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Research Article

Acute toxicity (LD_{50}) and histopathological effects of local insecticide (*Ota-piapia*) in male Wistar rats via dermal, inhalation and oral routes

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Ota-piapia, Ld_{50}, insecticide, toxicity.

Abstract

Insects in the home and environment are a constant menace that we contend with every day. Insecticides though a solution, is uneconomical to most households. *Ota-piapia* is a famous somewhat cheap locally produced insecticide. The acute toxicity (Ld_{50}) of this insecticide through dermal, oral, and inhalation routes in male Wistar rats was investigated. Toxicity indices included behavioral and clinical signs, body weight, and histology of selected organs. For oral and dermal routes, there were 33% to 100% mortality rates at 25%, 50%, and 100% administrations, while the inhalation route had 0% mortality. Toxicity signs observed ranged from slightly to moderate to high hazardous irrespective of the route of administration. The clinical signs for dermal and oral routes were aggressiveness, confusion, bristly hair and bulgy eyes, brown hair on the head and shaking of the tail. Fast breathing, coughing, and nose bleeds were observed for the inhalation route. Histopathology of selected organs revealed that the kidney tissue showed damage in the glomeruli and kidney tubules irrespective of the route of administration or concentration of insecticide applied. The liver tissue had areas of congested vascular channels in different ranges and also sighted areas of lymphocytic infiltration. For lungs (oral and dermal routes), the damage was not so pronounced but congested vascular channels were observed. For the testis (dermal route only), the mildest damage was seen, but with an increase in the percentage of *ota-piapia* administered, there was distortion in testicular morphology. However, these findings indicate that ota-piapia should be taken with utmost caution and the preferred route of administration is via inhalation route.

1. Introduction

The home and environment are constantly besieged by insects. The obvious answer to insects/pest control is the use of insecticides. The choice of insecticides varies and depends on the insect problem inherently dealt with. Most insecticides used today are either man-made or based on synthetic formulas like carbamates or pyrethroids, i.e. natural or chemical-based. These types are usually made from heavy materials like boric acid and silica gel. Insecticides that are inorganic in origin are mostly effective and are of different types, but care must be applied to their usage and application [1-2]. In contrast, systematic insecticides, contact insecticides, ingested insecticides, and herbicides are eco-friendly and cause no harm to crops because they are made from plant oils or fatty acids derived from plants or animals [3-6].

Nigeria is a third-world country where most households are of low-income status so these

insecticides are too expensive to buy. Also, the volume of insects in the environment creates a variety of insecticides to choose from [7]. The antidote or succor to this is the production of the local insecticide called *ota-piapia* (which translates to kill and dry). This local insecticide is produced all over the country, and surprisingly, some studies have found that it contains dichlorvos, the main insecticide ingredient, although in various concentrations about 1-10% [8-9]. The means of administration of this insecticide is through spraying in homes and environment, which somewhat causes the 'sprayer' to be in contact via the mouth (oral), skin (dermal), and nose (inhalation). Another issue is the formulations through which ota-piapia is administered, as it varies with the user.

Most pesticides are poisonous to humans or animals and toxicity levels are determined either acutely (one large dose) or chronically (repeated small doses). The most common bioassay used in assessing toxicity is known as LD_{50}, which is the lethal dose at which 50% of the population dies, within a given period under controlled laboratory conditions. The LD_{50} is usually an initial screening step in the assessment and evaluation of the toxic characteristics of a substance.
In Nigeria, the producers of insecticides indicate the effectiveness of their product by using the general name '*ota-piapia*' which translates to something that would completely eradicate their insect problem [10]. The acceptability of this insecticide and its supposed fame are due to its cheap production methods, ready accessibility, and relative cheapness [11]. The National Agency for Food Drug and Administration Control (NAFDAC), the accepted regulatory body in Nigeria has yet to register this product [12]. Despite this, this product has wide applicability as it has been used as an insecticide in the control of mosquito and insect infestations, and for preventing food storage. This product is mostly associated with Nigeria.

