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Synergistic Toxicity Studies and Effect of Sub-Lethal Doses of Locally Formulated Pesticides "*Ota-Piapia*" on *Wistar* Rats

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Abstract

The research measured the synergistic and toxic effect of Sub-lethal doses of locally formulated pesticide "*Ota-piapia*" on some liver enzymes activities and haemato-biochemical parameters in *Wistar* rats while extrapolate their likely effect on humans and the possibility of recovery after cessation of exposure. Twenty-four male and female *Wistar* rats were acquired from the animal house of Biological Sciences department of Nigerian Defence Academy Kaduna and were allowed to acclimatize for 4 weeks. They were assigned into four different groups (1, 2, 3 and 4) of four rats per group. Group1 served as the control thus no treatment was administered. Varying concentrations (w/v) of "*Ota piapia"* were administered to groups 2 (5 mL), 3 (8 mL) and 4 (10 mL) via sprinkling with 10 mL syringe three times a week for a period of 12 weeks. The rats were left for 4 weeks to recover from the effect of the pesticide. Both groups were mildly anaesthetized with chloroform before euthanasia and dissection and subsequently they were sacrificed. Blood were immediately collected and analyzed for activities of some liver enzymes and haemato-biochemical parameters. Results revealed increase in aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, red blood cell (RBC), white blood cell (WBC), as compared to the control ($P<0.05$). There was no significant level of recovery in the haematological and biochemical parameter when compared to the control group even after four weeks of cessation of exposure. The findings of the study showed that local formulation of *Ota-piapia* pesticide had significant ($P<0.05$) effect on the experimental animals at different sub-lethal concentrations. We therefore, recommend that such pesticide be handled with caution and its proliferation should be regulated.

Keywords: Dichlorvos, Haematological, Biochemical and Parameters.

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Introduction

Pesticides are group of substances used as insecticides, fungicides, herbicides, rodenticides, molluscicides and nematicides (Muyesaier *et al.,* 2021 and Bernardes *et al.,* 2015), hence, pesticides are being used in agricultural practice and to a lesser extend in domestic operations to control unwanted pests and disease vectors; insects and weeds (Muyesaier *et al.,* 2021; Bala *et al.,* 2019 and Harnandez *et al.,* 2003). The development of pesticides increase during World War II (1939-1945), consequently, from 1940s onwards, the sporadic increased in both synthetic and non-synthetic usage of pesticides in agricultural and domestic activities (Harnandez *et al.,* 2013).

Several chemicals used as pesticides are unselective and toxic to many non-target including humans and the environment (Muyesaier *et al.,* 2021, Harnandez *et al.,* 2010 and Murphy 1986). The major routes of insecticide exposure to agricultural workers include dermal and respiratory tracts (Islam *et al.,* 2022, Durham and Wolf, 1962). Exposure to these pesticides can include pathological changes in liver, kidney, heart, lungs and other non-target internal organs (Honda 2003, Gathwan, 2006) which can cause cytotoxic changes, genotoxicity, cell necrosis and changes in biochemical parameters of the brain, heart and lungs respectively (Amachree and Idam, 2021; Benjamin *et al.,* 2020 and Militovic *et al.,* 2006).

Hence dichlorvos also known as DDVP (O-O-dimethyl –O-2 2-dichlorvos-vynyl phosphate) (Tela *et al.,* 2018 and USEPA, 2007) is an organophosphate insecticide and have been applied in part of northern Nigeria as mosquitoes insecticides and preservatives on dried or smoked fish over the decades (Musa *et al.,* 2010a; Foll *et al.,* 1965; Foll and Pant, 1966) since its commercially manufacture started in 1961 (BCERF, 1999).

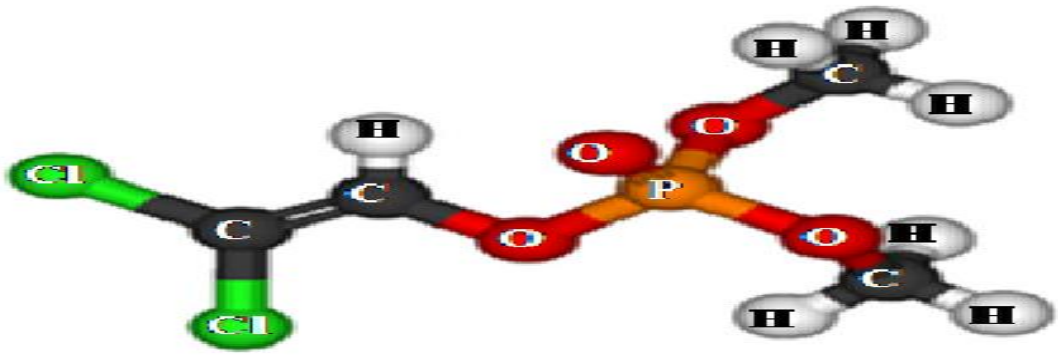

Figure 1: Chemical formula of Dichlorvos (CAS# 62-73-1, Musa *et al.,* 2010b).

