Macrothink Institute™

Journal of Agricultural Studies
ISSN 2166-0379
2022, Vol. 10, No. 4

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate DDVP (SNIPER)) Toxicity on Histological Organs of Wistar Rats Fed on Treated Cowpea Grains (*Vigna unguiculata* (L.) Walp)

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Received: July 1, 2022 Accepted: September 21, 2022 Published: October 16, 2022

doi:10.5296/jas.v10i4.20360 URL: https://doi.org/10.5296/jas.v10i4.20360

Abstract

Cowpea is an important grain legume that provides half of the plant protein and is consumed worldwide. The crop is usually affected by pests on the field and in storage which always result in huge losses, hence being controlled by using synthetic pesticides. However, consumption of cowpea grains treated with synthetic pesticides had resulted in bioaccumulation and several health-related problems. Therefore, this study examined the toxic effects of Dichlorvos (DDVP) treated cowpea grains fed to Wistar rats for four weeks. Sixteen male rats weighing 125±20g were used for this experiment. They were randomly divided into four groups of four rats each including the control and acclimatised for one week fed with Standard Feed Ratio (SFR). The DDVP (98% purity) was applied at the rates of 0 (control), 0.23, 0.33, and 0.43mL/kg directly to 1kg cowpea grains of IT 89k-391, respectively. Treated cowpea grains were milled (with a 2 mm sieve) and mixed with SFR and fed to the animals daily for four weeks. Data were collected weeks after treatment (WAT) on feed intake, body weight gain/loss, hematology, and, serum chemistry and analysed using ANOVA at $p<0.05$, while histopathology was examined. Results showed significant differences ($p < 0.05$) in all the parameters. There was a reduction in feed intake and body weights progressively in DDVP

cowpea treated animals, while the control animals increased. Hematological parameters showed 0.23 mL/kg DDVP recording the highest hemoglobin (g/dl) (17.00±1.16), while the lowest value in white blood cells (1100±5.78 x 10^3/µL), and 0.43 mL/kg recorded the lowest and highest red blood cells (10^3/µL) (5.34±0.78) and (8.83±0.23) all between 1 and 4 WAT. Serum parameters revealed Total protein having lowest values (5.22±0.01, 5.13±0.01, 5.18±0.01) from 0.23, 0.33, and 0.43 mL/kg DDVP treated animals. Alkaline phosphate, Aspartate aminotransferase, and Alanine transaminase recorded the highest values from 0.43 and 0.33 mL/kg treatments at 3 and 4 WAT, respectively. Histopathology at 1 to 4 WAT revealed capillary congestion with necrotic changes, severe cell infiltration of interstitial and parenchyma with inflammatory cells in the kidneys of 0.23, and 0.33 mL/kg DDVP cowpea fed animals. On liver organs, 0.23, 0.33, and 0.43 mL/kg treatments showed necrosis, vacuolar degeneration of hepatocytes, and congestion with distinct cysts and granular formation. The central portions of livers were pale and distinct patterns of arrangement in cords were absent. However, the control animals recorded the highest values in the aforementioned parameters with clear and normal liver and kidney organs in the experiment. In this study, varied changes observed on the hematological, serum biochemical, and histopathological parameters at 0.23, 0.33, and 0.43 mL/kg dichlorvos (DDVP) treated cowpea grains could result in impairment of vital organs, hence ill health issues. Continued use of dichlorvos for cowpea grains in preservation should be discouraged to prevent health problems and promote high-quality nutrition and safe food.

Keywords: toxicity, cowpea, Wistar rats, Dichlorvos (DDVP), hematology and serum biochemistry, histopathology

1. Introduction

In Africa, the use of synthetic chemical pesticides has mostly depended upon controlling pests and diseases which are of great threat during handling and storage processes. Synthetic pesticides, however, are still considered the most effective method of controlling stored product pests and diseases, particularly for large-scale storage; especially in developing countries; despite the public concern about their effects on human health and the environment (Isman, 2000; Gbaye and Holloway, 2011). The chemical control method is the most commonly used for pest management in Nigeria (Chedi and Aliyu, 2010) both in the field and in storage. Owing to the lack of education and high level of illiteracy among local farmers and post-harvest handlers of cowpea in Nigeria, pesticides are indiscriminately applied for insect pest control (Gbaye *et al*., 2016).

Synthetic chemicals' continual and indiscriminate uses have led to the development of resistant strains of pests, and the accumulation of toxic residues on food crops and animal products consumed by humans (Anon, 1974; Degri, 2008). Altogether these result in health problems such as cancer, birth abnormalities, hormonal imbalance, environmental pollution (Ofuya and Lale, 2001; Sahayaraj, 2008), and food poisoning for both humans and animals.

Dichlorvos (2, 2- dichlorovinyl dimethyl phosphate; DDVP) is an organophosphate compound with different brand names such as Nuvan, DD Force and Sniper, etc. It is one of the most widely used pesticides for the control of household pests, public health pests, and crops and

stored product insects (Celik *et al.*, 2008). The DDVP is an insecticide and fumigant with contact, respiratory, and stomach poison on stored product pests (Lotti, 2001; Nguegang *et al*., 2005; Booth *et al*., 2007; Gbaye *et al*., 2012; Perveen and Khan, 2014).

Contact of the general public to dichlorvos may occur via air, water, or food because it is readily absorbed through all routes of exposure (Raheja and Gill, 2002). Akoto *et al*. (2013) reported that an estimated 200,000 people every year dies because Dichlorvos pesticide is been used for self-poisoning, an important clinical problem in the developing world. Toxic effects from acute exposure include perspiration, nausea, vomiting, diarrhea, drowsiness, fatigue, headache, depressed motor function and respiration, seizures and at very high concentrations, convulsions, coma, and death (ATSDR, 1997; Chedi and Aliyu, 2010). Chronic dichlorvos exposures have resulted in severe motor incoordination and deterioration in memory functions (Verma *et al.*, 2009), oxidative stress; effects on blood cells; damage to the liver and heart (Mishra *et al*., 2013).

Cowpea, *Vigna unguiculata* is a staple grain legume of worldwide importance (Jackai and Asante, 2003). Cowpea provides more than half of the plant protein consumed by many poor people in the tropics and also it is a source of income (Fatokun *et al.*, 2002). However, huge post-harvest losses are usually recorded due to the damage caused by *Callosobruchus maculatus* (F.), Coleoptera: Bruchidae a major storage pest of cowpea grains (Singh and Ntare, 1985; IITA, 2010). According to Adamawa grain sellers (Grain markets of Adamawa State, Nigeria, 2014), pests and diseases pose the greatest threat to increased food production during storage and handling. Before harvest, insects cause about 15 – 100% crop damage, while storage losses in food grains range between 10 – 60%. Management of storage pests in cowpea grains in Nigeria however relies heavily on the use of chemical insecticides (Jackai and Asante, 2003). Application of these synthetic insecticides to control pests is directly done on cowpea grains which have been the usual practice and more intensive among merchants/ traders in Nigeria. Reports from the study of Yusuf *et al*. (2017) revealed misuse/ abusive application of pesticides on stored cowpea grains at Dawanau grains market (largest grains market in West Africa) in Kano State, the Northern part of Nigeria.

Consumption of cowpea grains treated with synthetic pesticides had resulted in bioaccumulation and several health-related problems. Analysis carried out in a laboratory and reported by Daily Trust Newspaper Nigeria (2020) showed that consumers of grains treated with pesticides including beans are at risk due to their harmful effects. The quality of chemicals in treated beans sold across the country (Nigeria) is unsafe for consumers with their effects leading to cancer and kidney-related diseases. Samples of beans were randomly obtained from markets in six states across the geopolitical zones and analysed in a laboratory at the University of Agriculture Makurdi, Benue State, Nigeria. Results revealed a high amount of pesticides residue on treated beans sold in the markets which were harmful to humans. Information on social media about the bean grains retailers showed that a particular insecticide sniper, DDVP was been used to preserve beans. It was gathered that some of the chemicals used in cowpea preservation are potentially injurious (Daily Trust Newspaper Nigeria, 2020).

A curious look made at Bodija International foodstuff market in Ibadan, Oyo State, Nigeria

showed how cowpea grains were treated with different synthetic pesticides. The cowpea grains were sold out immediately after pesticide application, without giving it proper time for storage/preservation. Studies had been carried out and reports had revealed residues of different pesticides used in stored cowpea grains, while toxic effects of consuming such crop products through toxicological studies of feeding to Wistar rats have a dearth of information. Also, this insecticide (Dichlorvos, DDVP) was chosen for this experiment because it is being widely used in Nigeria by cowpea grain farmers and retailers. There is a need, therefore, to assess the toxicity of Dichlorvos (DDVP) treated cowpea grains on Wistar rats in ensuring high food quality and safety.