Some studies have indicated that *Ota-piapia* is an unidentified insecticide and its application is seen as a dangerous practice since its chemical constituent is yet to be determined [12, 13-14, 15]. *Ota-piapia* is a vernacular (Igbo) of eastern Nigeria origin and a generally used household name for local insecticide/ rodenticide/pesticide, the word means "that which completely consumes/devours" [15]. Some researchers have looked at the constituents in these local formulations, Dawodu et al. [16] using GCMS, found 43 constituents of which Dichlorvos was a major fraction, Hati et al. [15], used Gas Chromatography-flame ionization detection (GC-FID) on 100 samples of *ota-piapia* formulations collected in Northern Nigeria, and also discovered dichlorvos as a major constituent in all formulations in varying quantities. Considering the economic status of most Nigerians, it is almost impossible to purchase registered and approved insecticides. Therefore, the alternative is to use this somewhat cheap alternative to control their household insect menace. For example, *ota-piapia* has been used to control bed bug infestations in Ilorin, Nigeria [17]. The administration of this insecticide is also not specified on these bottles, unfortunately, there are many of them with their compound name being mostly *ota-piapia*. The study aimed to determine the acute toxicity (LD_{50}) of the local insecticide (*ota-piapia*) via dermal, oral, and inhalation routes in male Wistar rats.

2. Materials and methods

2.1. Chemicals

All reagents, chemicals, and solvents used in this study were purchased from Kadlad Chemical Laboratory, Osogbo, Osun State, Nigeria. The local insecticide product (*ota-piapia*) used in this study was purchased from a local market in Ibadan, Oyo State, Nigeria. The choice of brand was based on its high consumption rate among those available in the market.

2.2. Research experimentation protocol

The research protocol was approved by the Animal Care Committee of the Department of Science Laboratory Technology, Federal Polytechnic Ede, Osun State. Animal usage followed the animal guidelines for the protection and usage of animals for experiments of the same institution, adapted from the animal care guidelines of the National Academy of Sciences-National Research Council (NAS-NRC) [18].

2.3. Behavioral evaluation

Behavioral parameters like drowsiness, bristly hair, aggressiveness, bulgy eyes, confusion, and weakness were assessed and recorded as signs of toxicity after insecticide exposure. They were scored and labeled using scales like slightly hazardous to indicate mild

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effects Animals were observed daily, with daily weight intake used as a form of physical assessment criterion [19-20].

2.4. Experimental design

Thirty-six healthy adult male Wistar rats (weighing about 100 – 150 g) were obtained from Obafemi Awolowo University, Osun State, Nigeria. The rats were housed in poly-acrylic cages, with no more than five animals per cage, and maintained under standard laboratory conditions (natural light/dark cycle, room temperature 22 ± 3^0C).

2.5. Animal handling

Wistar male albino rats 100-150 g were selected for this study. All animals were kept in a standard cage placed inside the animal house, with a controlled environment of air and temperature, with free access to water and feed. Animals were left for two weeks for acclimatization and fed with a standard dry rat pellet diet and tap water was provided *ad libitum*.

2.6. Sample Preparation

Preparation of 25% insecticide concentration: A 25 mL of the insecticide was measured and diluted with 75 mL of distilled water.

Preparation of 50% insecticide concentration: A 50 mL of the insecticide was measured and diluted with 50 mL of distilled water.

Preparation of 100% insecticide concentration: A 100 mL of insecticide (undiluted) was measured.

2.7. Animal grouping and insecticide administration

Control group: The control group was given normal feed and 100 mL normal saline).

Group A was exposed to insecticides through inhalation.

Group B was exposed to insecticides orally.

Group C was exposed to insecticides through the dermal.

These 3 groups were further divided into 3 subgroups 'a' to 'c' and exposed to different concentrations of insecticide as indicated below:

a= exposed to 25% (25mL insecticide/ 75mL distilled water v/v).

b= exposed to 50% (50mL insecticide /50mL distilled water v/v).

c= exposed to 100% (100 mL insecticide, undiluted).

