

Original Research Article

Toxicity Assessment of Sub Lethal Doses of Chlorpyrifos on the Liver and Kidney Organs of Male Wistar Rats

ABSTRACT

Chlorpyrifos, an organophosphate is an important neurotoxic and tissue damage agent. It is one of the most heavily used pesticides in domestic and agricultural applications globally. Repeated doses of chlorpyrifos have been able to cause significant disturbances on the biochemical and physiological functions of the blood, liver and kidney and toxicities on living organisms exposed to this insecticide. The toxicities of sub lethal oral administration of chlorpyrifos daily for 28 days were assessed using a completely randomized design. Twenty five albino Wistar rats weighing between 150-200g divided into five groups containing five rats each were used in the experimental animal house from the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan. Chlorpyrifos, was orally administered to male rats at the doses of 0 (control), 18.9, 25.9, 32.2 and 39.2mg/kg for 28 consecutive days (four weeks), between the months of May and June, 2014. At the end of the experimental period, the toxicities of chlorpyrifos were assessed in rats using haematology, serum liver enzymes and histopathological assays. Results revealed significant reduction in body weights compared to control. The packed cell volume (PCV), hemoglobin (Hb) and lymphocytes (Lymp) also showed significant reduction at 39.2, 25.9 and 32.2mg/kg (39.33, 45.40 and 44.80%), respectively compared to control (49.67%). Insecticide administration to rats resulted in significant elevation of serum transaminases (AST and ALT) and alkaline phosphatase (ALP) from 32.2, 39.2 and 25.9mg/kg dosages, (ranged from 294.94-542u/L for AST and from 96.25-130.77u/L for ALT), respectively. The total protein resulted in significant reduction in dosages of 18.9 (81.63g/dL) and 25.9mg/kg (75.78g/dL), this trend was also observed in albumin and globulin from 25.9(32.79g/dL) and 39.2mg/kg (42.06g/dL) doses, respectively compared to control. Histopathological studies of livers and kidneys revealed dose dependent increase (0 (control), 18.9, 25.9, 32.2 and 39.2mg/kg) of chlorpyrifos in all experimental animals. The livers showed moderate vacuolar change of hepatocytes, having a finely reticulated cytoplasmic and congestion of central veins. The sections of the kidneys showed mild focal sloughing off of tubular epithelium of renal cortex, fluid in tubular lumen, tubules appearing dilated and cystic (nephrosis) and proteinaceous fluid in Bowman's capsule and compressing the glomerulus tufts (Esinophilic). Different concentrations of chlorpyrifos including the lowest tested dose produced marked alterations in the exposed animals in this study and thereby affecting the overall performance in terms of health and wellbeing. Thus, these could cause similar health and environmental risks to humans even at lowest dose.

Keywords: *Chlorpyrifos, Toxicity, Biochemical, Haematology, Histopathology*

1. INTRODUCTION

Chemical pesticides are to improve the quantity and quality of food to feed the ever increasing world human and animal population and promote public health [1]. However, they become one

of the most widely available environmental contaminants that are deliberately released to the environment. Pesticides fall into numerous chemical classes, which have widely differing biological activities and thus differing potential to produce toxic effects in living organisms, including humans [2] and their poisonings in developing countries had been occurring as a result of their misuse. Unintended exposure to toxic pesticides can occur during their manufacturing, formulation and application or from environmental residues after application [3]. Chemical plant workers, transport workers and pesticide applicators (especially the farmers) [4] are the major occupational groups that might be exposed to different toxic pesticides. Also workers in industrial settings are at increased risk since they handle various toxic chemicals including pesticides, raw materials, toxic solvents and inert carriers.

Organophosphates (OP's) are the esters of pentavalent phosphorous acid, exhibiting wide range of toxicity in mammals [5]. Organophosphate insecticides are one of the most widely used chemicals in agriculture and public health [6]. The compelling needs to improve human and animal nutrition and promote public health has led to an increase in organophosphates usage in recent time as they are used extensively to control agricultural, household and structural pests [7].

Chlorpyrifos, (O, O-diethyl-O-(3,5,6-trichloro-2- pyridyl)-phosphorothioate) is a chlorinated organophosphate (OP) insecticide with a broad spectrum of activity such as acaricide and nematicide used on pests of plants, animals and humans. It has been widely used in domestic, agricultural and to control public health pests such as mosquitoes and fire ants [8]. Chlorpyrifos has been reported as one of the most widely used organophosphate insecticides, thereby accounting for more than 50% of global insecticidal use [9]. Chlorpyrifos' toxicity is typically manifested in the central and peripheral nervous system where it inhibits acetylcholinesterase by its active metabolite (Chlorpyrifos- oxon) leading to cholinergic hyper stimulation. Another associated mechanism implicated in the induced toxicosis includes the induction of oxidative stress by the organophosphate compounds [10].

Literatures have reported about sub chronic toxicological studies of chlorpyrifos in rats which revealed reduced weight gain and slight histopathological changes in adrenal gland with significant brain and plasma AChE inhibition [11]. The evidence that chlorpyrifos causes alteration changes in some histopathological, hematological and biochemical parameters in experimental animals were reported from the studies of Kazmi *et al.* (2003)[12]; [13] and [14]. Chlorpyrifos, an organophosphate has also been identified in showing a number of additional toxic effects which include hepatic dysfunction, haematological and immunological abnormalities, embryotoxicity, genotoxicity and neurobehavioral changes as reported by the earlier researchers: Heikal *et al.* (2012a)[15]; [16];[17]; [18] and [19].