2. Materials and Methods

2.1 Experimental Study Site

The experiment was carried out at the University of Ibadan, Ibadan, Nigeria, which lies between longitude 7°27.05`N and 3°53.74`E of the Greenwich Meridian at an altitude of 200 m above sea level. The typical temperature and relative humidity of the location are between 23-42°C and 60-80%, respectively (SMUI 2018) with a mean annual rainfall of 1110.1mm. The rainfall pattern is a bimodal type characterised by a peak in July and September. The experiment was commenced at the Department of Animal Science (Animal House) and Toxicology Research Laboratory of the Department of Crop Protection and Environmental Biology, Faculty of Agriculture and Forestry, and the Clinical Pathology and Histopathology Laboratories of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, all in the University of Ibadan, Ibadan, Nigeria.

2.2 Sources of Experimental Materials

Cowpea grains, *Vigna unguiculata* (L) Walp. (untreated) (IT 89k-391) were purchased from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. They were sorted to remove perforated/wrinkled grains and were put in an air-tight plastic container. Sixteen male *Rattus noreveigicus* male (Wistar) rats with a mean body weight of 125±20 g obtained from the experimental animal house of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, were used for this experiment. Dichlorvos (DDVP, 98% purity) used for this study was purchased from Saro Agroscience Chemicals at Oluyole Estate Ibadan, Oyo State, Nigeria.

2.2.1 Application of Dichlorvos to Treated Cowpea Grains

One kilogram of untreated cowpea grains of *Vigna unguiculata* (L) Walp. (IT 89k-391) each was weighed into a separate air-tight container for respective treatments. Dichlorvos (DDVP) was applied at the rates of 0.23, 0.33, and 0.43 mL/kg, based on Bodija market findings (Bodija Market Cowpea Sellers Association), while the control was without any pesticide application. All the treated cowpea grains were well-kept-up over a period of four weeks within the commencement of the treatments to the experimental animals.

2.3 Experimental Animals Set Up

Sixteen male rats were randomly divided into four experimental groups including the control of

four rats each and were labeled A1, A2, A3, and A4, they were subjected to acclimatisation for one week before the commencement of treatments. The animals were maintained at 27±2ºC, with 12-hour light, 12-hour dark cycles, and relative humidity of 75-80%, and kept in procured, cleaned, and disinfected individual cages for four weeks (28 days). Cages were fitted with drinkers that could comfortably drop water when imbibed by rats and the feeders were properly placed to eliminate feed spillage before the arrival of the Wistar rats. The experimental animals were fed with growers' mash feed (Standard Ration Feed) sourced locally and water was given to them *ad libitum* during acclimatisation. All animals were handled following the guidelines for care and use of laboratory animals as stated and approved by the University of Ibadan Ethical Committee which conforms to the Ethical use of Animals (Clarke *et al*., 1996).

2.3.1 Administration of Dichlorvos (DDVP) Treated Cowpea Grains to the Male Wistar Rats

The experimental animals (male Wistar rats) were randomly allocated to four treatments; individual treatment. Treated cowpea grains with respective rates (0, 0.23, 0.33, and 0.43 mL/kg as A1, A2, A3, and A4) of Dichlorvos (DDVP) were ground separately and added to the formulation of the Standard Feed Ratio (SFR) weekly. Treatments consisted of the following allotted rats` groups:

Group A1: rats fed with SFR 37g + 63 g untreated cowpea grains ground (Control, 0 mL/kg), Group A2: rats fed on SRF 37g + 63 g of DDVP at 0.23 mL/kg treated cowpea grains, Group A3: rats fed on SRF 37 g + 63 g of DDVP at 0.33 mL/kg treated cowpea grains and Group A4: rats fed on SRF 37 g + 63 g of DDVP at 0.43 mL/kg treated cowpea grains.

Rat representatives from each group of the treatments were sacrificed after 1, 2, 3, and 4 weeks (every week) after treatment (WAT), respectively.

Experimental animals` body weights were taken with a weighing scale on the first day of the experiment and were repeated weekly for four weeks (28 days) to evaluate the weight gain/loss. Also, the percentage (%) daily feed intake of rats was determined during the study trial using this formula:

$$\frac{\text{Weight of feed offered (g)} - \text{the weight of the leftover feed (g)}}{\text{Weight of feed offered (g)}} \times 100\%$$

Observations of DDVP treated cowpea toxicity were made on the physiological/ behavioural patterns of the rats on daily routine during feeding. These include shyness, signs of pesticide poisoning such as loss of appetite, weight gain/loss, tremors, convulsions, paralysis, and mortality as earlier reported by Fayinminnu *et al*. (2017).

2.4 Blood Hematology and Serum Biochemistry Analyses

On each termination in every week (1, 2, 3, and 4) of the experiment, all the surviving male rats were sacrificed through cervical dislocation, and organs (liver and kidney) of interest were harvested, weighed, and treated in 10% formalin for histopathological examination. A 2 mL of blood samples were taken for hematological analysis collected through the ocular orbit into Vacuum trainers (vacuum tainer) EDTAK3 tubes by heparinized capillary tubes. Another

blood sample of 3 mL was collected by heparinized capillary tubes into lithium heparinized tubes; a vacutainer bottle without anticoagulant for serum biochemical analysis. The above procedure was repeated weekly for the scarification of male Wistar rats fed on Dichlorvos treated ground cowpea grains, throughout the experiment.

Blood samples collected were determined for the blood hematology and serum biochemistry parameter indices using the following methods. Packed Cell Volume (PCV) determination was done using the microhematocrit procedure of Mitruka and Rawnsley (1997) and, Hemoglobin count (Hb) using the cyano methemoglobin method (Gibson and Harrison, 1945). Red Blood Cells (RBCs), White Blood Cells (WBCs), and platelets were determined using the improved Neubauer techniques (Schalm *et al*., 1975). Monocytes, lymphocytes, and neutrophils (leucocyte differentials) were determined by scanning Giemsa's-stained slides in the classic manner (Schalm *et al*., 1975). A drop of blood was placed at the end of a grease-free slide. Blood serum chemistry determination was also done to ascertain the enzymes: Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) by the method of Reitman and Frankel (1957).

2.5 Histopathological Examinations

The experimental animals` abdomen were dissected immediately after blood collection to harvest organs of interest, and the tissues (livers and kidneys) of each animal were weighed and treated in Bouin's fluid for 24 hours in universal bottles for histopathological examination. Later, the tissues were dehydrated at different stages of ethanol concentrations; 70, 95, and 100% ethanol, each for 2 hours. The tissues were moved to 100% xylene to clear the ethanol from them for one hour. The tissues were afterward placed in molten paraffin wax. The molten wax was applied three times for 2 hours each time in a wax oven of fairly high temperature before embedding. The tissues were positioned in molten paraffin wax within a cassette- mold from the wax dispenser and left to solidify.

The animal tissues were trimmed with a micro-tone and micro-tone blade in preparation for microscopic examination; histological procedures were followed in a stepwise protocol as follows: fixation, dehydration, clearing, infiltration, embedding, blocking, sectioning and staining. Tissue specimens were collected from rats` livers and kidneys and rapidly fixed in 10% neutral buffered formalin. After proper fixation, thin paraffin sections were routinely prepared and stained with hematoxylin and eosin (H & E) stain for microscopical examination according to Drury and Wallington (1980) through the light microscope (x400) and the readings were recorded. Hematological and serum biochemistry analyses and histopathological examinations were carried out by qualified Pathologists in the Clinical Pathology and Histopathology Laboratories of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

2.6 Statistical Analysis

Data collected were analyzed using analysis of variance (ANOVA) with Statistical Analysis System (SAS) software at a 5% ($p<0.05$) level of significance, while means were separated using the Duncan Multiple Range Test (DMRT). Results were presented as mean ± standard

error of the mean (SEM).

3. Results and Discussions

3.1 Behavioural / Morphological Observations of Dichlorvos (DDVP) Treated Cowpea Grains Fed to Male Wistar Rats

Signs observed in the physiological/ behavioral patterns of the male experimental rats include shyness, loss of appetite, reduced in feed intake, decreased body weight gain, and muscular tremor.

3.2 Toxic Effects of Different Concentrations of Dichlorvos (DDVP) Treated Cowpea Grains on Feed Intake of Male Wistar Rats

Results of feed intake as shown in Fig. 1 revealed increase in feed intake having 14.43 g for each treated animal group with 0.23, 0.33 and 0.43 mL/kg DDVP on cowpea grains including the control at one week after treatment (WAT). There was decrease in feed intake at 2 WAT with 0.23, 0.33, and 0.43 mL/kg of DDVP having 13.57, 12.00 and 12.71g, respectively, while the control had 14.71 g. Observations at 3 WAT revealed decrease in feed intake of cowpea treated DDVP at 0.23, 0.33, and 0.43 mL/kg to have 13.00, 12.29 and 12.57 g, respectively, while the control group increased in feed intake having 14.41 g. This followed the same trend at 4 WAT where all treated animal groups at 0.23, 0.33, and 0.43 mL/kg DDVP feed intake also reduced and gave 8.14, 6.86 and 6.29g, respectively in a progressively manner as doses increased. The control animals however, had increase in feed intake of 14.43g which showed throughout the study.