A number of evidence indicates that dichlorvos is most likely the major active pesticide ingredient of *Ota-pipia* formulation in north eastern Nigeria (Islam *et al.,* 2022 and Musa *et al.,* 2010). These consist of the fact that generally dichlorvos is used as an agricultural insecticide on crop, stored products and on animals. It is used as an insecticides for slow used as an anthelmintic botacide agent that kills fly larvae and mosquitos (Benjamin *et al.,* 2020 and USEPA, 1994), the latest being a major menace in northeastern Nigeria and hence, the observed large tonnage of various brands of dichlorvos in the open market.

Ota-piapia is a vernacular parlance (Igbo) of eastern Nigeria origin and a household name for insecticide/rodenticide/pesticide, which literally translates into "that which completely consumes/devours" Local pesticide makers emphasized the potency of their pesticides by the word "*Ota-pipia*" indicating that such products will completely take care of our little pest problems (Mortui, 2006). Its acceptance and wide spread proliferation in Nigeria have been due to sole clamed cheap production, efficacy, accessibility and affordability (Essiet, 2009), the product formulations is still unspecific as not registered with regulatory bodies;

Standard Organization of Nigeria (SON), National Agency for Food and Drug Administration and Control (NAFDAC) (Akunyili, 2007) but have been commonly used as insecticide, especially for mosquitoes (Mortui, 2006 and Foll *et al.,* 1965), food storage, such as grains and preventing insect infestation on smoked fish (Vardell *et al.,* 1973; Desmarchelier, 1977; FAO, 2001) thus, the trend of application in northeastern Nigeria.

The locally formulation of *Ota-piapia* is thought to entails repackaging into a small (about 10-15 mL) retail bottle of an active ingredient which include an unspecified quantities and measurements of pesticides from those imported, hence, cypermethrin, dichlorvos, gamalin 20, gammalin super, lindane, and capsidox 20 (Islam *et al.,* 2022 and PAN, 2007). Some contain homemade cocktail of kerosene, oil, alcohol and any suitable solvent with the pesticide. Therefore, the present study aimed at evaluating the synergistic toxicity effect of sub-lethal doses of locally formulated pesticide "*Ota-piapia*" on exposure to *Wistar* rats.

Materials and Methods

The study was conducted within Kaduna Metropolis, Kaduna State. The state has a total land mass of 48473.25Km^2 and is located West Geographical Zone of Nigeria. It lies between longitude 6° and 9° E and latitude 9° and 11° N (Ajaero *et al.,* 2023). The state has a distinct Wet Season (April – October) and Dry Season November-March), Vegetation type is the Guinea Savannah (Umar *et al.,* 2021and Dilli *et al.,* 2020).

Ethical Consideration

The use of rats for this study was in accordance with the internationally accepted principle for laboratory animal use and care (Benthai, 2019 and NIH publication No. 85-23, 1985).

Chemicals

One liter of a locally formulated *Ota-piapia* pesticide was purchased from central market Kaduna common farm chemicals are told and dispense both for retail and wholesale use in Kaduna.

Chemicals Used for the Study

One liter of locally formulated *Ota-piapia* pesticide was purchased from Central Market Kaduna, where farmer's chemicals are sold and dispense both for retail and wholesale use in Kaduna. Prior to use, all glasswares were washed and sterilized.

Animals Used for the Study

Wistar rats (n=24) weighing between 180-200g were obtained from the animal house, Department Biological Sciences of Nigeria Defence Academy (NDA), Kaduna.

Acclimatization

Rats were acclimatized in the Department of Biological Sciences Laboratory for four weeks. The rats were fed twice daily with standard pelletized animal feed and given water *ad libitum* throughout the study period (Famiyesin *et al.,* 2020). Unconsumed feeds were cleared every day to maintain standard hygienic environment (Benjamin *et al.,* 2020 and Benthai, 2019).