Humans and animals are occasionally and unintentionally exposed to lethal and sub-lethal doses of pesticides [20]. This stems from its continuous uses in-door, due to household use in the control of termites and as protectants in the preservation of kola, maize and yam flour chips, pesticide exterminator application and dietary (ingestion) exposure [21] thereby aggravating toxicity in human and ill health. Previous researchers have reported that repeated exposure to chlorpyrifos has been shown to cause severe damage to the vital organs. Therefore, due to the widely usage of chlorpyrifos domestically, in agriculture and public health continuously

necessitated the study of its toxicological effects. This present study was aimed at assessing the toxicity of sub lethal doses of Chlorpyrifos on haematology, biochemical and histopathology of kidney and liver organs of male wistar rats.

2. MATERIALS and METHODS

2.1 Experimental sites

The experiment was carried out in the Department of Crop Protection and Environmental Biology (CPEB) Toxicology laboratory, Faculty of Agriculture and Forestry; Central Animal house of College of Medicine, Clinical pathology and Histopathology Laboratories of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, all in the University of Ibadan, Nigeria. Male *Rattus norvegicus* (Wistar) rats were obtained from the experimental animal house of Department of Veterinary Anatomy and housed at the Central Animal house of College of Medicine, University of Ibadan

2.2 Experimental Animals

Twenty five male *rattus norvegicus* (wistar) rats of five weeks old weighing 150 – 200g used for this experiment; were obtained from the experimental animal house from the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan. Animals were kept under and maintained at $27 \pm 2^\circ\text{C}$, with 12 hour light, 12 hour dark cycles, relative humidity of 75-80%. They were kept in procured, cleaned individual plastic cages for four weeks (28 days). The rats were divided into five groups (A, B, C, D and E) with five rats in each cage, making it five treatments replicated five times and the rats were acclimatised for one week before commencement of treatments. Growers mash feed sourced locally and water were given to the animals during acclimatisation and treatments periods *ad libitum*. All animals were handled in accordance with the guidelines for care and use of laboratory animals.

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

2.3 Chlorpyrifos pesticide

Chlorpyrifos trade names include DursbanTM and LorsbanTM [22], 480g/l EC (Dorsban) used for this study was purchased from Saro Agroscience chemicals located at Oluyole Estate Ibadan, Oyo state, Nigeria. All other chemicals required for biochemical, haematological and histopathological estimation were of high quality and were provided by the Department of Pathology, Veterinary Medicine, University of Ibadan.

2.4. Chlorpyrifos pesticide sub lethal dilution

The Chlorpyrifos 480g/l stock solution used for this study is liquid and soluble in water. Dilution was carried out on the stock solution using the chemical formula as described by [23].

$$C_1V_1 = C_2V_2$$

Where C_1 = Initial Concentration of Stock

V_1 = Volume of Stock used

C_2 = Concentration desired

V_2 = Volume desired

Dilution of 100g/l was made from the stock solution.

The next dilution was made from 100g/l to prepare 10g/l of the insecticide. The following concentrations of 18.9mg/l, 25.9mg/l, 32.2mg/l and 39.2mg/l were prepared from 10g/l.

The treatments were 0mg/kg (Control), 18.9mg/kg, 25.9mg/kg, 32.2mg/kg and 39.2mg/kg of chlorpyrifos. All the treatments were administered daily. Oral administration of the treatment doses was done through gavaging for 28 days (four weeks).

2.5 Experimental Design

The experimental was a completely randomized design with five treatments replicated five times.

2.6 Experimental Toxicological Study

A total of 25 male albino (wistar rats) were used and divided into five groups with five rats in each cage. The various levels of Chlorpyrifos formed the treatments with a control group (0mg/kg; no pesticide). The treatment levels were 18.9mg/kg, 25.9mg/kg, 32.2mg/kg and 39.2mg/kg. All the treatments were administered daily (early in the morning) before feeding by oral gavage using 2ml of syringe and cannula for a period of 28 days. Weights of the animals were taken weekly and at the end of the experiment.

Observations on toxicity were made on the behavioural patterns of the rats during dosing. These include shyness, signs of pesticide poisoning such as loss of appetite, tremor, jerking of the muscles, constricted pupils, aggressiveness, skin irritation, convulsion, vomiting, weight gain / loss, paralysis and death [24].

On the termination day, 2ml of blood samples was collected through the ocular orbit from all the surviving rats into vacuutainer EDTAK3 tubes by heparinized capillary tubes, according to [25]. The blood samples for serum biochemical analysis were collected by heparinized capillary tubes into lithium heparinized tubes. The rats were sacrificed through cervical dislocation and organs of interest were harvested which include the kidney and liver for histopathological examination.

The hematological and serum biochemistry analyses and histopathological examinations were carried out by the technology experts in the Clinical pathology and Histopathology laboratories of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

2.7 Hematological and Serum Biochemical Analyses

Blood samples collected for packed cell volume (PCV) and Haemoglobin (Hb) count were determined using the micro haematocrit and cyanmethemoglobin methods, respectively as described by [25]. The Red blood cells (RBC) and White blood cells (WBC) were determined using improved Neubauer haemocytometer after appropriate dilution [26]. The differential lymphocyte counts were determined by scanning Giemsa's stained slides in the classic manner [26]. Blood chemistry analysis was also done to ascertain Total protein (TP) using the serum total as estimated by Biuret method [27]. Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) serum were obtained using the procedure of [28].