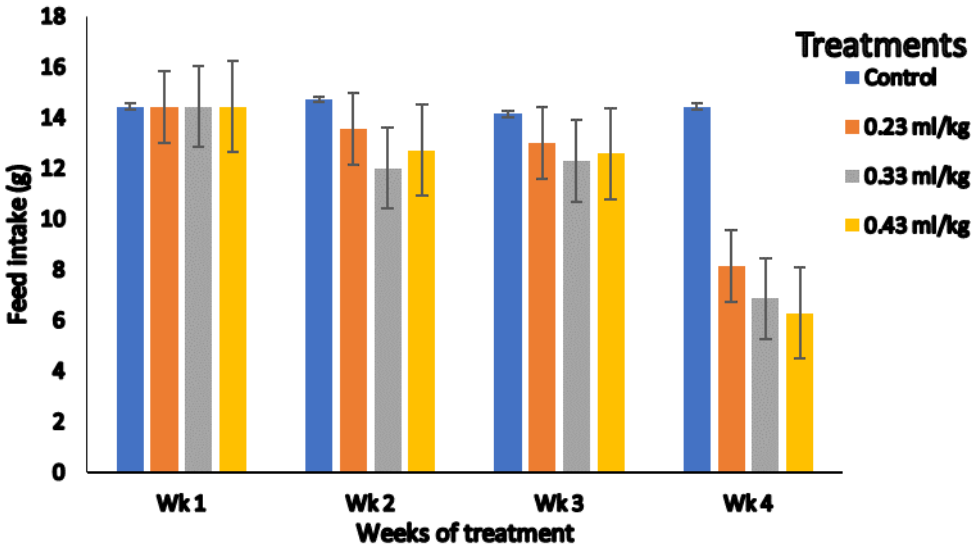

Figure 1. Toxic effects of different concentrations of Dichlorvos (DDVP) treated cowpea grains on feed intake (g) of male Wistar rats

3.3 Toxic Effects of Dichlorvos (DDVP) Treated Cowpea Grains Feed on Body Weights of Male Wistar Rats

The body weights ranged from 100.0-132.0 g of all treated and control experimental animals

at one week after treatment (WAT) (Fig. 2). Results showed rats fed to treated cowpea grains decreased in body weights 94.0, 82.0, and 72.0 g for DDVP at 0.23, 0.33, 0.43 mL/kg, respectively at 2 WAT. At 3 and 4 WAT, the same trends were observed: results of 77.0, 69.0 and 62.0 g at 0.23, 0.33 and 0.43mL/kg, respectively were at 3 WAT, also, 51.0, 43.0 and 35.0 g at 0.23, 0.33, and 0.43mL/kg, respectively at 4WAT. This revealed that an increase in DDVP dosages/levels in the feed resulted in a decrease in body weights progressively in treated animals. However, control animal groups had increased body weights of 152.0 and 158.0 g at 3 and 4 WAT, respectively, and throughout the study, as shown in Fig. 2.

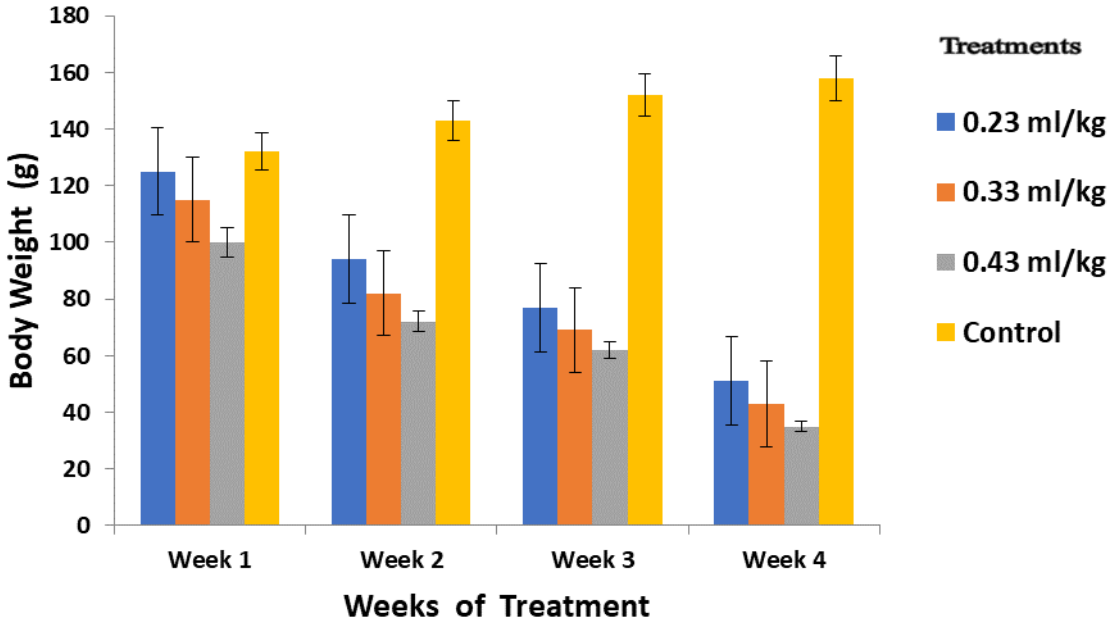

Figure 2. Toxic effects of different concentrations of Dichlorvos (DDVP) treated cowpea grains feed on body weights (g) of male Wistar rats

3.4. Toxic Effects of Different Concentrations of Dichlorvos (DDVP) Treated Cowpea Grains Feed on Hematological Parameters of Male Wistar Rats

The hematological parameters: packed cell volume (PCV), hemoglobin counts (Hb), white blood cell counts (WBC), red blood cell counts (RBC), Platelets (PLT), Lymphocytes (LMPH), Neutrophils (NUT) and Monocytes (MON) on male Wistar rats are presented in Tables 1a and 1b.

Results showed no significant differences ($p>0.05$) among the treatments on packed cell volume (PCV) parameter throughout the experiment (Table 1a).

Hemoglobin (Hb) results as presented in Table 1a revealed significant differences ($p<0.05$) throughout the study. Highest value (17.0 g/dl) of Hb at one week after treatment (WAT) was obtained from treated animals at 0.23 mL/kg DDVP, while all other treated animals revealed similar values (13.0; 14.3 and 14.5 g/dl) of Hb for 0.33 and 0.43mL/kg DDVP and control, respectively. At 2WAT, the control and treated animals at 0.33 mL/kg DDVP showed the

highest values (15.7 and 15.5 g/dl) of Hb, while 0.23mL/kg recorded the lowest value (12.7 g/dl). Results at 3WAT revealed 0.43 mL/kg DDVP having highest value (16.3g/dl), while the lowest value (11.7 g/dl) was from 0.23 mL/kg. At 4 WAT, 0.43mL/kg also revealed the highest Hb value (15.3 g/dl), while 0.23 mL/kg and 0.33 mL/kg of DDVP recorded the lowest values (10.7 and 10.3 g/dl), respectively.

White blood cells (WBC) showed significant differences ($p<0.05$) throughout the experiment (Table 1a). Control of treated animals had the highest WBC value (7950 $x10^3/\mu L$), while 0.33 mL/kg DDVP recorded the lowest value (5900$x10^3/\mu L$) one week after treatment (WAT). At 2WAT, DDVP at 0.23 mL/kg showed the highest value (5750 $x10^3/\mu L$) of WBC, while 0.43 mL/kg DDVP had the lowest value (4700$x10^3/\mu L$). Results at 3WAT revealed animals treated with 0.33 mL/kg having WBC maximum value (9600 $x10^3/\mu L$), closely followed by 0.43 mL/kg (8300$x10^3/\mu L$), while the minimum value (1100$x10^3/\mu L$) was recorded from 0.23 mL/kg DDVP treated cowpea. Control animals recorded the highest value (5700$x10^3/\mu L$) of WBC at 4WAT, while 0.33mL/kg DDVP treated animals had the lowest value (3750 $x10^3/\mu L$).

There were significant differences ($p<0.05$) in the results shown among the animal treated groups in Red blood cells (RBC) as presented in Table 1a. The highest value (8.66$x10^3/\mu L$) was obtained from 0.23 mL/kg DDVP, while 0.43 mL/kg and control had similar lowest values (7.27 and 7.31$x10^3/\mu L$), respectively at one week after treatment (WAT). At 2WAT, the control recorded the highest value (8.12 $x10^3/\mu L$), while 0.43 mL/kg DDVP had the lowest (5.34 $x10^3/\mu L$). The control animals had the highest value (7.71 $x10^3/\mu L$) of RBC, while 0.43 mL/kg DDVP treated animals gave the lowest value (5.58$x10^3/\mu L$) at 3WAT. Results at 4 WAT showed that animals treated with 0.43 mL/kg DDVP had the highest RBC (8.83$x10^3/\mu L$), while the 0.33 mL/kg DDVP treated group recorded the lowest value (6.12$x10^3/\mu L$) of DDVP treated cowpea.