Experimental Design

Range Finding Assay (LD_{50})

A range finding assay was carried out to determine the lethal dose from sub-lethal dose on the animals. Eight animals were set apart and exposed to (20 mL, 16 mL, 12 mL and 10 mL) of the pesticide. About 16 mL to 20 mL was instantaneously lethal to the animals while 12 mL to 10 m dose was experienced to be less stressful on the animals. Therefore, 10 mL, 8 mL, and 5 mL respectively were considered as sub-lethal dose for the experiment.

Treatment Phase

Four identical rooms (four by four square meters) were used as exposure chambers each with internal volume. Each room contains six rats and labeled Groups 1, 2, 3 and 4 respectively.Group1 was used as control without any treatment administered. Animals in groups 2, 3 and 4 were treated in the exposure room with sub-lethal doses of 5 mL, 8mL and 10mL exposed for four hours with the locally formulated pesticide *Ota-piapia* every three days. A modified Lorke's, method of exposure was adopted for the experiment (Lorke *et al.,* 1983). The *Ota-piapia* was administered using hypodermic

syringes by sprinkling it inside the exposure room and the animals inhaled it through breathing and surface skin absorption. The treatment lasted for twelve weeks after which the animals were divided into two phases; (treatment and recovery phase).

Recovery Phase

The s00econd phase (recovery phase) was allowed to see if there will be any possible recovery after cessation of exposure treatment for the next four weeks. The first set of the animals (exposed phase) were humanely euthanized 24 h after the last treatment through cervical dislocation, while the last set of the recovery phase were euthanized four weeks later. After which the brain, heart and lungs were harvested, placed in 10 % buffered neutral formalin and processed using standard histopathology procedure as described by Benjamin (2020).

Blood Sample Collection

About 5 mL blood was immediately collected from the inferior vena cava into a 2.5 mL heparinized tubes. The blood samples were centrifuge at 4000x g for 10 minutes. The plasma was collected into a 50 mL Eppendorf bottles using a Pasteur pipette and stored at -20°C until analyzed.

Determination of Liver Enzyme Activities,

Blood samples were collected from all anaesthetized animals into appropriate tubes and serum was obtained by centrifugation at 5000rpm for 10 minutes. Serum samples were analyzed using Selectra Pro S Auto analyzer (ELITech, 6003-500) using the prescribed kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP). All parameters were determined calorimetrically according to Reitman and Frankel modified method (Frankel *et al.*, 1995 and Femiyesin *et al.*, 2020).

Determination of Haematological and Biochemical Parameters

The blood samples were collected into a plain tube and were inoculated into different cuvette and inserted into an automated auto analyzer machine model Sysmex KX21N made in Germany. Pack cell volume (PCV), red blood cell (RBC), white blood cell (WBC), HBC, HB, MCV, MCH, MCHC, Neutrophil, Basophil, Lymphocyte, Menocyte, Eosinophil, and Total Protein were determined (Frankel *et al.*, 1995).

Statistical Analysis

The data for liver enzyme activities, haematological and biochemical parameters were analyzed using statistical package for social sciences (SPSS) version 20.0. It was subsequently express as mean ± (SD) standard deviation comparing the plasma mean values between control group and various groups exposed to *Ota-piapia* including those that were allowed for possible recovery after cessation of exposure. P-value (<0.05) were considered statistically significant.

Results

The values of biochemical parameters variation in of *Wistar* rats after exposure to locally formulated *Ota-piapia* pesticide at different concentration is presented in Table 1. The result thus shows that, there is a significant increase ($P<0.05$) in bilirubin, total bilirubin when expose to 5 mL and 10 mL concentration (group 2 and 4) as compared to the control group. No increase was observed in group 3 as compare to the control animal. ALP shows no significant increase across all the groups while AST shows an increase in group 3 and 4 but decrease in group 2 to the control an. However, the concentration of *Ota-piapia* shows no increase in the level of Albumin across all the groups and there was not statistically significant ($P>0.05$). With respect to the total protein, the result shows an increase in all the treated groups compared to the control group. It is therefore concluded that locally formulated *Ota-piapia* pesticide has significant effect in protein and bilirubin level.

When compared with the control, the experimental animals had gradual increase in the mean values over the period of exposure in RBC, WBC, PCV, (except in group 4), MCH, HB (except in group 3) and lymphocyte across the groups; but in Neutrophil, there was a decrease across all the groups. Furthermore, Monocyte Eosinophil and Basophil, the mean value

almost remained steady throughout the period of the experiment. RBC, WBC and HB were not statistically significant (P>0.05) but statistically significant in other blood parameters (P<0.05), (Table 2). It is therefore concluded that locally formulated *Ota-piapia* has significant effect on the blood parameters except for Platelet, Neutrophil Monocyte, Eosinophil and Basophil. Monocyte however, shows a fluctuation at different concentration.