2.8 Histopathological study

Preparation of experimental animal tissues (kidney and liver organs) of each rat for microscopic examination, the histological procedures were followed in a stepwise protocol such as fixation,

dehydration, clearing, infiltration, embedding, blocking, sectioning and staining. Tissue specimens were collected from rats livers, kidneys and testis 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 [29] through the light microscope (400x). The histopathological examination was conducted in Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

2.9 Statistical Analysis

Data obtained in this study were analysed with one way analysis of variance (ANOVA), group means were compared for significance at 95% confidence level ($p < 0.05$) by Fischer's Least Significant Difference (LSD). Results were presented as mean $\pm$ standard error of the mean (SEM).

3. RESULTS

Effects of different concentrations of chlorpyrifos on the feed intake of wistar rats from this study showed significant differences ($p < 0.05$) as presented in Fig.1. Treated rats with 25.9mg/kg dose of chlorpyrifos had the least feed intake (72g) followed by treatment 18.9mg/kg in having (73g). Feed intake of experimental rats dosed with 39.2mg/kg chlorpyrifos also decreased progressively (82g). However, animals from the control (0mg/kg) had significant higher feed intake (96g) over other treatments. All the animals treated reduced progressively on feed intake with no significant difference ($p > 0.05$) from each other except from the control

The results on toxicity of different concentrations of chlorpyrifos on body weights showed the treated animals with no significant difference ($p > 0.05$) amongst each other from week zero (0) to week three (3). However, a highly significant differences ($p < 0.05$) between the control (212g).

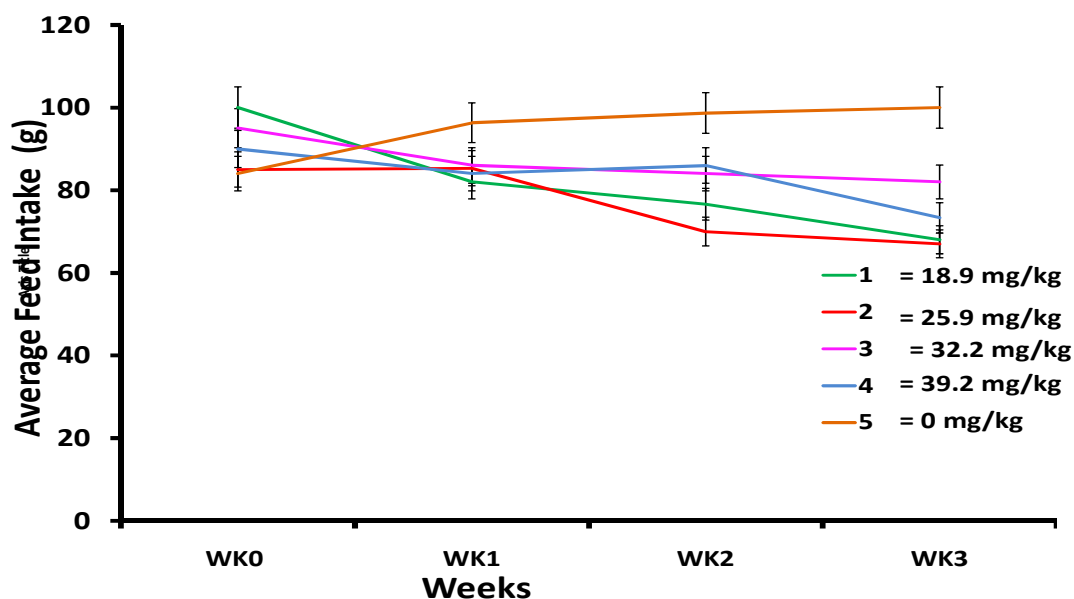
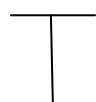


Figure 1: Effects of varying doses of chlorpyrifos on feed intake of wistar rats.

 = Standard Error Bar, WK= Week

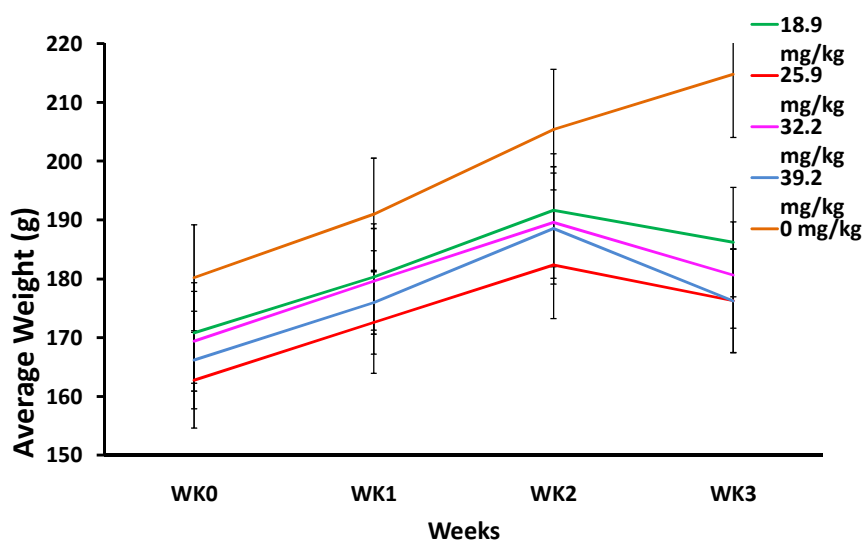


Fig 2: Effects of varying doses of Chlorpyrifos on the average weekly weight (g) gain of wistar rats

The results showed the toxic effects of chlorpyrifos on hematological parameters: packed cell volume (PCV), heamoglobin counts (Hb) with significant differences ($p < 0.05$) while the white blood cell counts (WBC) revealed no significant difference ($p < 0.05$) as presented in Table 1.