Table 1a. Toxic effects of different concentrations of Dichlorvos (DDVP) treated cowpea grains feed on hematological parameters of male Wistar rats

Treatment	Week	PCV (%)	HB(g/dl)	WBC($x10^3/\mu L$)	RBC($x10^3/\mu L$)
0 mL/kg	1	44±0.58a	14.5±0.01b	7950±1.16b	7.31±0.01b
	2	48±0.58a	15.66±0.01a	5700±2.89f	8.12±0.23a
	3	45±1.73b	15±1.73ab	9500±5.78g	7.71±0.07a
	4	45±0.58a	13±0.58c	5700±5.78b	7.22±0.11bc
0.23mL/kg	1	39±1.16a	17±1.16a	6900±1.73d	8.66±0.59a
	2	38±1.16a	12.66±0.58c	5750±1.73b	6.35±0.38cd

	3	35±0.58b	11.66±0.01d	1100±5.78a	6.08±0.27bc
	4	32±1.16a	10.67±0.01d	5000±5.78d	7.06±0.32c
0.33mL/kg	1	40±1.45a	13±0.58b	5900±0.58g	6.34±0.98b
	2	46±0.58a	15.51±0.19a	4700±2.89f	7.51±0.73ab
	3	43±1.16b	14.33±b0.58c	9600±2.89c	6.88±0.43ab
	4	31±0.58a	10.33±0.01d	3750±5.78f	6.12±0.89c
0.43mL/kg	1	43±0.58a	14.3±0.58b	7000±1.16c	7.27±0.28b
	2	35±1.16a	14.±0.01c	5050±2.31e	5.34±0.78d
	3	49±1.16b	16.33±0.58a	8300±2.31d	5.58±0.54c
	4	46±0.58a	15.33±0.02a	5150±1.73c	8.83±0.78a

Means followed by the same letters within a column are not significant at ($p<0.05$) level of probability from each other using the Duncan Multiple Range Test

PCV= packed Cell Volume, HB= Hemoglobin, WBC= White Blood Cell counts, RBC=Red Blood Cell counts

Significant differences ($p<0.05$) in results were revealed on the platelets of the animals fed on DDVP treated cowpea in this study (Table 1b). The highest value (538000 $x10^3$/UL) of platelets was obtained from the control animals, while treatment 0.33 mL/kg DDVP recorded the lowest value (244000 $x10^3$/UL) one week after treatment (WAT). Experimental animals fed to 0.23 mL/kg DDVP treated cowpea had the highest value of platelets (286000 $x10^3$/UL), while the control and 0.33 mL/kg treated animals had the same lowest value (189000$x10^3$/UL) of platelets, respectively at 2WAT. Results at 3WAT showed the highest value (254000 $x10^3$/UL) of platelets from 0.23 mL/kg DDVP, while the lowest value (130000 $x10^3$/UL) was from the control animals. The 0.43 mL/kg DDVP treatment produced the highest value (368000 $x10^3$/UL) of platelets at 4WAT, while the lowest value (240000$x10^3$/UL) was obtained from 0.23 mL/kg DDVP.

Results on Lymphocytes showed significant differences ($p<0.05$) in the experimental animals (Table 1b). Treatment 0.43 mL/kg DDVP recorded the highest lymphocytes (75 $x10^3$/UL), while the lowest value (61 $x10^3$/UL) was from 0.23 mL/kg DDVP one week after treatment (WAT). At 2WAT, treatments 0.23 and 0.33 mL/kg and control had similar highest values (63, 65, and 65 $x10^3$/UL) of lymphocytes, respectively with no significant differences ($p>0.05$). While the lowest value (57 $x10^3$/UL) was from 0.43 mL/kg DDVP with a significant difference

($p<0.05$) from other treatments. The highest value (73 $x10^3$/UL) of lymphocytes was obtained from the control animals at 3WAT, while treatment 0.43 mL/kg DDVP recorded the lowest value (58 $x10^3$/UL). Results showed animals group from 0.33 mL/kg treatment having the highest value of lymphocytes (67 $x10^3$/UL), while the control and 0.23 mL/kg DDVP treated animals gave similar lowest values (55 and 56 $x10^3$/UL), respectively at 4 WAT.

Significant differences ($p<0.05$) were observed in Neutrophils in the treated animal groups in this study. The control animals recorded the highest value of neutrophils ($35x10^3$), while the lowest value (21 $x10^3$) was observed from treatment at 0.43 mL/kg DDVP one week after treatment (WAT). Results at 2 WAT revealed the highest value of neutrophils (38 $x10^3$) from the animals fed to treated cowpea grains with 0.43 mL/kg, while the control, 0.23 and 0.33 mL/kg DDVP treatments recorded the same lowest values (32, 32, and 32 $x10^3$) of neutrophils, respectively. The highest value (37 $x10^3$) of neutrophils was obtained from 0.43 mL/kg DDVP, while the lowest value (23 $x10^3$) of neutrophils was recorded from the control animals at 3WAT. Results at 4WAT revealed the highest value of neutrophils (40 $x10^3$) from the control animals, while the lowest value ($29x10^3$) was observed from 0.33 mL/kg DDVP.

There were no significant differences ($p>0.05$) shown in the results on Monocytes and Bilirubin in the experimental animals throughout the study.

Table 1b. Toxic effects of different concentrations of Dichlorvos (DDVP) treated cowpea grains feed on hematological parameters of male Wistar rats

Treatment (mL/kg)	Week	PLT	LYM	NEUT	MN	Bilirubin	GLOB
0 mL/kg	1	538000±1.73g	61±1.73a	35±1.16e	3.0±0.58b	0.2±0.06a	5.35±0.01f
	2	189000±5.77b	65±0.58d	32±1.16a	1.0±0.00a	0.3±0.06a	2.75±0.01a
	3	130000±0.58a	73±0.58d	23±0.58b	3.0±0.58ab	0.30±0.06ab	24.49±0.01e
	4	340000±3.46e	55±1.16a	40±0.58de	2.0±0.58ab	0.2±0.06ab	13.45±0.01e
0.23mL/kg	1	305000±0.58d	72±1.16cd	24±0.58b	1.0±0.00a	3.0±1.16b	2.58±0.01d
	2	286000±2.31e	63±1.73cd	32±1.16a	2.0±0.58ab	0.2±0.06a	5.13±0.01c
	3	254000±3.46e	69±0.58c	35±1.16c	3.0±0.58ab	0.3±0.06ab	10.63±0.01b
	4	240000±5.77b	56±1.16a	38±0.58d	2.0±0.58ab	0.3±0.06b	16.61±0.01f
0.33mL/kg	1	244000±1.16c	66±0.58b	28±0.58c	2.0±0.58ab	2.0±0.00ab	2.13±0.01c
	2	189000±2.89b	65±0.58d	32±0.58a	1.0±0.00a	0.3±0.12a	7.66±0.01g
	3	203000±5.77c	68±0.58c	25±1.16b	5.0±0.58b	0.2±0.06ab	12.52±0.01c

	4	274000±5.77c	67±1.16c	29±0.58b	1.0±0.00a	0.2±0.06ab	10.43±0.01c
0.43mL/kg	1	362000±1.73f	75±0.58d	21±0.58a	2.0±0.58ab	3.0±1.16b	1.82±0.01b
	2	213000±3.46c	57±1.16b	38±0.58c	2.0±0.58ab	7.0±1.16b	5.87±0.01f
	3	140000±2.89b	58±1.16b	37±1.16cd	5.0±1.16b	0.1±0.00a	8.99±0.58a
	4	368000±2.89f	63±0.58b	33±0.58c	2.0±0.58ab	0.2±0.06ab	10.6±0.12d

Means followed by the same letters within a column are not significant at ($p<0.05$) level of probability from each other using the Duncan Multiple Range Test

Platelets (PLT), Lymphocytes (LMPH), Neutrophils (NEUT), Monocytes (MN), and Globulin (GLOB)

3.5 Toxic Effects of Different Concentrations of Dichlorvos (DDVP) Treated Cowpea Grains Feed on Serum Biochemical Parameters of Male Wistar Rats

Results of serum biochemical parameters from different concentrations of Dichlorvos: Total Protein (TP), Alanine Amino Transferase (ALT), Alkaline phosphate (ALP), and Aspartate aminotransferase (AST) of experimental Wistar rats are presented in Table 2.