Tables 3: give the result on effect of locally formulated *Ota-piapia* pesticide on blood parameters of wistar albino rats after four weeks of cessation of exposure. It is seen that the inhalation of the locally formulated *Ota-piapia* resulted in significant decrease (p<0.05) in all the biochemical parameters in all the treated groups except in group 4 of Bilirubin and AST, group 2 of total Bilirubin and ALT. Total Protein concentration showed no significant difference among treated groups of albino rats compared to control group but was statistically significant (P<0.05). The result thus shows no significant recovery in most of the biochemical parameters of the experimental animals.

Tables 5 present the number of total haematological parameter at different count in the experimental albino rats at different concentration after four weeks cessation of treatment. Total RBC, Total platelet, WBC (except group 1), PCV, HB and neutrophil number decreased in the rats treated groups and reached a significant level (p<0.05) across the groups. On the other hand, MCH, MCHC, MCV showed a significant difference compared with control in group 1 and 2. Neutrophils values were decreased in all treated. No recoveries were observed in high dose (10 mL) (group 4), of MCHC, MCV, HB, Neutrophil, and Lymphocyte. However, the number of monocytes presented a fluctuating pattern in rats, the level decreased for the group treated with low dose and increased for the group treated with high dose.

Table 1: Effect of Different Concentration of *Ota-piapia* Pesticide on Liver Function Enzymes of *Wistar* Rats mean ± Standard Deviation

Biochemical Indices	**TREATMENT GROUPS**			
	Group1 Control	**Group 2 (5 mL)**	**Group 3 (8 mL)**	**Group 4 (10 mL)**
Bilirubin (µmol/L)	2.00±2.00	2.77±1.53	2.00±2.00	4.60±2.00
Total Bilirubin (µmol/L)	6.00±2.00	10.20±2.00	2.10±2.00	8.0±2.00
ALP (µ/L)	110±2.00	108±2.00	60±2.00	35±2.00
AST (µ/L)	265±2.00	243±2.00	301±2.00	267±2.00
ALT (µ/L)	118±2.00	110±2.00	153±2.00	179±2.00
Albumin (g/L)	31±2.00	28±2.00	29±2.00	29±2.00
Total Protein (g/L)	66±2.00	70±2.00	78±2.00	60±2.00

Data presented as the mean ± SD for three rats in each group, Significant difference from the control at P<0.05. N = Not significant, S =Significant.

Keys: AST- aspartate aminotransferase**,** ALT- aminotransferases**,** ALP- alkaline phosphatase, RBC- red blood cell, WBC- white blood cell, PCV, packed cell volume, HB- haemoglobin, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, MCV- mean corpuscular volume.

Table 2a: Effect of Different Concentration of *Ota-piapia* Pesticide on Haematological Parameters (Full Blood Count) of *Wistar* Rats

Blood Parameters	**Group 1 Control**	**Group 2 (5 mL)**	**Group 3 (8 mL)**	**Group 4 (10 mL)**

Total RBC ($X10^9$/L)	8.27±2.00	8.39±2.00	9.68±2.00	8.48±2.00
Total Platelet ($X10^{12}$/L)	1035.±2.00	873.00±2.00	926±2.00	679±2.00
Total WBC ($X10^9$/L)	4.20±2.00	5.80±2.00	8.10±2.00	7.10±2.00
Hb (g/dl)	15.20±2.00	15.80±2.00	17.10±2.00	15.7±2.00
PCV (%)	52.0±2.00	53.00±2.00	58.±2.00	51±2.00
MCH (pg)	1.87±1.80	18.80±2.00	17.70±2.00	18.5±2.00
MCHC (g/dl)	291.0±2.00	298.00±2.00	295±2.00	304±2.00
MCV (fl)	63.0±2.00	63.00±2.00	59±2.00	61.±2.00

Table 2b: Effect of Different Concentration of *Ota-piapia* Pesticide on Haematological Parameters (Differential Count) of *Wistar* Rats

