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# Histopathological Changes in the Brain, Heart and Lungs of Wistar Rats Exposed to Sub-Lethal Dose of a Locally Formulated Pesticide Ota-Piapia

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## Abstract

*This study presented histopathological changes in the brain, heart and lungs of Wistar rats exposed to sub-lethal doses of a local pesticide Ota-piapiaused to control mosquitoes in Nigeria. A total of 24 rats of both sexes weighing between 180-200g were used for this study. The rats were acclimatized for four weeks, fed twice daily and water givenadlibitum. After acclimatization, the rats were randomly divided into four groups; 1, 2, 3, and 4, with six rats per group. Group1 served as control with no treatment administered. Rats in groups 2, 3 and 4 were exposed to 5 ml, 8 ml and 10ml sub-lethal doses (for 4 hours) of Ota-piapiarespectively for twelve weeks at interval of three days. Twelve of the rats were kept for additional four weeks after ceasation of exposure. Twenty four hours after the last exposure, the animals were anesthetized using chloroform and euthanized by cervical dislocation. Sections of the brain, heart and lungs were harvestedin 10 % buffered neutral formalin and processed for histopathology. Result revealed congestion, necrosis of neurons and vacuolation in brain; congestion, haemorrhage and necrosis of cardiac muscle fibres. In the lung, there were thickened inter-alveolar walls, massive infiltration of the bronchiole by inflammatory cells, congestion and haemorrhages. There were no significant observable differencesin the histopathological findings in the animals kept for the additional four weeks without treatment. Therefore, it was concluded that locally formulated Ota-piapiapesticides are hazardous to the health of the test animals and the use of insecticides treated mosquito nets should be used rather than pesticides.*

Keywords: *Histopathological changes, sub-lethal,Ota-piapia, congestion, haemorrhage*

## Introduction

Malaria is a common disease in tropical and subtropical regions, with an approximate index of 350-500 million caseswith majority of the incidents among children in Sub-Saharan Africa (Snow *et al.,* 2001). Malaria is cause by any of the four species of *Plasmodium falciparum, P. vivax, P.malariae* and *P.ovale.* Signs and symptoms include fever, headache, back pain, chills, sweats, myalgia, nausea, vomiting, diarrhea, and cough while coma, renal failure, pulmonary edema and death have been reported in untreated *P. falciparum* infection (CDC, 1997).

Humans label 'pests' as any plants or animals that endanger food supply, health, or comfort and pesticides have been used to manage these pests(Benbrook, 1991). Pesticide is "any substance or mixture of substances intended for preventing, destroying or controlling any pest; including vectors of human or animal diseases" (FAO, 2010).Pesticides can be chemical or biological in nature and their biological

activity is determined by its active ingredient (AI- also called the active substance). The active ingredient are formulated with other materials commonly sold, but it may be further diluted with other substances such as oil, alcohol, kerosene,water and other compatible soluble solvent. The formulation improves the handling, storage and application of pesticides and may influence effectiveness and safety (Burges, 1998). Also, most pesticides formulation includes carrier substances that improve absorption in addition to the active ingredients.

Local pesticides manufacturers in Nigeria emphasize the potency of their pesticides by the word *"Ota-piapia"* indicating that such products will completely take care of our little pest problem (Mortui, 2006). Their acceptance and wide spread proliferation in Nigeria have been due solely to their efficacy, accessibility and affordability (Essiet*et al*., 2009). The locally formulated products are still not registered with the National Food, Drug and Control Agency (NAFDAC) (Akunyili, 2007), but have been commonly used as insecticides, especially against mosquitoes the vector of plasmodium parasites (Foll*et al.,* 1965). They have also been employed in food preservation, on grains for prevention of insect infestation (FAO, 2001). The local formulation of *Ota-piapia* entails dispensing/repackaging into small (about 10-15 ml) retail bottles of the pesticide which is unspecified from those imported, with trade names as dichlorvos, cypermethrin, gammalin 20, gammalin super, lindane, capsitox 20 (PAN, 2007).Some locally made pesticides have caused the death of many individuals in Nigeria (Olebunne, 2009), and worldwide (USEPA, 2007), especially through food contamination from acute or chronic exposures (Akunyili *et al*., 2007). Children have been reported to beprone to accidental poisoning by these products (Okeniyili*et al*., 2007).

