvos. This finding is in tandem with another work (Holy *et al.*, 2015) which showed dose-dependent behaviour of white blood cell and platelet count under different concentrations of dichlorvos. Consequently, the immuno-protective apparatus of the experimental animals see the residues of dichlorvos as foreign bodies and rises to offer a defensive and protective shield against these xenobiotics. White blood cells which is the fulcrum of the immuno-protective system is therefore produced in increased numbers to face the challenges. From the result, it is observed that group II whose feed was not parboiled before storage had the highest increase of WBC and all other immuno-protective parameters

analyzed in this study. The increase in WBC is in agreement with a previous work (Agina *et al.*, 2017) which reported a severely high WBC count in rats exposed to dichlorvos. However, Fayinminnu *et al.* (2022) reported a contrary result where WBC and lymphocytes were decreased due to the destructive effect of dichlorvos on WBC and lymphocytes.

The relationship established between the groups showed a significant difference between groups II and III ($p < 0.05$). The lowest values for WBC, LYM, MID, and GRA were obtained in group III as compared to group II. This can be explained by the fact that the bean samples used for group III were parboiled before their storage and subsequent administration to the rats. Parboiling is known to reduce the toxic effect of dichlorvos (Milani, 2023). Parboiling was shown to significantly reduce the toxic effect of dichlorvos in stored bean samples as evidenced by the low toxicity level recorded in the group fed with parboiled bean diets compared to the group fed with un-parboiled bean diets (Nwauzobilon *et al.*, 2020). The elevation in lymphocyte values obtained in this result may have emanated from the effect of DDVP on lymphoid tissues thus leading to increased generation of lymphocytes. Gastric irritants cause tissue damage and disturbances of the general

immune system with a consequent spike in leukocyte production (Gbakon *et al.*, 2018).

The use or misuse of dichlorvos either from agricultural exposure or any other means can cause structural abnormalities in many organs of the body system. Frequent clinical examinations of these organs is therefore a good step for the assessment of environmental contamination and consequent organ distress (Deord *et al.*, 2022).

In this work, clinical examinations of the spleen revealed a wide spectrum of histological changes in the integrity of the spleen varying from severe atrophy of follicular lymphoid cells, moderate congestion of red pulp, lymphoid hyperplasia, and paler germinal center. This result follows an earlier work (Mfaume *et al.*, 2023) where exposure of rats to dichlorvos caused abnormalities in the structure of the spleen. The spleen which consists of immunological defensive cells (splenocytes) as B-cells, T-cells, natural killer (NK) cells, and monocytes (Aliyu *et al.*, 2021), plays crucial roles in both innate and adaptive immunological systems. It is heavily exposed to a large influx of xenobiotics making it highly vulnerable to many toxicants. The consequences of this vulnerability are manifested in the various histo-architectural distortions observed in this current work. The alterations in the splenic structure were seen to be dose-dependent with the high doses in group II producing more severe histopathological abnormalities. The result obtained here is in agreement with a previous work (Seferoglu *et al.*, 2013) where dose-dependent responses were recorded in the spleen and kidney of juvenile *Sparusaurata* (Sea Bream) exposed to different concentrations of dichlorvos.

In this work, the splenic distress recorded may be because dichlorvos is a known potent agent for lipid peroxidation thus causing electrolyte imbalance affecting several other membranous functions of the spleen. Results of an earlier work conducted on the effect of diazinon on the spleen of fish give credence to the result obtained in this work (Urgulu *et al.*, 2022). Photomicrographic examinations of the spleen show that all the groups exposed to dichlorvos exhibited some degree of structural distortions though the level of damage was seen to be less severe as the dosage of dichlorvos was reduced. A toxicant has the potential to generate reactive oxygen species thereby causing oxidative damage in the spleen (Sarkar *et al.*, 2011). Toxicants such as nano-Cu can cross the gastrointestinal lumen into the lymphocyte

nodes of the spleen (Jani *et al.*, 2011) where eventually they are captured by splenic macrophages and form cytotoxic aggregates (Kaewamatawong *et al.*, 2005).

CONCLUSIONS

After 6 months of treatment and preservation of beans with dichlorvos, the findings of this research confirmed the toxicity potentials of DDVP in the treated rats as indicated by the increased values of all the white blood cell components in the treated groups. The work also revealed a compromise in the structural integrity of the spleen in the groups treated with dichlorvos as corroborated by the observed severe follicular atrophy, lymphoid hyperplasia, moderate congestion of the red pulp, and paler germinal center. Conclusively, the research proved that treatment of beans with dichlorvos and subsequent storage for 6 months did not eradicate its residual harmful nature. Toxicity was however seen to be reduced in the groups fed with the parboiled beans diets.

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