2.8. Estimation of the dose range and percentage of mortality

An approximate LD_{50} can be determined by the so-called "up and down" or the "staircase method" using two animals and increasing the doses of the insecticide [21]. Four doses were chosen which were given via the 3 routes to four groups of rats, with three rats in each group, for the determination of the LD_{50} of the insecticide from 0% mortality to 100% mortality [22]. The animals were observed for 24 h and then on the 4^{th}, $6^{th,}$ and 14^{th} day, for any toxic signs and symptoms. After 24 h, the numbers of deceased rats in each group were counted, and the percentage of mortality was calculated using the graphical method of Miller and Tainter, [23] and Welkos and O'Brien [24].

2.9. Route of administration of insecticide to the rat

Three distinct routes of administration were used in this study namely oral, dermal, and inhalation routes. For oral administration, the cannula was inserted into a desired syringe containing the solution of drug or fluid test substance and held with the right (dominant) hand [25], while for dermal administration, the fur on the back of each rat was shaved and each group was exposed to the insecticide by applying it to the shaved part according to the designed concentration explained [26]. For inhalation administration, an inhalation chamber [27-29]. The insecticide was placed in the cage where the experimental animal was and it was observed. The chamber was a wooden box and the insecticide was kept in an inhaler format. The animals were placed in this chamber group-wise to inhale the insecticide for 5 min.

3. Results

Table 1 shows the acute toxicity screening results of *ota-piapia* in male Wistar rats. The insecticide was lethal via the oral and dermal routes, with a 100% mortality rate observed, while no mortality was observed irrespective of the dose administered via inhalation. Table 2 shows the behavioral toxicity signs. It was observed that all groups irrespective of route, expressed all levels of toxicity signs, with a slight difference at 25% insecticide application. Table 3 shows the body weight changes observed in the Wistar rats following insecticide application. There was a slight decrease in weight during the duration of the experiment, irrespective of the route of administration, when compared with the controls.

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Table 1. Acute toxicity screening (LD_{50}) of local insecticide in Wistar rats.

Insecticide (%)	Volume administration	Mortality (%)	
Control (saline)	1 mL	0	0
Oral 25%	1 mL	3/3	100
50%	1 mL	1/3	33
100%	1 mL	3/3	100
Dermal 25%	1 mL	3/3	100
50%	1 mL	3/3	100
100%	1 mL	1/3	33
Inhalation 25%	*	0/3	0
50%	*	0/3	0
100%	*	0/3	0

* indicates that 1 mL of respective concentrations was expressed in an inhaler and the animals were made to inhale in the chamber for 5 min.

Table 2. Toxicity signs following oral, dermal, and inhalation routes of insecticide applications.

Treatment Group	Oral					Dermal					Inhalation				
	T	A	B	C	D	T	A	B	C	D	T	A	B	C	D
Control	None	0	0	0	0	None	0	0	0	0	None	0	0	0	0
25%	S-H	3/3	0/3	3/3	3/3	S-H	3/3	0/3	3/3	3/3	S-H	3/3	0/3	3/3	3/3
50%	M-H	3/3	3/3	3/3	3/3	M-H	3/3	3/3	3/3	3/3	M-H	3/3	3/3	3/3	3/3
100%	H=H	3/3	3/3	3/3	3/3	H-H	3/3	3/3	3/3	3/3	H-H	3/3	3/3	3/3	3/3

T-Toxicity, A- Aggressiveness, B- Bristly hair, C- Confusion and D- Drowsiness, S-H—slightly hazardous. M-H—moderately hazardous, H-H—highly hazardous.

Table 3. Body weight changes following administration of ota pia pia via oral, dermal and inhalation routes.