The PVC revealed no significant difference ($p > 0.05$) between the treatments 18.9mg/kg, 25.9mg/kg, 32.2mg/kg and control, while there was a significant difference ($p < 0.05$) between treated animals of chlorpyrifos at 39.2mg/kg and the control. Experimental rats from the control had significant higher mean value (49.67%) for PVC, while the lowest mean value (39.33%) was obtained from animals treated with 39.2mg/kg chlorpyrifos. The haemoglobin results in this study showed no significant difference ($p > 0.05$) from animals treated with 18.9mg/kg, 32.2mg/kg, 39.2mg/kg (15.64, 14.60 and 14.78%) chlorpyrifos and control (15.91%). While, the animals treated with 25.9mg/kg differed significantly ($p < 0.05$) in having (12.63%) from 18.9mg/kg (15.64%) and the controlled animals (15.91%). The highest mean (15.91%) for

haemoglobin was recorded from the control while lowest value (12.63%) was from 25.9mg/kg chlorpyrifos treated animals. The results on toxic effects of chlorpyrifos on WBC revealed no significant difference ($p > 0.05$) amongst all the treatments and control. Although the control had highest mean values (8300 cells/mm^3) for WBC, while the experimental animals treated with 18.9mg/kg chlorpyrifos had the lowest mean value (4460 cells/mm^3).

Table 1: Effects of varying levels of sub lethal of Chlorpyrifos on haematological parameters of wistar rats

Dosages of Chlorpyrifos	PVC (%)	Haemoglobin (Gg/dl)	WBC (cells/mm^3)
18.9mg/kg	48.8 ± 2.35	15.64 ± 0.71	4460 ± 1301.77
25.9mg/kg	45.4 ± 1.57	12.63 ± 1.86	6400 ± 635.09
32.2mg/kg	44.8 ± 0.37	14.6 ± 0.2	6380 ± 1304.37
39.2mg/kg	39.33 ± 5.78	14.78 ± 0.37	7440 ± 2537.24
0mg/kg	49.67 ± 1.40	15.91 ± 0.41	8300 ± 4788.53
LSD	6.98	2.12	6318.89
			NS

Means separation using Least Significant Difference (LSD) at $p < 0.05$

NS = Not Significant, PVC = Packed Cell Volume, WBC = White Blood Cell Counts

The results observed from the toxicity of chlorpyrifos on lymphocytes showed significant difference ($p < 0.05$), while the Red blood cell, neutrophiles and monocytes revealed no significant difference ($p > 0.05$) of haematological parameters as shown in Table 2.

The lymphocytes showed no significant difference ($p > 0.05$) between the control and treated animals with 18.9mg/kg, 25.9mg/kg and 39.2mg/kg. However, 32.2mg/kg treatment differed significantly ($p < 0.05$) when compared with the control. The control had the highest mean value (62.33%) for the lymphocytes while animals treated with 32.2mg/kg chlorpyrifos had the lowest value (45.40%). No significant differences ($p > 0.05$) were observed amongst all the treated and control animals in the RBC, neutrophiles and monocytes of haematological parameters. However, animals treated with 32.2mg/kg had highest mean value ($5.80 \times 10^9/\text{L}$) and lowest ($3.10 \times 10^9/\text{L}$) was from 25.9mg/kg in RBC. The neutrophiles had highest mean value (50.00%) in animals treated with 32.2mg/kg and lowest value (32.67%) in the control. The trend in monocytes revealed highest mean value (6.20%) in animals treated with 39.2mg/kg and lowest (4.00%) in 25.9mg/kg as shown in Table 2.

Table 2: Effects of varying levels of sub lethal of Chlorpyrifos on haematological parameters of wistar rats

Dosages of Chlorpyrifos	RBC ($\times 10^9/\text{L}$)	NEUT (%)	LYMPH (%)	MONO (%)
18.9mg/kg	5.26 ± 1.65	36.4 ± 5.95	58.2 ± 4.64	5.40 ± 1.96

25.9mg/kg	3.10±1.25	46.67±8.35	49.33±5.90	4.00±2.52
32.2mg/kg	5.80±1.15	50.0±4.24	45.4±3.54	5.00±1.48
39.2mg/kg	5.50±1.03	43.4±6.34	50.4±4.91	6.20±2.99
0mg/kg	4.73±0.85	32.67±3.37	62.33±2.33	5.00±2.11
LSD	NS	NS	14.17	NS

Means separation using Least Significant Difference (LSD) at $p<0.05$, NS = Not Significant
RBC= Red Blood Cell Counts, NEUT= Neutrophiles, LYMPH = Lymphocytes,
MONO =Monocytes

Results of toxic effects of chlorpyrifos on serum biochemical parameters on experimental animals revealed significant differences ($p<0.05$) in this study as presented in Table 3.