Therefore, this study presented the histopathological effects of sub-lethal dose of a locally formulated pesticide *Ota-piapia* on brain, heart and lungs of Wistar rats.

## Materials and Methods

### Ethical Approval

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

### Chemicals Used for the Study

Locally formulated *Ota-piapia* pesticide was purchased from Central Market Kaduna, Nigeria. 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. Unconsumed feeds were cleared every day to maintain standard hygienic environment.

### Experimental Design

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 mlto 10 ml 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.

Four identical rooms (four by four square metres)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 roomwith sub-lethal dosesof 5ml, 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 usinghypodermic syringes by sprinkling it inside the exposure roomand 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). The second phase (recovery phase) was allowedto 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 formalinand processed using standardhistopathology procedure as described by Luna (1968).

## Results

**Table 1.** *Clinical signs and physical evaluation of exposed animals to a locally formulated pesticideOta-piapia*

| Treatment Group | Group 1 | Group 2 (5ml) | Group 3 (8ml) | Group 4 (10ml) |
|---|---|---|---|---|
| Clinical signs | -No activities | -Mild drowsiness<br>-Pruritus itching and salivation<br>-Bulging of the eyes | -Moderate drowsiness and weakness of the body<br>-Moderate ruritus itching and salivation<br>-Bulging of the eyes | - Severe drowsiness and continues weakness of the body<br>-Continues and severe pruritus itching and salivation<br>-bulging of the eyes and pale mucus membrane |

**Table 2.** *Clinical signs and physical evaluation of animals after exposure to a locally formulated pesticide Ota-piapiarecovery phase*

| Treatment Group | Group 1 | Group 2 (5ml) | Group 3 (8ml) | Group 4 (10ml) |
|---|---|---|---|---|
| Clinical signs | -No activities | -No activities | -No activities | - No activities |