Significant differences ($p<0.05$) were observed in the Total protein (TP) of the treated animal groups fed on cowpea grains with DDVP at 0.23,0.33 and 0.43 mL/kg in the study (Table 2). At one week after treatment (WAT), the highest TP value (8.40 g/dL) was observed from the control, while the lowest value (5.13 g/dL) for all other treated animals was from 0.33 mL/kg DDVP. The group of animals treated with 0.33 mL/kg DDVP recorded the highest TP value (12.14 g/dL) at 2WAT, while the lowest value (6.64 g/dL) was obtained from the control. Results at 3 WAT showed highest TP value (26.29 g/dL) for control, while 0.43 mL/kg DDVP treated animals had the lowest value (12.05 g/dL). At 4 WAT, 0.23 mL/kg DDVP produced the highest TP value (20.24 g/dL), while treatments of 0.33 and 0.43 mL/kg recorded lowest values 13.00 and 13.65 g/dL, respectively.

Alanine Amino Transferase (ALT) revealed significant differences ($p<0.05$) throughout the study. The highest ALT value (8.32 U/L) was observed from 0.43 mL/kg DDVP at one week after treatment (WAT), while lowest value (1.76 U/L) was obtained from cowpea treated with 0.33 mL/kg. At 2WAT, 0.33 mL/kg recorded the highest ALT value (32.81 U/L), while 0.23mL/kg treatment produced the lowest value (15.81 U/L). Experimental animals at 3 WAT, recorded highest ALT value (32.81 U/L) from 0.33 mL/kg DDVP, while lowest value (7.01 U/L) was observed from the control. At 4 WAT, the highest value (24.00 U/L) of ALT was observed from 0.33mL/kg, while 0.43mL/kg DDVP recorded the lowest value (5.76 U/L) (Table 2).

Alkaline Phosphate (ALP) revealed significant differences ($p<0.05$) in the study (Table 2). Treated experimental animals at one week after treatment (WAT) showed highest value (125.00 U/L) of ALP at 0.23mL/kg DDVP with a significant difference from other treatments,

while other treatments revealed no significant differences ($p>0.05$). However, the control group animals recorded the lowest value (112.00 U/L). At 2 WAT highest value (114.00 U/L) was recorded from animals fed with cowpea treated with 0.23mL/kg DDVP, while treatments from 0.33 and 0.43 mL/kg had similar lowest values (106.00 and 108.00 U/L), respectively with no significant differences ($p>0.05$). Results at 3 WAT revealed animals treated with 0.33 and 0.43 mL/kg DDVP having the highest ALP values (124.00 and 125.00 U/L), respectively with no significant differences ($p>0.05$), while 0.23 mL/kg DDVP recorded the lowest value (104.00 U/L). The highest value of ALP (114.00 U/L) was obtained from the animals fed on cowpea treated with 0.33 mL/kg DDVP, while the similar lowest values (106.00 and 108.00 U/L) with no significant differences were recorded from the control and 0.43 mL/kg DDVP, respectively at 4WAT.

Aspartate aminotransferase (AST) revealed significant differences ($p<0.05$) in the experimental study as presented in Table 2. The highest value of AST (104.71 U/L) was observed from animals fed on cowpea treated with 0.23 mL/kg DDVP, while 0.33 mL/kg DDVP recorded the lowest value (52.00 U/L) at one week after treatment (WAT). At 2 WAT, it was observed that the highest AST value (147.64 U/L) was obtained from 0.33 mL/kg, while 0.23 mL/kg recorded the lowest value (113.61 U/L). Results on experimental animals at 3WAT showed the highest value of AST (25.31 U/L) from 0.23 mL/kg, while 0.33 mL/kg recorded the lowest value (20.70 U/L). The maximum value of AST (113.61 U/L) was obtained from animals fed on cowpea treated with 0.23 mL/kg, while the minimum value of AST (39.51 U/L) was from 0.43 mL DDVP at 4 WAT.

Table 2. Toxic effects of different concentrations of Dichlorvos (DDVP) treated cowpea grains feed on serum biochemical parameters of male Wistar rats

Treatment	Week	TP(g/dL)	ALT(U/L)	ALP(U/L)	AST(U/L)
0 mL/kg	1	8.4±0.02a	4.16±0.01d	112±1.16d	93.71±0.01f
	2	6.64±0.01ef	17.3±0.06e	111±0.58a	127.60±7.74b
	3	26.29±0.58a	7.01±0.58b	107±1.16c	23.48±0.57c
	4	17.49±0.01b	15.98±0.01c	106±0.58c	41.00±18.88e
0.23mL/kg	1	5.22±0.01c	7.2±0.06b	125±1.16a	104.71±17.94c
	2	7.33±0.01de	15.81±0.01f	114±1.16bc	113.61±11.35e
	3	14.93±0.01d	8.32±0.01f	104±1.16d	25.31±1.58d
	4	20.24±0.01a	15.98±0.58c	103±1.16d	113.61±12.90a
0.33mL/kg	1	5.13±0.01c	1.76±0.06e	114±0.58cd	52±15.18g
	2	12.14±0.01a	32.81±0.01a	106±1.16d	147.64±7.87b
	3	15.57±0.01d	10.56±0.01de	124±0.58a	20.7±0.92f
	4	13.56±0.01c	24±0.58b	114±0.58a	76±12.85c
0.43mL/kg	1	5.18±0.01c	8.32±0.01a	114±0.58cd	99.99±15.54e
	2	11.31±0.58b	21.33±0.01d	108±1.16d	124.08±30.24d
	3	12.05±0.01e	13.43±0.01e	125±0.58a	23±6.18e
	4	13.65±0.01c	5.76±0.01f	108±1.16bc	39.51±11.12f

TP= Total Protein, ALT= Alanine aminotransferase, ALP= Alkaline Phosphate, AST= Aspartate aminotransferase

Means followed by the same letters within a column are not significant at ($p<0.05$) level of probability from each other using the DMRT.

3.6 Toxic Effects of Different Concentrations of Dichlorvos (DDVP) Treated Cowpea Grains Feed on Histopathology of Male Wistar Rats

Results of histopathological examinations were carried out on the experimental rats over a period of four weeks. The liver and kidney organs of the control animal groups showed no visible lesions throughout the experiment (four weeks) as shown on Plates 1 and 2. They showed healthy hepatocytes arranged in cords.

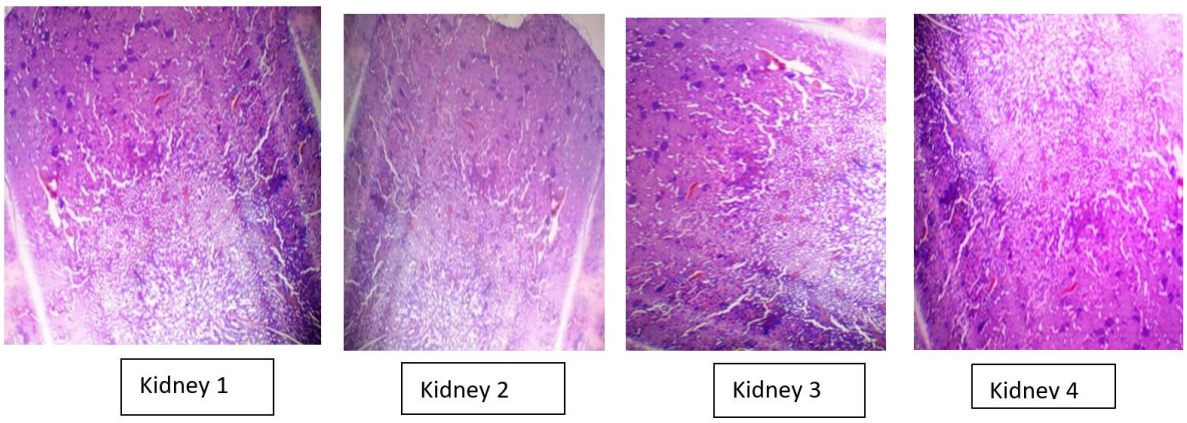

Plate1. Photomicrographs Sections of Kidneys of Control Male Rats Exposed to Standard Ration Feed containing no pesticide (DDVP)

Kidney 1 = at one week after treatment (WAT) (no inflammation), Kidney 2 = at 2WAT (no visible lesion), Kidney 3 = at 3 WAT (no visible lesion) Kidney 4 = at 4 WAT (no visible lesion). H& E Magnification x400.