There was a significant difference ($p<0.05$) in alkaline phosphatase (ALP) of rats treated with 25.9mg/kg chlorpyrifos and control. Although no different significance ($p>0.05$) was observed amongst the other treatments and the control. However, the treatment 25.9mg/kg had the highest mean value (417.9u/L), while the lowest (173.97u/L) was from 32.2mg/kg treatment. The aspartate amino transferase (AST) results on experimental rats showed that there were significant differences ($p<0.05$) between the treatments 18.9mg/kg and 25.9mg/kg when compared with the control. No significant differences ($p>0.05$) were observed between the control and the chlorpyrifos treatments 32.2mg/kg and 39.2mg/kg. Although, 32.2mg/kg treated animals had highest mean value (542.23u/L) while 18.9mg/kg chlorpyrifos had the lowest (262.00u/L).

There were no significant differences ($p<0.05$) in the total and direct bilirubins in all the chlorpyrifos treatments when compared with the control. However, in total bilirubin 39.2mg/kg treatment had the highest mean value (0.57mg/dL) and the same lowest value (0.47mg/dL) was from the control and other treatments. In direct bilirubin, 32.2mg/kg treatment had highest mean value (0.20mg/dL) while the lowest mean value (0.1mg/dL) was from 18.9mg/kg treatment.

Table 3: Effects of varying levels of sub lethal of Chlorpyrifos on serum biochemical parameters of wistar rats

Dosages of Chlorpyrifos	TBmg/dL	DBmg/dL	ALPu/L	ASTu/L
18.9mg/kg	0.47±0.03	0.1±0.00	284.73±19.9	262.0±38.70
25.9mg/kg	0.47±0.03	0.1±0.00	417.9±126.27	294.94±42.06
32.2mg/kg	0.49±0.20	0.2±0.00	173.97±11.48	542.23±26.68
39.2mg/kg	0.57±0.03	0.13±0.03	239.1±21.89	454.87±37.52
0mg/kg	0.47±0.03	0.13±0.03	185.97±11.92	461.80±70.81
LSD	0.30	6.64	142.71	111.32
	NS	NS		

Means separation using Least Significant Difference (LSD) at $p<0.05$, NS = Not Significant
AST=Aspertate Amino Transferase, TB=Total Bilirubin, DB = Direct Bilirubin,
ALP = Alkaline Phosphate

The results from toxic effects of different concentrations of chlorpyrifos on alanine amino transferase, total protein, albumin and globulin on the experimental animals showed significant differences ($p < 0.05$) as presented in Table 4.

Significant difference ($p < 0.05$) was observed in Alanine amino transferase (ALT) between 39.2mg/kg treatment and control animals. Significant differences ($p < 0.05$) was also shown between 39.2mg/kg and 18.9mg/kg chlorpyrifos treated animals as well as 25.9mg/kg treatment. Meanwhile, no significance difference ($p > 0.05$) was observed between 32.2mg/kg, 25.9mg/kg and 18.9mg/kg chlorpyrifos treated animals when compared with the control animals. Although, 39.2mg/kg treatment had the highest mean value (130.77u/L) while control 0mg/kg had the lowest mean value (83.70u/L). The trend in total protein estimation showed significant difference ($p < 0.05$) between 18.9mg/kg and 25.9mg/kg chlorpyrifos treated animals and the control. However, 32.2mg/kg and 39.2mg/kg chlorpyrifos treatments showed no significant difference ($p > 0.05$) with the control. However, the control had the highest mean value (86.10g/dL) and lowest mean value (75.78g/dL) was from 25.9mg/kg chlorpyrifos treated animals. The result for albumin in the experimental animals showed significant difference ($p < 0.05$) between 25.9mg/kg chlorpyrifos and the control while other treatments 18.9mg/kg, 32.2mg/kg and 39.2mg/kg showed no significant difference ($p > 0.05$) with the control. The highest mean value (42.30g/dL) was observed in 39.2mg/kg chlorpyrifos treated animals and the lowest mean value (32.79g/dL) was from 25.9mg/kg treatment. Globulin ratio result revealed no significant differences ($p > 0.05$) amongst all the chlorpyrifos treated animals and the control. However, highest mean value (45.31g/dL) was recorded from the control and lowest mean value (42.06g/dL) was from 39.2mg/kg treatment.

Table 4: Effects of varying levels of sub lethal of Chlorpyrifos on serum biochemical parameters of wistar rats

Dosages of Chlorpyrifos	ALTu/L	TPg/dL	ALBg/dL	GLBg/dL
18.9mg/kg	95.8±8.78	81.63±1.59	37.58±0.81	44.05±1.25
25.9mg/kg	96.25±14.84	75.78±16.85	32.79±3.47	43.22±3.82
32.2mg/kg	115.73±6.21	84.03±1.24	40.90±1.41	43.14±0.21
39.2mg/kg	130.77±4.75	84.37±0.88	42.30±2.06	42.06±2.06
0mg/kg	83.7±3.91	86.10±1.51	40.79±1.21	45.31±2.32
LSD	21.1	3.45	4.94	1.96
				NS

Means separation using Least Significant Difference (LSD) at $p < 0.05$, NS = Not Significant
ALT=Alanine Amino Transferase, TP=Total Protein, ALB = Albumin, GLB= Globulin

Results on the toxic effects of chlorpyrifos on the histopathological examinations (Photomicrographs sections) carried out on the experimental rats (*Rattus norvegicus*) were shown in Plates 1- 2.