Days	Oral				Dermal				Inhalation			
	Control	25%	50%	100%	Control	25%	50%	100%	Control	25%	50%	100%
1	140.1	122.3	168.3	170.3	160.3	169.0	169.5	198.2	143.3	154.5	180.2	237.10
3	155.2	125.4	131.4	166.4	170.1	131.0	168.3	198.3	150.2	157.3	183.4	168.3
5	166.2	126.3	167.2	166.2	178.3	165.4	165.3	180.4	155.2	152.2	188.3	229.4
7	168.3	130.2	166.2	168.5	160.4	170.2	168.4	185.5	160.3	146.4	190.0	190.3
9	170.1	123.1	162.4	165.5	168.0	180.0	165.2	170.4	166.2	160.3	180.2	199.2
11	173.3	125.3	170.4	171.4	172.3	173.3	170.1	199.2	170.4	167.2	170.3	188.4
14	175.2	124.4	168.3	169.2	180.1	168.2	173.2	186.5	180.5	157.2	170.3	188.4
Mean	164.7	125.2	161.0	168.2	169.9	165.3	168.6	188.4	160.9	156.4	180.4	200.2
+/-	±	±	±	±	±	±	±	±	±	±	±	±
SD	13.1	2.5	13.	2.0	7.8	15.8	2.8	10.9	12.6	6.5	7.8	24.5

Table 4. Clinical Signs observed via local insecticide ota-pia pia application.

Route of administration	Clinical signs
Oral	Drowsiness, bristly hair, aggressiveness, bulgy eyes, confusion, weakness
Inhalation	Fast breathing, coughing, blood coming from the nose.
Dermal	Bulgy eyes, drowsiness, bristly hair, brown hair on the head, weakness, confusion, shaking of the tail.

The clinical signs observed in the rats after insecticide administration were peculiar to each route, as shown in Table 4. The histopathological effects of ota-piapia on selected organs are shown below. Plates 1-3 represent cross-sections of liver tissue at 25, 50 and 100% local insecticide applications via oral, dermal, and inhalation routes. Plates 4-6 represent cross sections of the kidney, tissue at 25, 50, and 100% local insecticide

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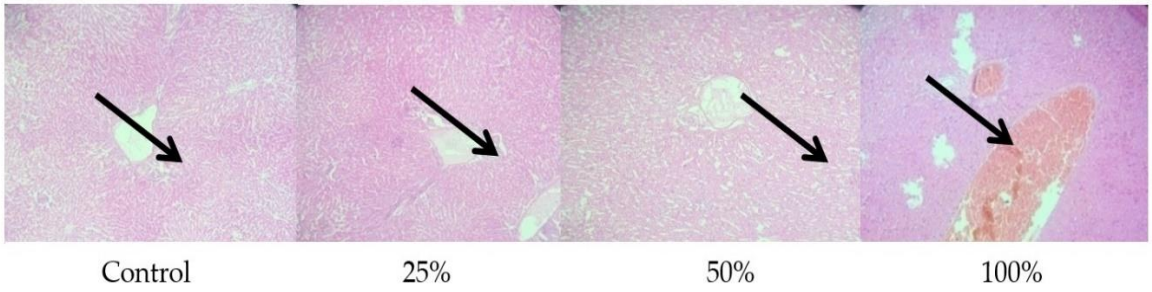

Plate 1. Photomicrographs of cross sections of rat liver via oral route (H & E, X 100).
Control: Section shows hepatic tissue with preserved lobular architecture. The portal tract and rachating sinusoids are also seen.
25%: Section shows hepatic tissue with normal histo-architecture. The portal tract is well differentiated. There are however slightly congested vascular channels.
50%: Section shows hepatic tissue. The vascular channels appear congested while there are local areas of edema. Also seen is lymphocytic infiltration in areas while there is noticeable cytoplasmic degeneration.
100%: Section shows hepatic tissue with distorted architecture. There is marked fibrosis of some portal tracts with areas of lymphocytic infiltration. Also seen are areas of necrosis and vascular degeneration.