Parameter	Group1 Control	Group2 (5 mL)	Group3 (8 mL)	Group4 (10 mL)
Neutrophil (%)	54.0±2.00	12.0±2.00	35.±2.00	44.±2.00
Lymphocyte (%)	41.0±2.00	85.0±2.00	65.±2.00	64.±2.00
Monocyte (%)	1.0±0.00	1.0±0.00	0.0±0.00	1.00±0.00
Eosinophil (%)	1.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Basophil (%)	0.00±0.00	0.0±0.00	0.0±0.00	0.0±0.00

Table 3: Effect of Different Concentration of locally formulated *Ota-piapia* Pesticide on Liver Function Enzymes of *Wistard* Rats (Recovery Phase) mean ± Standard Deviation

Parameter	Group 1 Control	Group 2 (5 mL)	Group 3 (8 mL)	Group 4 (10 mL)
Bilirubin (µmol/L)	2.00±2.00	1.50±0.00	1.00±0.00	2.82±2.71
Total Bilirubin (µmol/L)	6.00±2.00	7.70±2.00	4.10±2.00	3.90 ±2.00
ALP (µ/L)	110.00±2.00	99.00±2.00	33.33±4.16	88.00±2.00
AST (µ/L)	265.00±2.00	251.00±2.00	265.00±2.00	326.00±2.00
ALT (µ/L)	118.00±2.00	154.00±2.00	84.00±2.00	117.00±2.00
Albumin (g/L)	31.00±2.00	21.00±2.00	28.00±2.00	28.00±2.00
Total Protein (g/L)	66.00±2.00	45.00±2.00	63.00±2.00	63.00±2.00

Table 4: Hematological Parameters of Rats exposed to Different Concentration of *Ota-piapia* (Recovery Phase)

Blood Indices	Group 1 Control	Group 2 (5 mL)	Group 3 (8 mL)	Group 4 (10 mL)
Total RBC ($X10^{12}$/L)	8.3±2.00	7.7±2.00	6.8±2.00	1.0±1.41
Total Platelet ($X10^9$/L)	1035±2.0	781.7±1.5	573.7±1.5	0.67±1.15
Total WBC ($X10^9$/L)	4.2±2.00	5.5±2.00	3.1±2.00	0.67±1.15
PCV (%)	52±2.00	49±2.00	46±2.00	0.67±1.15
MCH (pg)	18±2.00	19.4±2.00	20.6±2.00	0.67±1.15
MCHC (g/dl)	291±2.00	303±2.00	296±2.00	0
MCV (fl)	63±2.00	64±2.00	68.9±2.00	0
Hb (g/dl)	15.2±2.00	14.9±2.00	14±2.00	0

Table 5: Effect of Different Concentration of *Ota-piapia* Pesticide on Haematological Parameters (Differential Count) of *Wistar* Rats (Recovery Phase) after Four Weeks of Cessation of Treatment

Parameter	Group 1 Control	Group 2 (5 mL)	Group 3 (8 mL)	Group 4 (10 mL)

Neutrophil (%)	54±2.00	29±2.00	45±2.00	0
Lymphocyte (%)	45±2.00	70±2.00	54.7±1.15	0
Monocyte (%)	1	0	1	0
Eosinophil (%)	0	0	0	0
Basophil (%)	0	0	0	0

Discussion

The liver is the main organ most commonly associated with the intense metabolic breakdown of endogenous and xenophobic substance. It is the primary site of detoxification and is therefore susceptible to various disorders arising from exposure to toxins of extrinsic as well as intrinsic form. Result of this study indicated that *Ota-piapia* pesticide had hepatocellular changes in the liver of Wistar albino rats on and after exposure to the pesticide at different concentrations. The measurement of the level and activities of various enzymes in liver plays a significant role in disease investigation and diagnosis (CEPA, 1996). Liver transaminase enzymes, AST, ALP and ALT are usually used as biomarkers of liver injury because they are released by hepatocytes into the extracellular space. They are considered to be more precise and sensitive measures in assessing liver function and damage (Sarhna *et al*., 2011). There was an increase in the level of AST in group 3 and 4 but decrease in group 2 and control group. Increase in AST level is in agreement with the findings of Uthman *et al* (2013) who found elevation in transaminase enzyme (AST and ALT) in rat when treated with diazinon, a component of pesticide. Elevation in the serum level of this enzyme could be attributed to acute hepatocellular damage or extrahepatic dysfunction or persistent stress (Okeniyi, 2007); hence *Ota-piapia* is hepatoxic. Contrary to the report of Uthman *et al.* (2013), the level of ALP in this study showed no significant increase in all the groups of animals. From the study of Fawzy *et al.* (2007), they are of opinion that decrease in ALP level could be as a result of the effect on absorptive and secretory surface of the cell membrane causing cellular leakage and thus reduced activity. Increase in ALT level could also be due to hepatoxicity causing permeability alterations (Okeniyi, 2007).