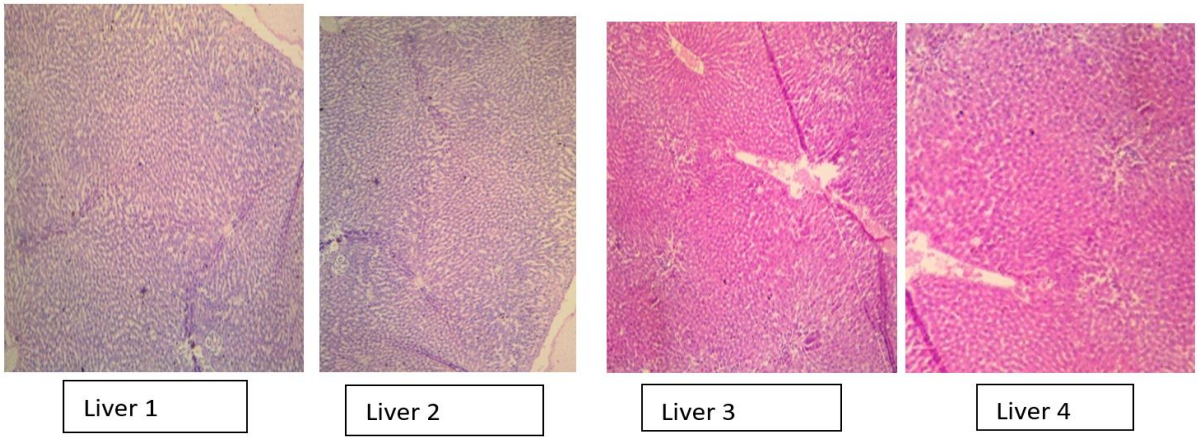

Plate 2. Photomicrographs Sections of Livers of Control Male Rats Exposed to Standard Ration Feed containing no pesticide (DDVP)

Liver 1 = at one week after treatment (WAT) (liver showing healthy hepatocytes arranged in cords), Liver 2 = at 2 WAT (no visible lesion), Liver 3 = at 3WAT (no visible lesion) and Liver 4 = at 4WAT (no visible lesion). H& E Magnification x400.

There were no distinct inflammatory/ necrotic changes in the kidney of some animals

exposed to 0.23 mL/kg of DDVP cowpea grains treated, while some animals showed moderate congestion (Plate 3). Results in Plate 4 showed moderate diffuse, vacuolar degeneration of hepatocytes, and the presence of clear circular space in some liver organs, while others showed moderate multifocal. Severe cell infiltration of interstitions and parenchyma of the liver, while others revealed mild and locally extensive.

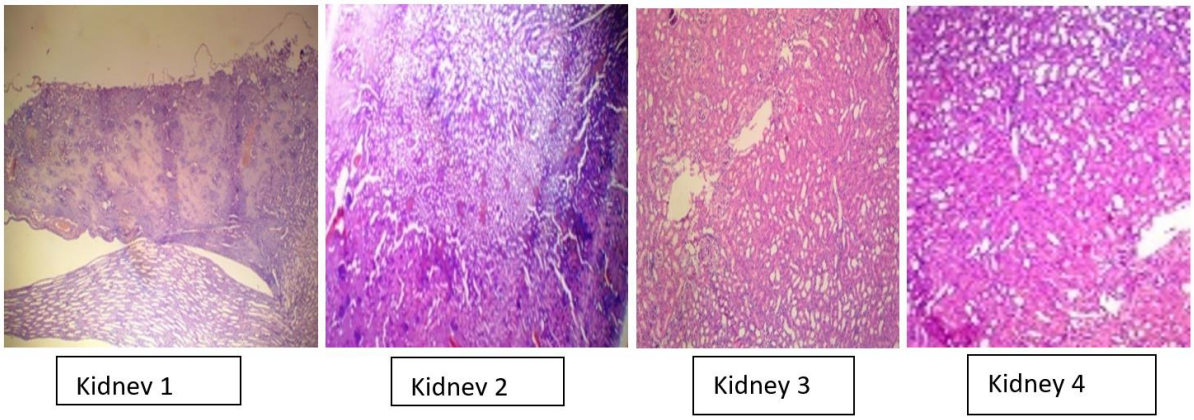

Plate 3. Photomicrographs Sections of Kidneys of Male Rats Exposed to Standard Ration Feed mixed with 0.23 mL/kg of dichlorvos (DDVP) treated cowpea grains

Kidney 1 = at one week after treatment (WAT) (moderate capillary congestion in the cortex), Kidney 2 = at 2WAT (no distinct inflammatory/ necrotic changes in kidney), Kidney 3 = at 3WAT (mild lesion) and Kidney 4 = at 4WAT (no inflammation). H& E Magnification x400.

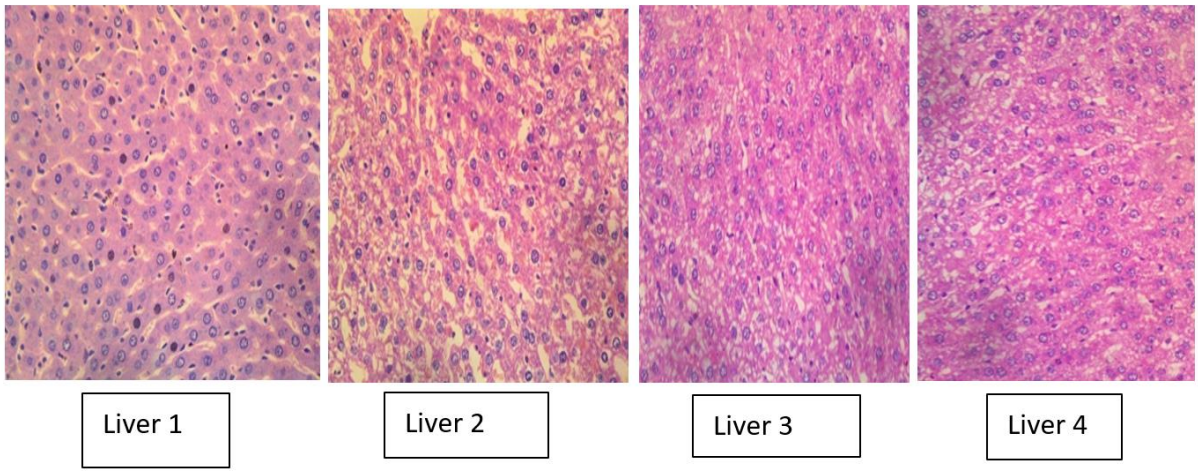

Plate 4. Photomicrographs Section of Livers of Rats Exposed to Standard Ration Feed mixed with 0.23 mL/kg of dichlorvos (DDVP) treated cowpea grains

Liver 1 = at one week after treatment (WAT) (single cell necrosis of hepatocyte), Liver 2 = at 2 WAT (moderate diffuse, vacuolar degeneration of hepatocytes, presence of clear, circular spaces in the liver cells), Liver 3 = at 3WAT (moderate multifocal) and Liver 4 = at 4 WAT (moderate multifocal). H& E Magnification x400.

Photomicrographs in Plate 5 showed normal kidneys of experimental animals treated with 0.33 mL/kg of DDVP, while others showed no visible lesion of the kidney. There was no inflammation of the kidney but mild multifocal and severe diffuse congestion. Vacuolar degeneration was observed in the liver of experimental animals treated with 0.33 mL/kg of DDVP. There were also distinct cysts fibrotic wall, while others showed moderate diffuse (Plate 6).

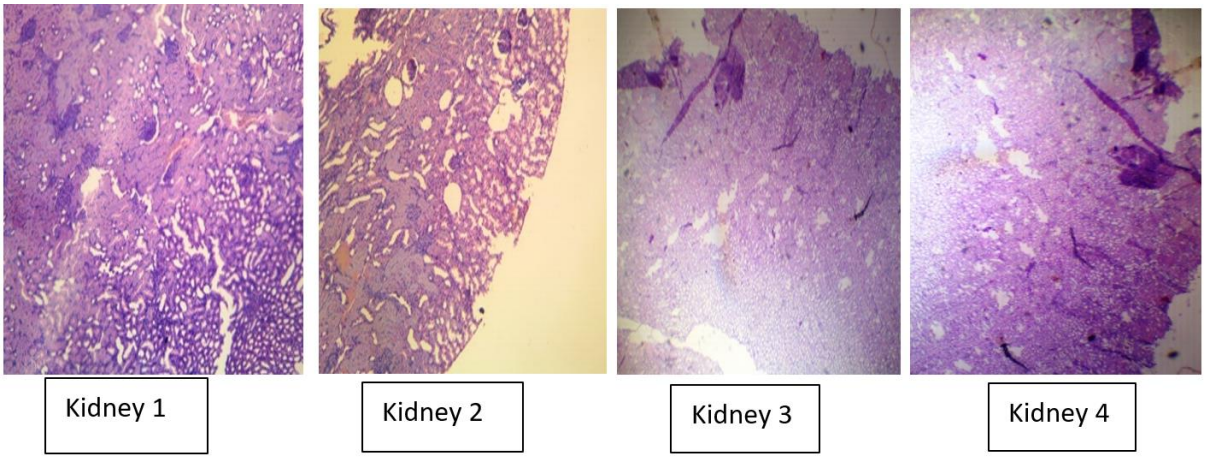

Plate 5. Photomicrographs Sections of Kidneys of Male Rats Exposed to Standard Ration Feed mixed with 0.33 mL/kg of dichlorvos (DDVP) treated cowpea grains

Kidney 1 = at one week after treatment (WAT) (severe cell infiltration of interstitions and parenchyma with inflammatory cell), Kidney 2 = at 2WAT (no structural changes present), Kidney 3 = at 3WAT (mild localized) and Kidney 4 = at 4WAT (locally extensive). H& E Magnification x400.