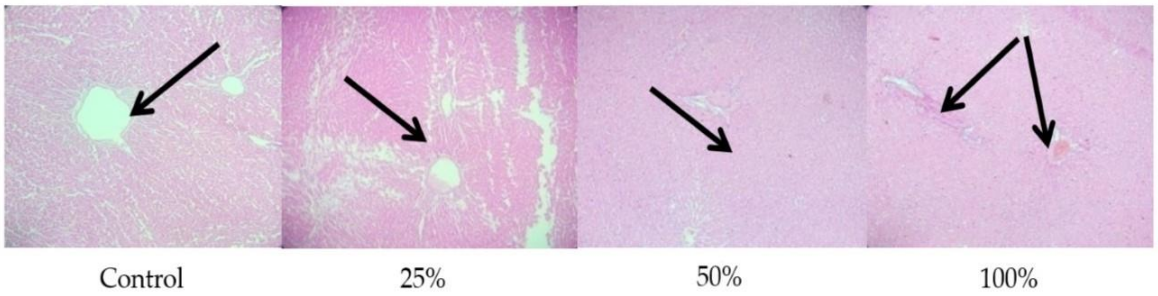

Plate 2. Photomicrographs of cross sections of rat liver via dermal route (H & E, X 100).
Control: Section shows hepatic tissue with well-preserved architecture. The hepatic lobules are well arranged with well-differentiated sinusoids. The portal trod is intact.
25%: Section shows hepatic tissue. The vascular channels are slightly congested while there are areas of edema.
50%: Section shows hepatic tissue. The portal tract is unremarkable. Also seen are areas of hemorrhage.
100%: Sections show hepatic tissue. There is mild to moderate edema within the limited plates and sinusoids.

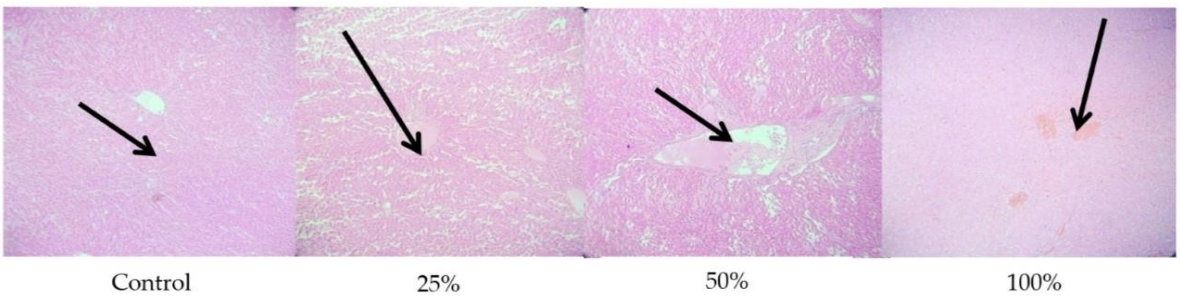

Plate 3. Photomicrographs of cross sections of rat liver via the inhalation route (H & E, X 100).
Control: Section shows hepatic tissue with normal histo-architecture. The portal tract is intact with regular radiating sinusoids.
25%: Section shows hepatic tissue. The portal tract is unremarkable while there are noticeably congested vascular channels and prominent areas of edema.
50%: Section shows hepatic tissue histo-architecture. There is mild to moderate edema within the sinusoids and areas of lymphocytic infiltration.
100%: Section shows hepatic tissue histo-architecture which is slightly distorted. There is mild to moderate edema within the limiting plates sinusoids and areas of vacuolar degeneration.

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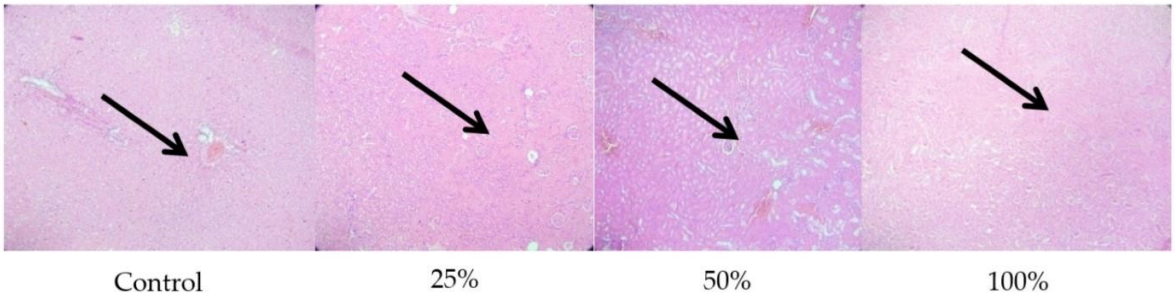

Plate 4. Photomicrographs of cross sections of rat kidney via oral route (H & E, X 100).