Total proteins and albumin, which help in maintaining blood osmotic pressure, are a measure of synthetic function of the liver (Sarhna *et al*., 2011). The signification elevation in total proteins in all the treated groups when compared to the control group indicates a reduction in the synthetic function of the liver. Significant decrease in the total protein level in the experimental group 4 animals might be due to the catabolism of protein or malfunctioning of liver (Harper *et al.*, 1977). Swamy *et al*., (1992) have similarly reported that the decrease in total proteins indicates their metabolic utilization. Total bilirubin formed from the breakdown of red blood cells by hepatocytes, are used to determine the extent of hepatocellular damage (Sarin and Gill, 1998). The result of this study suggests that the *Ota-piapia* caused some significant hepatocellular damage in the treated groups when compared with the control group, as there was a noticeable increase in bilirubin level of the treated groups. This result is in conformity with the findings of Uthman *et al.* (2013), who noticed an increased level of bilirubin when rat was treated with pesticides.

Following administration of chemical compounds, toxicants are transported by the blood to various organs including the liver, where they may exert their effects. A good pathological and physiological indicator of animal health is the blood (Hamid *et al.*, 2013). In this study, a decline in the level of RBC, PCV and haemoglobin levels in the course of administration of *Ota-piapia* is indicative of anaemia. This is in agreement with the findings of Holy *et al* (2015) following intraperitoneal administration of

dichlorvos. Red blood cell indices reflect the level of haemoglobin content of the red blood cells and aids in the diagnosis of the cause of anaemia. The haematological indices of the experimental animals when compared with the control showed that the experimental animals had gradual increase in the mean values over the period of exposure in RBC, PCV, (except in group 4) and MCH, Hb (except in group 3). The MCH, MCV and HB values suggest that normocytic normochromic anaemia might have occurred. This could be attributed to the destruction of the red blood cells by *Ota-piapia* beyond the production capacity of the bone marrow and the fall in the level of iron content (Holy *et al*., 2015). Hamid *et al.* (2013) reported that low values of RBC, Hb and PCV could be a result of possible disruption of haematopoiesis. However, the anaemia caused by *Ota-piapia* alleviated during the recovery phase or withdrawal of *Ota-piapia*, resulting in the restoration of RBC, PVC and haemoglobin.

During the administration of *Ota-piapia,* an elevation in lymphocytes and decrease in neutrophils which was observed in this study is indicative of leukocytosis (Celik *et al*., 2009). This finding is in concordance with the observation of Holy *et al*. (2015) where he similarly recorded an increase in the level of lymphocytes following the administration of dichlorvos. Leukocytosis may be caused by benign conditions such as infections, inflammation-tissue necrosis, stress and haemolytic anaemia (Hamid *et al.*, 2013). In this study, there was a noticeable decline in the level of blood platelets, which is contrary to the result of Celik *et al.* (2009); they opined that their experimental rats had secondary thrombocytosis at day 21 and 28 on administration of dichlorvos and this was due to an increase in the level of blood platelets. The mean values of monocyte, eosinophil and basophil were almost steady constant throughout the period of the experiment, indicating that they were not affected by the *Ota-piapia*.

Conclusion

The findings of the study shows that locally formulated *Ota-piapia* pesticide have significant effect on experimental *wistar* rats at different concentration (low and high dose). When the experimental albino rats were expose to the locally formulated *Ota-piapia* (Exposure phase) on biochemical parameters, the result shows a decrease in almost all the biochemical parameters except protein. With regards to the blood parameters, the result shows an increase and decrease in the blood parameters on the experimental animals. Thus, no significant recovery was observed in both high dose on MCHC, MCV, HB, neutrophil and lymphocyte after four weeks of cessation of treatment as well as all the treated levels of Eosinophil and basophil shows no recoveries.

Recommendation

We therefore, recommend following the outcome of the research thus:

- That it is a matter of public health concern to regulate and monitor pesticide residues in foods and human in order to assess the food safety risk and population exposure to pesticides.
- The illegal production and use of *Ota-piapia* by traders and consumers with little or no knowledge of public health policy must be checked through and adequate control of the trade and the enforcement of appropriate sanctions by government agency.

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