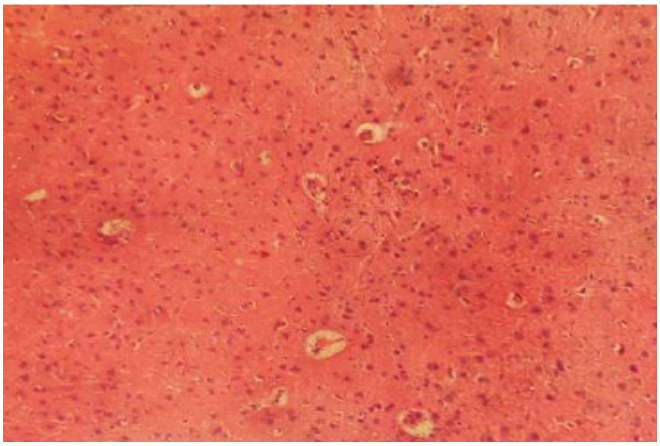

**Plate I.** *Photomicrograph of brain of Wistar rat(Group 1) showing normal tissue architecture.H&E x200.*

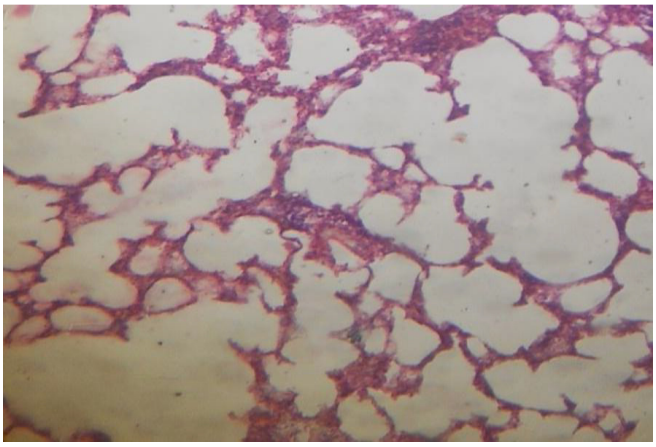

**Plate II.** *Photomicrograph of lung of Wistar rat(Group 1) showing normal tissue architecture.H&E x200.*

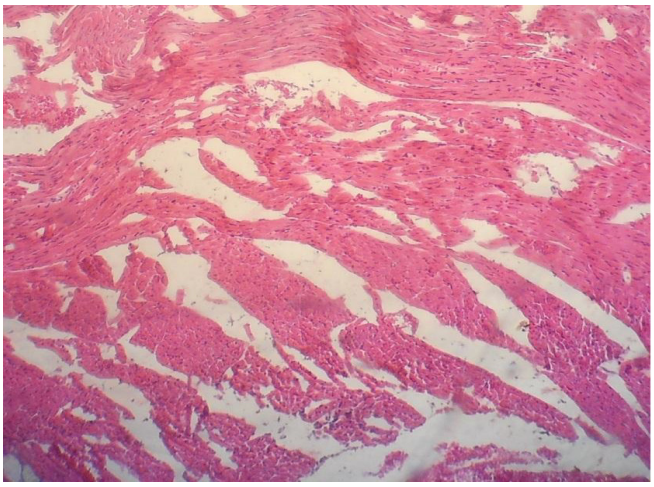

**Plate III.** *Photomicrograph of heart ofWistar rat (Group 1) showing normal tissue architecture. H&E x200.*

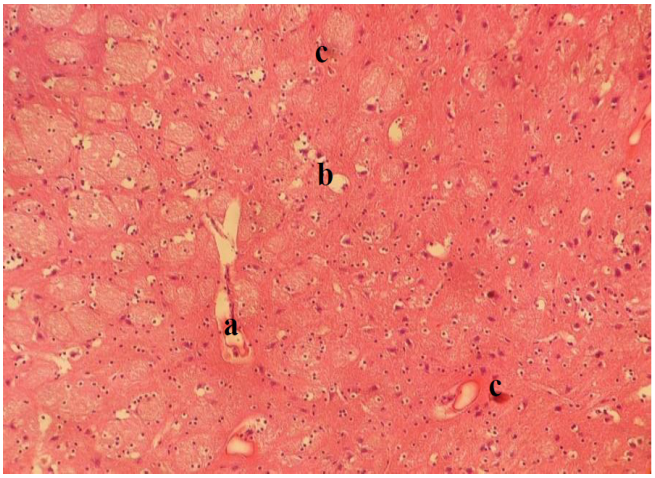


**Plate IV.** *Photomicrograph of brain ofWistar rat administered with 5mls of locally formulated Ota-piapia pesticide. Note congestion (a), vacuolation (b) and necrosis of neuron (c).H&E x200.*

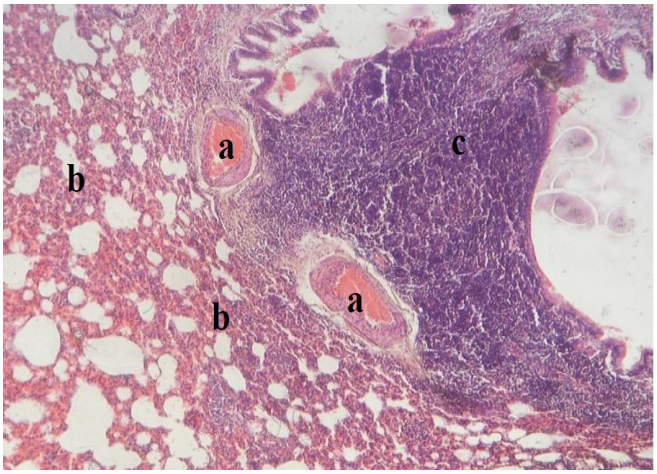


**Plate V.** *Photomicrograph of lung ofWistar rat administered with 5mls of locally formulated Ota-piapia pesticide. Note congestion (a), thickened interalveolar walls (b) and massive inflammatory cellular infiltrations of the bronchiole (c).H&E x200.*