Control: Sections show renal parenchyma tissue. The glomeruli and bowman's capsule and remarkable and the renal corpuscle is intact.

25%: - Section show renal tissue. There are marked areas of edema and lymphocytic infiltration.

50%: Section shows renal tissue. There is necrosis of the proximal convoluted tubules and infiltration of mononuclear cells. Also seen are areas of hemorrhage within the interstices.

100%: Section shows renal parenchyma tissue. There is moderate necrosis of proximal convoluted tubules. Also seen are areas of amorphous cellular eosinophillic secretion. There are also infiltrations of mononuclear cells.

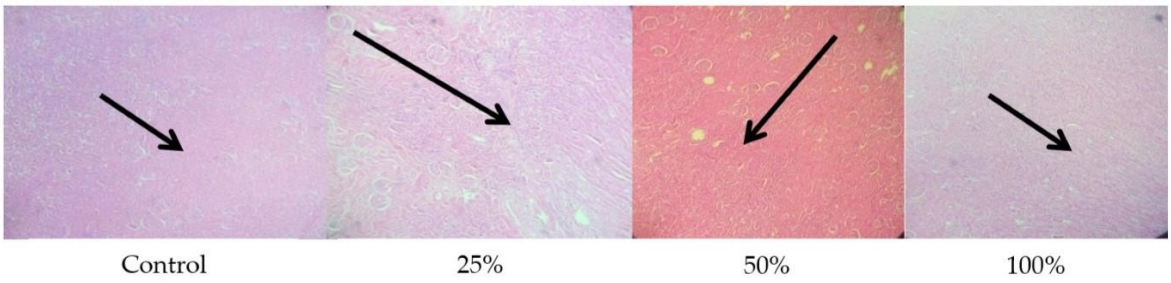

Plate 5. Photomicrograph of cross sections of rat kidney via inhalation route (H & E, X 100).

Control: Section shows renal tissue with normal histo-architecture. The glomeruli and Bowman's capsule are intact. The proximal and distal tubules are distinct.

25%: Section shows renal tissue. There are areas of necrosis within the distal tubules.

50%: Section shows renal tissue histo-architecture. There are areas of extensive lymphocytic infiltration and necrosis within the distal tubules.

100%: Section shows renal tissue histo-architecture. There are distorted areas of extensive lymphocytic infiltration. Congested vascular channels and coupled with areas of necrosis.

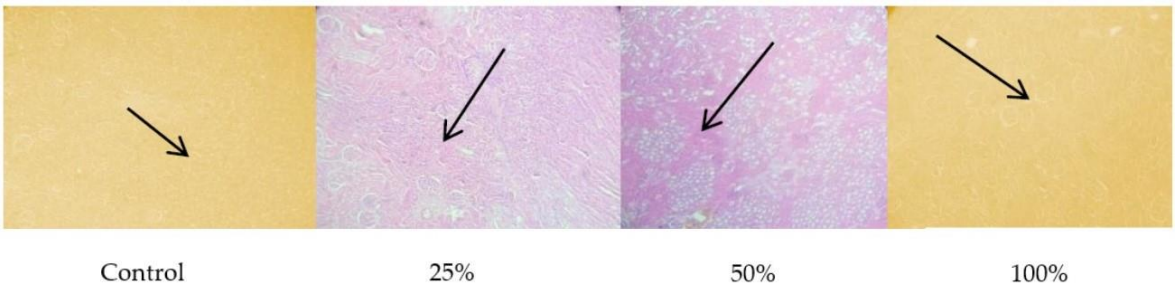

Plate 6. Photomicrograph of cross sections of rat kidney via dermal route (H & E, X 100).