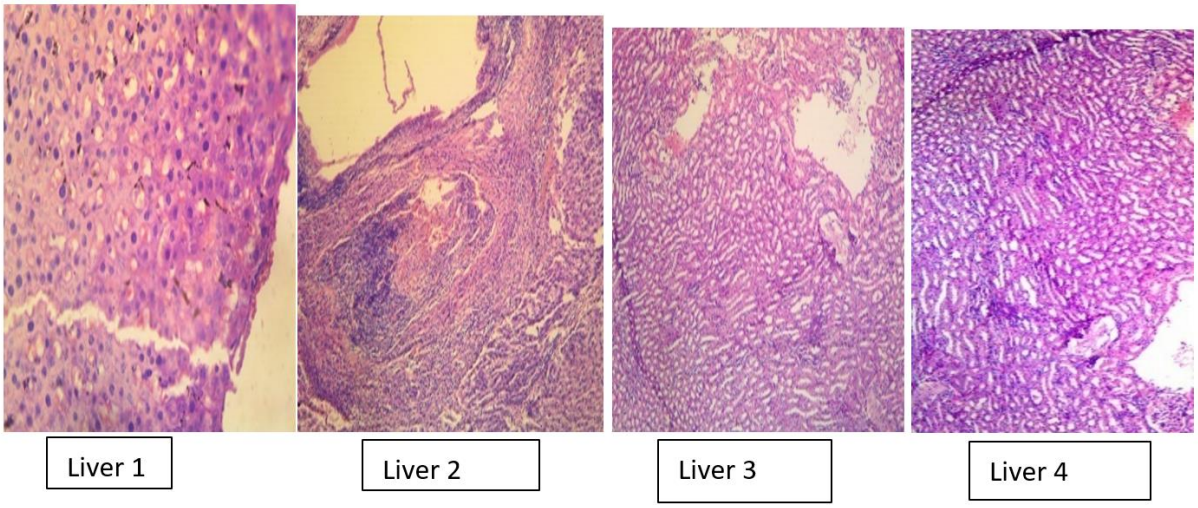

Plate 6. Photomicrographs Sections of Livers of Rats Exposed to Standard Ration Feed mixed with 0.33 mL/kg of dichlorvos (DDVP) treated cowpea grains

Liver 1 = at one week after treatment (WAT) (hepatocytes with vacuolar degeneration), Liver 2 = at 2WAT (moderate vascular congestion and moderate increase in cellular proliferation).

There is a distinct cysts fibrotic wall with evidence of granuloma formation in the liver), Liver 3 = at 3WAT (mild multifocal) and Liver 4 = at 4WAT (moderate diffuse). H& E Magnification x400.

Histopathology examinations revealed normal, no inflammation, and visible lesion in the kidneys of treated animals with DDVP at 0.43 mL/kg cowpea grains (Plate 7). In the liver, no inflammation was noticed in treated animals, while the central portion of the liver sections was pale staining, no distinct pattern of arrangement in cords, and mild multifocal, severe diffuses were revealed in other treated animals as shown in Plate 8.

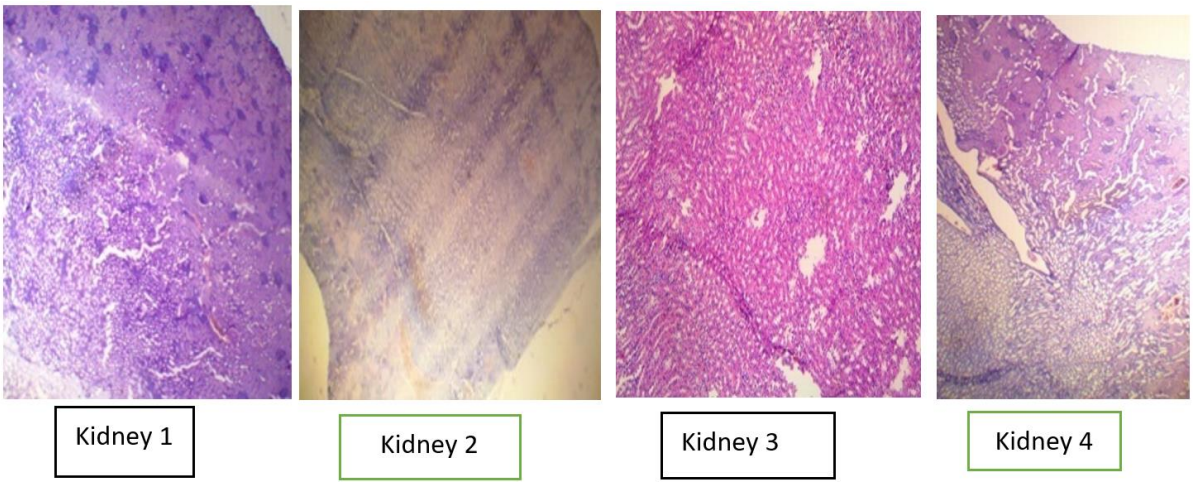

Plate 7. Photomicrographs Sections of kidneys of Rats Exposed to Standard Ration Feed mixed with 0.43 mL/kg of dichlorvos (DDVP) treated cowpea grains

Kidney 1 = at one week after treatment (WAT) (no inflammation), Kidney 2 = at 2WAT (normal), Kidney 3 = at 3WAT (no visible lesion) and Kidney 4 = at 4WAT (no visible lesion). H& E Magnification x400

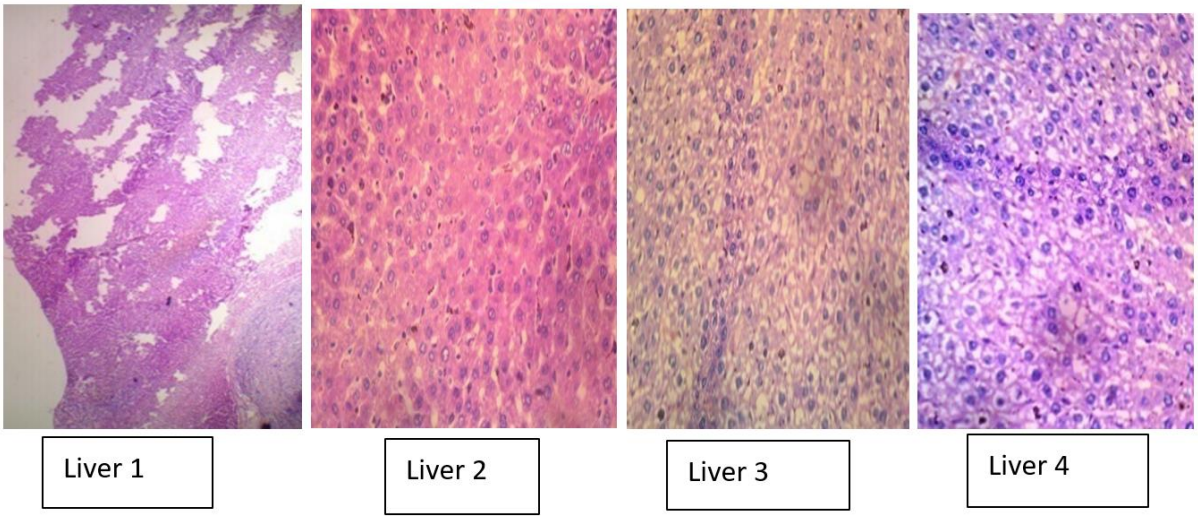

Plate 8. Photomicrographs of a Section of Livers of l Rats Exposed to Standard Ration Feed mixed with 0.43 mL/kg of dichlorvos (DDVP) treated cowpea grains

Liver 1 = at one week after treatment (WAT) (no inflammation), Liver 2 = at 2WAT (central portion of the liver section is pale staining and distinct pattern of arrangement in cords is absent), Liver 3 = at 3WAT (mild multifocal) and Liver 4 = at 4WAT (severe diffuse). H& E Magnification x400

4. Discussion

This study revealed the extent of Dichlorvos **(**DDVP; Snipper) at different concentrations` toxicity that people especially consumers could be exposed to when used in treating/ preserving cowpea grains in storage.

4.1 Behavioural/ Morphological Observations

Behavioral poisoning signs detected in this study on the male Wistar rats fed on treated cowpea grains at 0.23, 0.33 and 0.43 mL/kg Dichlorvos **(**DDVP) were shyness, loss of appetite, decreased feed intake, reduced body weight gain, and muscular tremor. This finding is in agreement with the work of Ajiboso *et al*. (2012) and Olaoye *et al*. (2015) who reported similar observations of dichlorvos toxicity in rats.