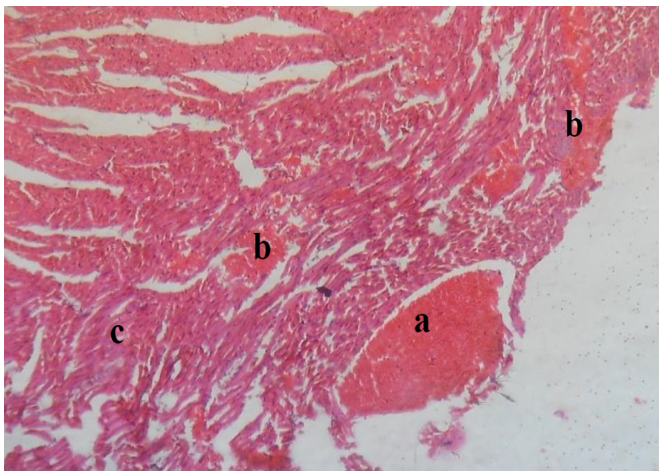


**Plate VI.** *Photomicrograph of heart of Wistar rat administered with 5mls of locally formulated Ota-piapia. Note congestion (a), haemorrhage (b) and necrosis of muscle fibres (c).H&E x200.*

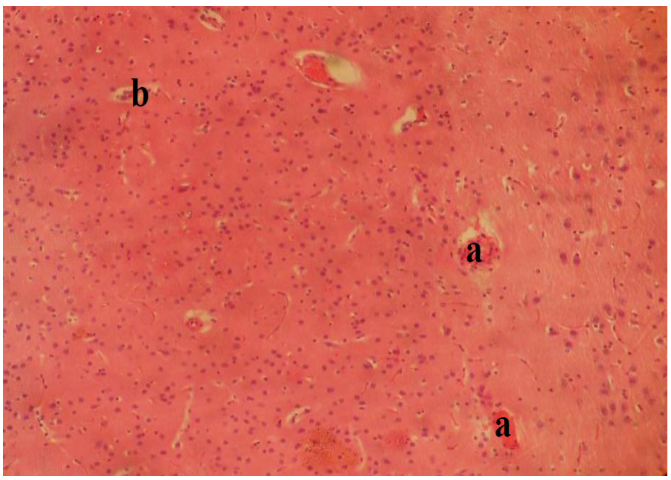


**Plate VII.** *Photomicrograph of brain ofWistar rat administered with 8mlsof locally formulated Ota-piapia pesticide. Note congestion (a) and necrosis of neuron (b).H&E x 200.*

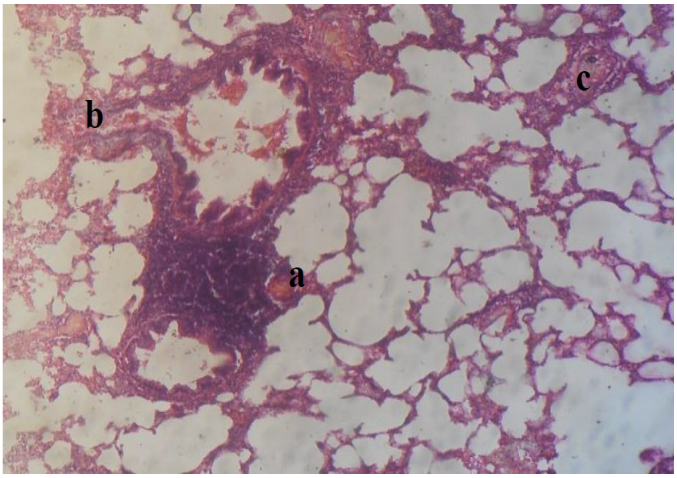


**Plate VIII**. *Photomicrograph of lung ofWistar rat administered with 8mlsof locally formulated Ota-piapia pesticide. Note congestion (a), haemorrhage (b) and thickened interalveolar wall (c).H&E x200.*