The 0mg/kg dosage (control) in organs examined (liver and kidney) showed no visible lesions with closely packed hepatic plates in the liver. There were no apparent morphological changes in

the liver and kidney of the control rats. The control group showed no symptoms of any gross abnormalities (Plates 1 and 2).

Observations from 18.9mg/kg chlorpyrifos treated experimental rats showed that the animals had mild multifocal sloughing off of tubular epithelium in renal cortex and cortico medullary junction of the kidney. The kidney of experimental rats exposed to 25.9mg/kg dosage chlorpyrifos expressed widespread presence of eosinophilic (proteinaceous) fluid in tubular lumens; multifocal moderate sloughing off of tubular epithelial cells while 32.2mg/kg dosage of chlorpyrifos showed nephrosis. The kidney of the animals exposed to 39.2mg/kg dosage of chlorpyrifos showed eosinophilic (proteinaceous fluid in the Bowman's capsule and compressing the glomeruli tufts) (Plate 1).

The animals treated with 18.9mg/kg and 25.9mg/kg chlorpyrifos showed moderate to severe multifocal thinning of hepatic plates in the liver. The histopathological examination result from 39.2mg/kg chlorpyrifos treated animals showed that the hepatocytes in the liver were finely reticulated with cytoplasmic appearance. However, there were moderate widespread of vacuolar changes of hepatocytes in the liver of some rats exposed to 39.2mg/kg dosage of chlorpyrifos (Plate 2).

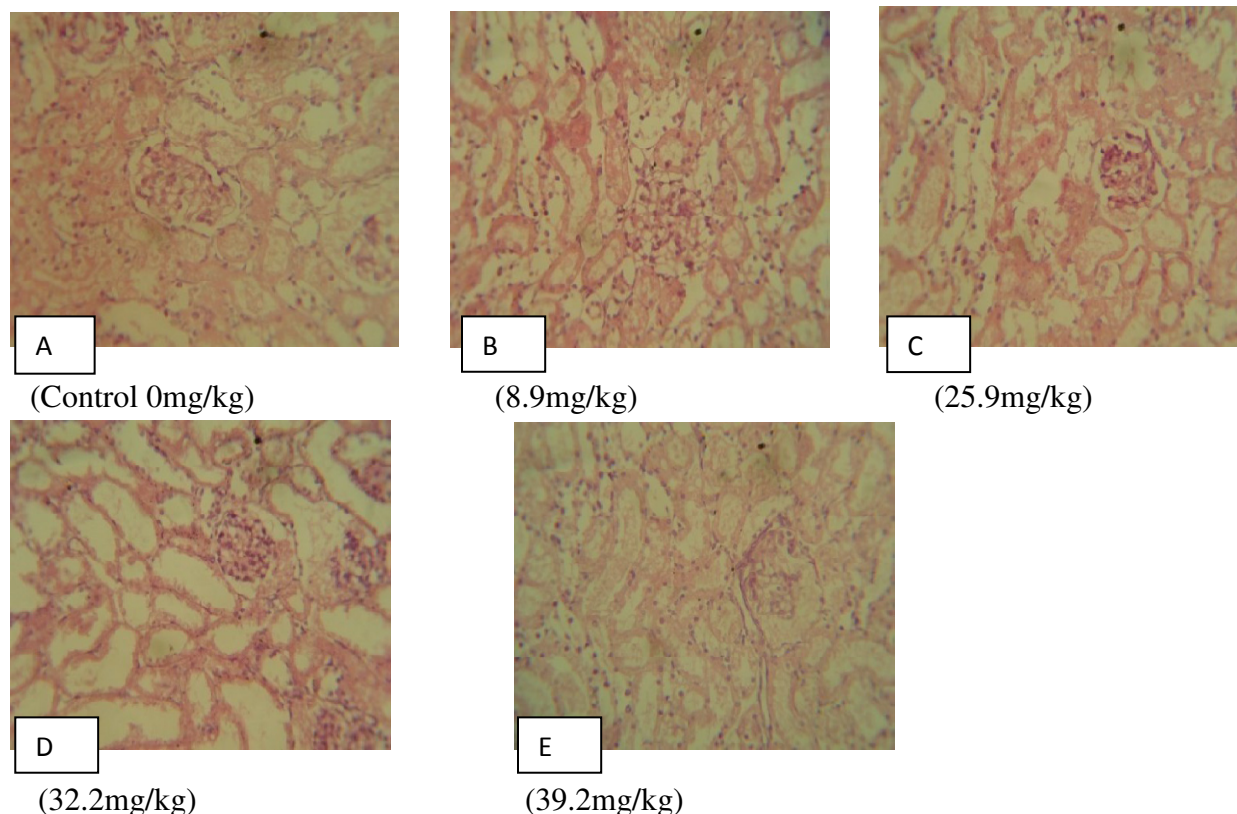


Plate 1: Photomicrographs sections of kidneys of *Rattus norvegicus* groups exposed to different sub lethal concentrations of chlorpyrifos and control.