Control: Sections of the kidney show well preserved histo-architecture with accurate number of glomeruli within the Bowman's capsule. The proximal and distal tubules is also distinct

25%: Sections of the kidney shows normal histo-architecture. The Bowman's capsule is adequate. There are areas of edema within the stroma.

50%: Sections of the kidney shows renal histo-architecture. The renal capsule and interstitial spaces are edematous. The proximal and distal tubules are unremarkable. Also seen are areas of hemorrhage.

100%: Section shows renal tissue histo-architecture. There are areas of necrosis and areas of lymphocytic infiltration.

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applications. Plates 7-8 represent cross-sections of lung tissue at 25, 50 and 100% local insecticide applications via oral and dermal routes, while plate 9 represent cross-sections of testis tissue at 25, 50 and 100% local insecticide applications via dermal route only.

4. Discussion

Pests in home and the environment are a common menace to many households. The effective control of these 'vermin' is an ongoing fight in many homes. Unfortunately, the choice of pesticide is determined by the economic capacity of many households. *Ota-piapia* is a cheap alternative to dealing with the pest problem. The relative safety of this local pesticide has not been considered hence, the effects of *ota-piapia* on selected organs of male Wistar rats via dermal, inhalation, and oral routes of administration were studied.

Body weight changes were reduced appreciably with an increase in the concentration of *ota-piapia* administered. Regarding the symptoms shown by the animals after insecticide exposure, these results indicated that weight gain was a good qualitative parameter to evaluate toxicity, as shown in the results obtained with pyrethroid administration [30]. The effects of inhalation, oral and dermal administration in male rats showed that, there was an increase in the weight of the control and it can be clearly seen that a high concentration of the insecticide affected the rat's body weight in the opposite way. The significant increase in the body weights of the control group throughout the experiment may have been due to an increase in feed intake. This was not the case for the groups exposed to insecticides, their respective weights only slightly deviated from the initial weight prior to insecticide administration.

The clinical signs observed in rats administered the local insecticide *Ota-piapia* varied with the route of administration. Fast breathing, coughing, and blood from nose were observed during inhalation. Dermal and oral routes of administration had some common signs such as drowsiness, bristly hair, confusion, bulgy, and weakness, with only aggressiveness distinct to oral administration, while brown hair on the head and shaking of the tail were peculiar to dermal administration.

Mortality rates observed within the experimental duration varied in all the groups administered different doses of insecticide. For oral and dermal routes of administration, there was a 33.3% to 100% mortality rate at 25, 50 and 100% administrations. While inhalation, it was found to be 0% mortality, irrespective of the concentration of insecticide administered. Toxicity signs observed ranged from slightly hazardous to highly hazardous, irrespective of the route of administration.

Histological investigations revealed marked areas of edema in the stroma for the oral and dermal routes, whereas with the inhalation route, there were areas of extensive lymphocytic infiltration in the kidneys. These findings are in agreement with Gupta [31], who reported extensively on the effect of organophosphate and carbamate on glomeruli, and necrosis of the renal tubule section shows hepatic tissue with distorted architecture. Marked fibrosis of some portal tracts with areas of lymphocytic infiltration was also observed. This finding is similar to that of Biernacki et al. [32] who studied cypermethrin, a synthetic pyrethroid used to control insect pests in the hom. There was mild to moderate edema was observed within the limited plates and sinusoids. These results are in accordance with those of Majumdar et al. [33], who reported glomerular and tubular necrosis in Wistar rats treated with oral, dermal, and inhalation doses of organophosphorus insecticides. This section shows renal tissue histo-architecture, with distorted areas of extensive lymphocytic congested vascular channels coupled with areas of necrosis. These results are consistent with those of Majumdar et al. [33]. The findings are also following Creasy et al. [34] who reported extensive lymphocytic infiltration with congested vascular channels coupled with areas of necrosis. Necrosis of the proximal convoluted tubules was also observed.