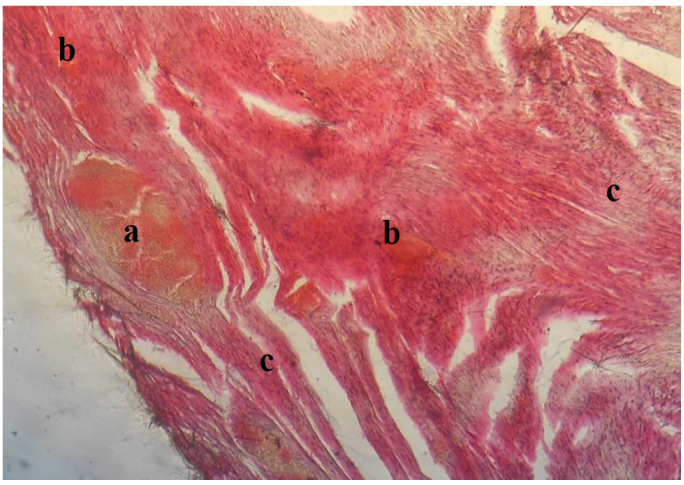


**Plate IX.** *Photomicrograph of heart ofWistar rat administered with 8mlsof locally formulated Ota-piapia pesticide. Note congestion (a), haemorrhage (b) and necrosis of muscle fibres (c). H&E x200.*

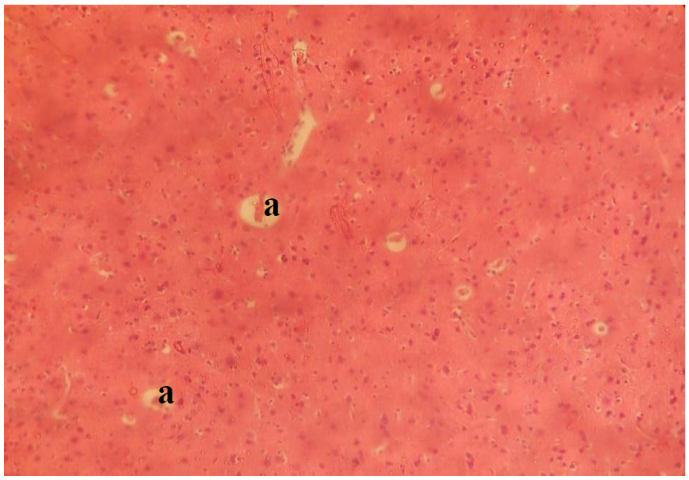


**Plate X.** *Photomicrograph of brain of Wistar rat administered 10mlsof locally formulated Ota-piapia pesticide. Note vacuolations (a).H&E x200.*

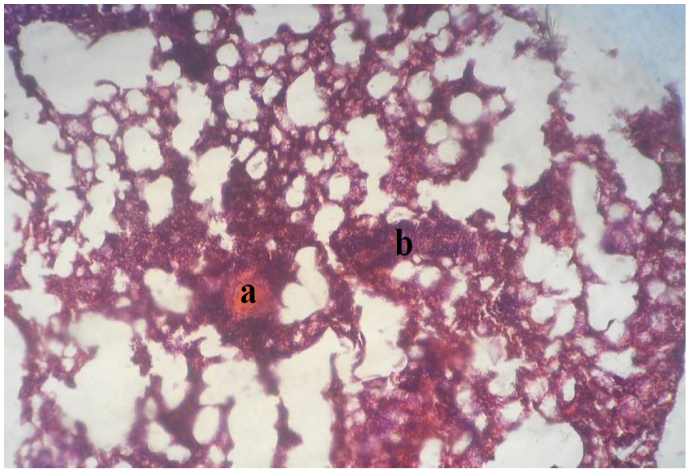


**Plate XI.** *Photomicrograph of lung of Wistar rat administered with 10mlsof locally formulated Ota-piapia pesticide. Note congestion (a) and thickenedinteralveolar walls (b).H&E x200.*

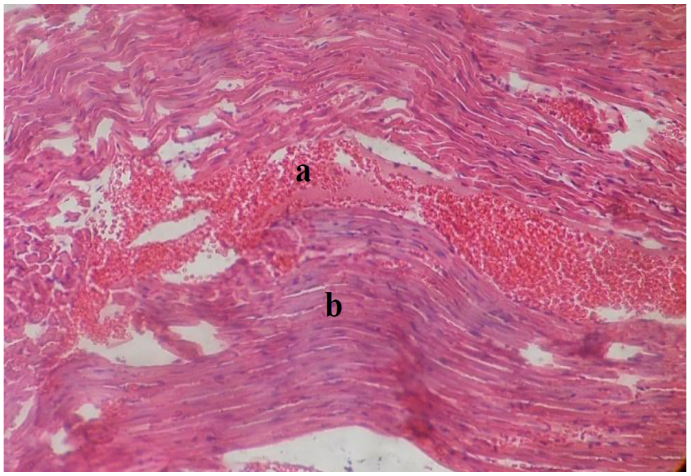


**Plate XII.** *Photomicrograph of heart of Wistar rat administered 10mlsof locally formulated Ota-piapia pesticide. Note haemorrhages (a) and necrosis (b) of muscle fibres.H&E x200.*