A= No treatment of chlorpyrifos (control 0mg/kg). No visible lesions. B = Treated with 18.9mg/kg of chlorpyrifos. Mild multifocal sloughing off of tubular epithelium in renal cortex and corticomedullary junction. C = Treated with 25.9mg/kg of chlorpyrifos. Widespread presence of eosinophilic (proteinaceous) fluid in tubular lumen; multifocal moderate sloughing off of tubular epithelial cells. D = Treated with 32.2mg/kg of chlorpyrifos. Multiple foci of moderate sloughing off of tubular epithelial cells; some tubules appearing dilated and cystic (nephrosis). E = Treated with 39.2mg/kg of chlorpyrifos. Eosinophilic (proteinaceous) fluid in Bowman's capsule and compressing the glomeruli tufts). (Hematoxylin and Eosin (H &E), 400x)

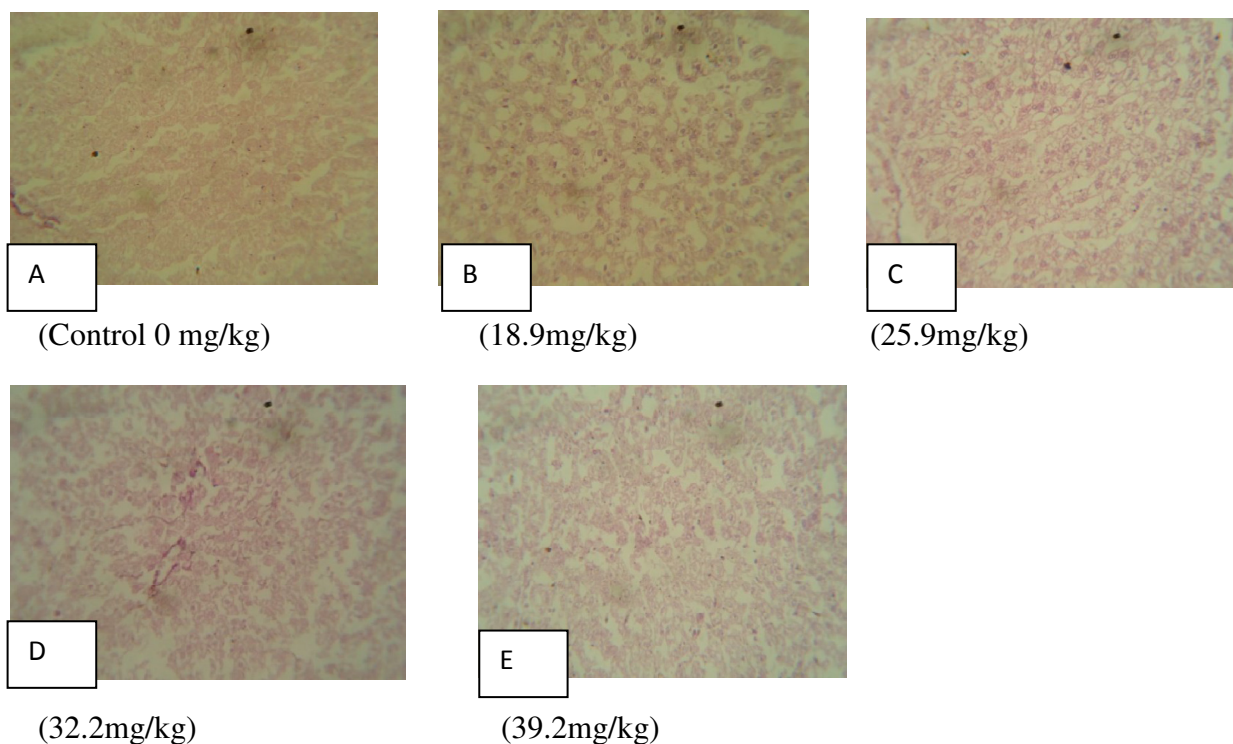


Plate 2: Photomicrographs sections of livers of *Rattus norvegicus* groups exposed to different sub lethal concentrations of chlorpyrifos and control.

A= Treated with 0mg/kg of chlorpyrifos (control). Closely packed hepatic plates with no visible lesions. B = Treated with 18.9mg/kg of chlorpyrifos. Moderate vacuolar change of hepatocytes; severe multifocal thinning of hepatic plates. C = Treated with 25.9mg/kg of chlorpyrifos. Widespread moderate vacuolar change of hepatocytes; moderate thinning of hepatic cords. D = Treated with 32.2mg/kg of chlorpyrifos. Multifocal mild thinning of hepatic plates; hepatocytes have a finely reticulated cytoplasmic appearance. E = Treated with 39.2mg/kg of chlorpyrifos. Moderate widespread vacuolar change of hepatocytes and congestion of central veins. (H &E, 400x)

4. DISCUSSION

The widespread and extensive use of organophosphate pesticides such as chlorpyrifos (CPF) in developing nations raises the likelihood of inadvertent exposure to the pesticide either from

short-term or long-term exposures with consequent toxic effects [30]. In most of these countries, the magnitude of pesticide residue in food of both plant and animal origin is largely unknown, hence, increasing the risk of exposure [31] and ill health.

In this study, the chlorpyrifos caused a decrease in body weight of rats exposed to different concentrations of the pesticide. Although, the effects did not show in the first week of administration to the animals, but it became apparent on the body weights from the second week to the fourth week of treatment with respect to dosages administered at 18.9mg/kg to 39.2mg/kg. All experimental rats treated with chlorpyrifos showed a marked decrease in body weights while untreated (control) rats continued to gain weight progressively during the period of treatments.