4.2 Feed Intake and Body Weight

The DDVP caused a decrease in body weight of rats exposed to different concentrations of 0.23, 0.33, and 0.43 mL/kg. Results from this study clearly indicated a decrease in the average body weight gain in the Dichlorvos treated stored cowpea grains on male Wistar rats. Reduction revealed general toxicity of the insecticide (Dichlorvos) which agrees with the work of Kemabonta and Akinhanmi, (2013) and Olaoye *et al*. (2015). The toxic effects did not show in the first week but were reflected from the second, third, and fourth weeks of treatment (WAT)/ administration with respect to dosage administered at 0.23, 0.33, and 0.43ml/kg, this decrease may occur as a consequence of the reduction in feed intake. A significant increase in body weight gain in the control and treated experimental animal groups at early treatment (1 WAT) may be attributed to an increase in feed intake. A decrease in body weights of the DDVP cowpea grains treated and fed to the animals at 2- 4 WAT and weekly fell in a reduction in feed intake. This finding also conforms with the reports of earlier researchers Ajiboso *et al*. (2012), El-Hilaly *et al*. (2004), Hamed *et al*. (2012) and Shobhit *et al*. (2012) that changes in body weights and feed intake serve as indicators of toxic effects of chemicals. Kalender *et al.* (2007) and Uzunhisarcikli *et al.* (2007) also concluded that organophosphate insecticides caused a reduction in body weights in experimental animals. Severe toxicity was observed at 3 and 4 WAT of continuous feeding of the animal groups to DDVP treated feed.

4.3 Hematological Parameters

Hematology (blood) analysis is very vital in toxicology research and environmental monitoring because it serves as a pointer to physiological or pathological changes under investigation. Various changes in the blood parameters occur in warm-blooded animals due to injuries to some tissues or organs which could have led to their dysfunctions (Ajiboso *et al*., 2012). The study thus revealed that DDVP treated cowpea grains fed to male rats showed abnormalities and reduction in the hematological parameters.

In DDVP treated animals, packed cell volume (PCV) showed marked reductions throughout the four weeks of the experiment but was not statistically different. On the other hand, hemoglobin (Hb) at 0.23 mL/kg and 0.43 mL/kg at 2 WAT were compared to the control. Red Blood Cells (RBC) reduction was obvious for all the treated animals throughout the study. These observations could be due to the toxic effects of the synthetic pesticide (dichlorvos) dosages, especially with continuous feeding (nutrition) on the treated stored cowpea grains; thereby having deleterious effects on the blood parameters. These findings could also be related to the report of Saliu *et al.* (2012). A decrease in hemoglobin levels observed in the treated animals could result in anemia due to its low count (Kemabonta and Akinhanmi, 2013). This is because hemoglobin measures the volume of oxygen-carrying proteins in the blood. Similarly, the destruction of matured red blood cells could lead to low Hb counts accompanied by a fall in the RBC and PCV.

White blood cell (WBC) differentials are indicators of the ability of an organism to eliminate infection. An increase in the number of circulating leukocytes is rarely due to an increase in all the types of leukocytes (Sahng *et al.*, 2005). Decreased WBC and Lymphocytes recorded in the treated animal groups could result to Leukopenia and Lymphocytopenia, respectively which may be attributed to destruction of WBC and lymphocytes as stated by Gu (1998) and Ajiboso *et al*. (2012) due to continuous feeding exposure to DDVP.

Exhibition of significant reduction in the RBC showed that anemia could likewise occur to the treated male Wistar rats (Kemabonta and Akinhanmi, 2013). The RBC decrease/reduction level in the animals fed on Dichlorvos treated stored cowpea grains could be a useful biomarker of an Organophosphate exposure and poisoning in humans. This corroborates with the report from the experiment of Kemabonta and Akinhanmi (2013) that biological (life) marker used as a monitor to blood serum (fluid) cholinesterase and cholinesterase enzymes such as RBC can have access to the Organophosphate insecticide actual exposure.

Presence of Monocytes in the experimental treated animal groups in this study could suggest alteration in lipid metabolism, especially in the high concentration of 0.43 mL/kg DDVP. Although this parameter revealed no significant difference in the experiment which conforms with the report of Olaoye *et al*. (2015).

4.4 Serum Biochemical Parameters

Serum biochemistry parameters revealed reduction of serum levels of total protein for cowpea grains DDVP treated animals at 0.23, 0.33 and 0.43 mL/kg at early stage to treatments, this correlates with the findings of Das and Mukherjee (2000) which indicated that toxicants may cause stress-mediated utilization of protein to cope with the harmful condition so imposed. The findings in this study revealed increase in ALP and AST, Organophosphate insecticides may cause increase in the enzymatic activities of ALP, AST and ALT activities (Altuntas and Delibas, 2002; Khan *et al.*, 2005; Kalender *et al.*, 2005; Mahajan, 1997). If the liver is damaged, the hepatocytic cell membrane becomes more permeable and some of the liver enzymes could leak out into the bloodstream. The significant rise in ALP observed in the experimental group suggests the presence of cholestatic injury. Actual high levels of ALT and AST could suggest severe liver damage such as hepatitis, liver

injuries from lack of blood flow, or from toxins (Ajiboso *et al.*, 2012). The ALT is a liver cytosol enzyme more specific to the liver so a rise only occurs with liver disease (Poli *et al.*, 1987). A significant rise observed in DDVP treated animals signified some degree of cytotoxicity. The toxic effects were more pronounced in the animal groups that were exposed to higher (0.43 mL/kg) DDVP concentration indicating dose-dependent activity in this study.

4.5 Histology Examinations of Liver and Kidney Organs

Histopathological organs (liver and kidney) of the animals that fed on the different doses of 0.23, 0.33, and 0.43 mL/kg Dichlorvos treated stored cowpea grains showed various pathological changes/variations. However, the liver and kidney organs of the control animal groups displayed no visible lesions throughout the study, the organs showed healthy hepatocytes arranged in cords.

The kidney sections of the DDVP experimental animals revealed interstitial nephritis, a kidney disorder in which spaces between the kidney tubules become inflamed, while the liver sections showed serious chronic inflammation. These observations in the results with necrosis of liver hepatocytes, inflammation, highly vacuolated hepatic cells, inflammatory cell infiltrate in the kidney, and thickening of blood vessels in the kidney were in accordance with the reports of Matthew (2010); Kemabonta and Akinhanmi (2013).

The induced histological alterations in the liver of male Wistar rats fed on treated stored cowpea grains of DDVP concentrations might be an indication of injured hepatocytes due to their toxicities. Also, the inability of the liver to deal with the accumulated toxic residues resulting from its metabolic products (Abdelhalim and Jarrar, 2012). The appearance of hepatocyte degeneration and destruction leading to necrosis may be due to the production of reactive oxygen species caused by dichlorvos (Sharma and Singh, 2011). This in turn might have induced oxidative stress in the hepatocytes initiating them to undergo necrosis.

5. Conclusion

(1). The four weeks (28 days) sub-chronic toxicity study of continuous feeding to cowpea grains treated with 0.23, 0.33, and 0.43 mL/kg Dichlorvos (DDVP, Snipper) revealed dose-related toxic effects on the assessed parameters.

(2). It was observed in this study that cowpea grains treated with Dichlorvos can lead to a decrease in feed intake, a decrease in body weight gain, and muscular tremor.

(3). Reduction in Red Blood Cells, Hemoglobin, and Pack Cell Volume revealed abnormalities that could lead to anemia and different histopathological lesions in the liver and kidney organs of the male Wistar rats.

(4). These abnormalities from the above could lead to health implications such as impairment of vital organs leading to malfunctioning, causing life threatening and affect the well-being of humans/populace that consume cowpea grains treated with Dichlorvos.

Recommendations

(1). Spraying this insecticide (Dichlorvos) directly on cowpea grains should be stopped.

(2). Dichlorvos should be applied in corners of the room away from foodstuff

(3). Avoid usage of dichlorvos in kitchens, stores, and in contact with food items

(4). Frequent monitoring of the National Agency for Food and Drug Administration and Control (NAFDAC) Agency, Nigeria, great awareness and enlightenment to cowpea grains sellers should be well encouraged.

(5). Also, dissemination of the outcome of research likened to this insecticide to the cowpea grains farmers and retailers (target audience) is very important for food quality and safety.

Acknowledgments

The authors appreciate the Cowpea Sellers Association at Bodija International Foodstuff Market, Bodija, Ibadan, Oyo State, Nigeria for their information and assistance. Also, the Toxicology Research Laboratory of the Department of Crop Protection and Environmental Biology and Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria for technical assistance and providing the housing units for the experimental animals during this study.

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