## Discussion

The major constituents and active ingredients of locally formulated pesticide *Ota-piapia*used in this study were dichlovors and lambda-cyhalothrin, as the entire sample run revealed the peaks of dichlovors and lambda-cyhalothrin standard (Ofordile, *et al.,* 2014). The locally formulated pesticides sold in Nigerian market contain dichlovors and Lambda-cyhalothrinas reported by Ofordile*et al* (2014). Musa *et al*(2010) reporteddichlovors as a major constituent of *Ota-piapia*. The findings of Khan *et al* (2005) revealed that *Ota-piapia*pesticides contain dichlorvos, cypermentrin, kerosene, alcohol and other organic solvents; and these are known to have adverse effects on the liver and kidney. *Ota-piapia* could cause toxicity in non-target species of animal (Cantalamessa, 1993).

In this study, exposure of Wistar rats to locally formulated *Ota-piapia* induced clinical symptoms such as drowsiness, blurred vision, severe salivation, pruritus itching, weakness of the body and vomiting. WHO (1990) and UNEP (2004) have earlier stated that pesticides containing dichlovors and organophosphate could cause such symptoms. Dichlovors has been reported to be rapidly absorbed by the skin, gastrointestinal and respiratory tracts (CERI, 2007) and inhibit acetylcholinesterase (Harlin and Dellinger, 1993). Lambda-cyhalothrin (LTC),a synthetic pyrethroid insecticide very similar in structure to pyrethrins, but more toxic has been reported to cause damages in the lungs (El-din *et al*., 2014), kidney (Fetoui*et al*., 2014) and thyroid gland (Al-Amoudi, 2018) of rats through DNA destruction. The lesions (vacuolations and necrosis of neurons) observed in the brain of Wistar rats in this study were due to the inhibition of acetyl cholinesterase by dichlovors. Also, the lesions observed in the heart were due to the cardiotoxic effects of dichlovors (Naidu *et al*., 1987) and LTC (Al-Amoudi, 2018). The thickened interalveolar walls resulted from the response of the lungs to the toxin characterized by erosions of alveolar surfaces, inflammatory cellular infiltrations and increased mucus production.

Since pesticides are chemicals meant to kill or ward off unwanted living organisms, it is not surprising that they could produce adverse health impacts in man and animals. Report of several researches by Eisler (1989), Nebeker*et al* (1992), Menegaux*et al* (2006) and Ofordile*et al* (2014) showed that exposure to pesticides constitutes sources of potential hazard in both man and animals and that prolonged exposure through inhalation caused changes in tissue architecture, cellular activities, liver damage, respiratory arrest and brain malfunction.

## Conclusion

It was concluded from this study that exposure of animals to sub-lethal doses of locally formulated pesticide*Ota-piapia* can lead to tissue damage which may pose health risk in laboratory animals and its ameliorationcould be difficult to achieve even after cessation of exposure. Hence, its use and misuse domestically or occupationally should be with caution and subjected to closer regulation by environmental and health protection agencies.

## Limitations

Tissue concentrations of the insecticide were not ascertained. Also the effects on haematological parameters were not determined.

## Recommendations

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

I. We therefore call on Government agencies whom are at the forefront of translating research findings to the nation through various workshops to help in creating more awareness to the general public on some of the long term effect of misuse of such locally formulated pesticides in humans and the environment.

II. There is therefore, the need for mass education of the general public to effectively address the issues involved in pesticide use, misuse, and abuse in the country.

III. Finally, we recommend that the general public employ the use of insecticides treated mosquito nets than residual pesticides and other forms of insecticides use (CDC 1996).

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