This result agreed with [32] who concluded that chlorpyrifos caused a significant change in rats' body weights. The obtained result also conformed with [33] findings that, there were adverse effects on exposure to chlorpyrifos on male rats with a significant decrease in body weight. Jaiswal and Verma (2012)[34], [35] and [36] also reported that the most profound rat stress was the reduction of body weight after administration of chlorpyrifos. Studies conducted by [37]; [38] and [39] gave decreased body weights in rats treated with chlorpyrifos with no effects on physical or reflex development.

This present study also revealed leucopenia in packed cell volume (PCV) which was apparently due to lymphopenia, neutropenia, and monocytopenia in the chlorpyrifos animal treated groups. The marked reduction in packed cell volume and hemoglobin in the rats' population exposed to varying concentration of chlorpyrifos dosages 39.2mg/kg, 32.2mg/kg, 25.9mg/kg and 18.9mg/kg resulted in leucopenia and low hemoglobin counts. These findings corroborated previous studies of [6] and [40] that attributed chlorpyrifos induced leucopenia to neutropenia and lymphopenia. Ambali *et al.* (2010)[6] also reported neutrophilia following chlorpyrifos exposure, in contrary to neutropenia recorded in the present study. [41] attributed monocytopenia recorded in workers exposed to chlorpyrifos experienced inhibition of a monocyte esterase, (alpha)-naphthyl butyrate esterase.

It was shown in this present study that chlorpyrifos caused a decrease in the white blood cell in the rats' population exposed to it with marked reductions observed in 39.2mg/kg and 32.2mg/kg treatments, respectively. This induced immune toxicity is in support of [42] and [43] that chlorpyrifos has been shown to induce immune toxicity in rats either via the induction of apoptosis or necrosis. Chlorpyrifos exposure has been shown to induce immune toxicity via the induction of apoptosis partly mediated through the activation of caspase as reported by [44]. This was found in this study as differential leucocytes which were greatly reduced thereby making the rats susceptible to varying degree of abnormalities like paralysis observed in treatment 32.2mg/kg. Chronic chlorpyrifos exposure has been associated with abnormality of the immune system including depression of T-lymphocytes [45].

Immunotoxicity of white blood cell (WBC) in chlorpyrifos has been associated with either inhibition of serine hydrolases or esterases in components of the immune system, through oxidative damage to immune organs, or by modulation of signal transduction pathways controlling immune functions [46].

The reduction of serum levels of protein for all the rats dosed with the chlorpyrifos at all dosages of treatment correlates with the findings of [47], this indicated that toxicants may cause stress-mediated mobilization of protein to cope with the detrimental condition so imposed. The protein mobilized is one of the strategies employed to meet the energy required to sustain increased physical activity, biotransformation and excretion of the toxicants. Aminotransferases (ALT and AST) are produced in the liver and are good markers of damage to liver cells but not necessarily the severity of the damage [48]; [49]. The reduced levels of AST, ALB and ALT in chlorpyrifos treated groups at 39.2mg/kg, 32.2mg/kg and 25.9mg/kg could be as a result of suppression of production by the liver. They are normally present at low levels in the blood so if the liver cells are damaged, it would be expected that some of the enzymes leak into the blood and increase the levels [14]. Also, apparently earlier damaging effect before progression to cell death and leakage of enzymes.

In contrast to serum enzymes, albumin apart from being a useful indicator of the integrity of glomerular membrane is also an important indicator in determining the severity of disease [50]; [51]. Decrease albumin may be due primarily to reduction in synthesis by the liver and secondarily to reduced protein intake which further confirms hepatic damage [52]; [53] as observed with 39.2mg/kg and 32.2mg/kg in the study. Decreased albumin observed in this work revealed that chlorpyrifos administration may be attributed to hepatic dysfunction.

The chlorpyrifos administered orally to the animals treated showed degeneration in some of renal corpuscles, sloughing off of tubular epithelium, congestion of glomeruli and compression in size of glomeruli tufts of the kidneys. These descriptions agree with the studies of those described by [54]; [55]; [56] and [14]. The toxicity of chlorpyrifos on the kidneys indicated a dose dependency.

There was loss of cell architecture and increased degeneration of hepatic cells in the livers of the experimental animals administered with chlorpyrifos. Most of hepatic cells were necrotic and the cytoplasm was vacuolar with denoting degenerating of hepatocytes. This finding collaborates with the studies of [57]; [56]. The changes in treated animal livers indicated that the degeneration of livers increased with the doses of chlorpyrifos to exposure which could be related to dose-dependent increase in the liver function enzymes.

The toxic effects of different sub doses of chlorpyrifos on livers and kidneys on treated animals in this study is also in agreement with the report from the work of [58] that administration of sub lethal doses of chlorpyrifos resulted in altered enzyme activities of liver and renal (kidney) damage to experimental animals.

5. CONCLUSION

The varied changes in the levels of the haematology, biochemical parameters and histopathology as a result of different sub lethal concentrations of chlorpyrifos in the treated groups relative to the control group affect the overall performance of the rats in terms of health and well being. From the outcome of this study, the parameters can be used as bio indicators of exposure and effects in instances where chlorpyrifos is used as a pesticide.

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