Irrespective of the route of administration, the degree of liver damage ranged from mild to moderate edema, congested tubules, and hemorrhages, with areas of vascular degeneration. The previous worker [35-39] observed areas of damage to the liver via diclorvos administration. Dawodu et al. [16] looked at the GCMS of this same *ota-piapia* brand and found

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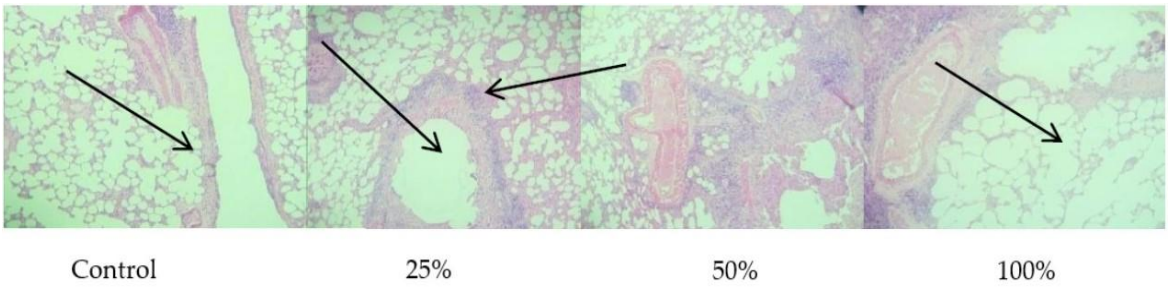

Plate 7. Photomicrograph of cross sections of rat lungs via oral route (H & E, X 100).

Control: Section shows pulmonary parenchyma tissue with well-arranged alveolar spaces. The bronchioles are also distinct and well-differentiated.

25%: Section shows pulmonary tissue. The alveolar septa and bronchioles architecture are slightly distorted. There are areas of mononuclear cell infiltration.

50%: Section shows pulmonary tissue histo-architecture. There are congested vascular channels while there is an increase in the lymphoid tissues layer. There are also areas of lymphocytic infiltration.

100%: Sections show pulmonary tissue. The alveolar spaces are lined by benign cuboidal epithelial cells. There are noticed congestion of the bronchioles while alveolar septa are collapsed in some areas also seen are prominent areas of lymphocytic infiltration.

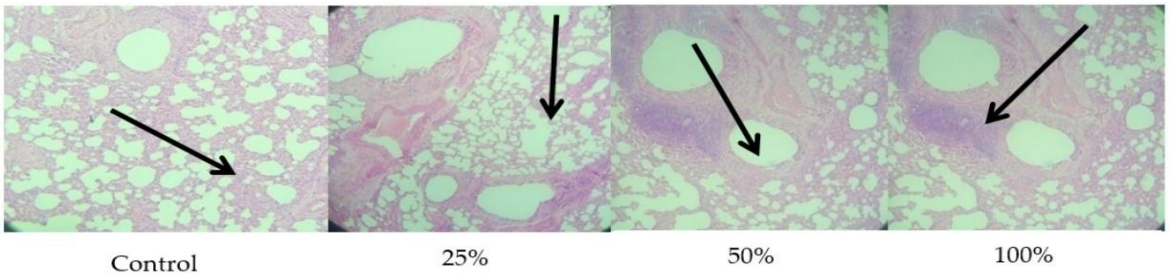

Plate 8. Photomicrographs of cross sections of rat lungs via dermal route (H & E, X 100).

Control: Sections show pulmonary parenchyma tissue composed of well-arranged alveolar and bronchioles, which are remarkable and distinct. Also seen are thick-walled vascular channels.

25%: Section shows pulmonary tissue with normal histo-architecture. The alveoli are well branched while the bronchioles are also distinct no visible damage is seen.

50%: Section shows pulmonary tissue with normal his architecture, bronchiolar and alveoli septa are remarkable also seen are congested vascular channels.

100%: Section shows pulmonary tissue with normal histo-architecture. The bronchiolar and alveoli septa are remarkable and also seen are congested vascular channels.

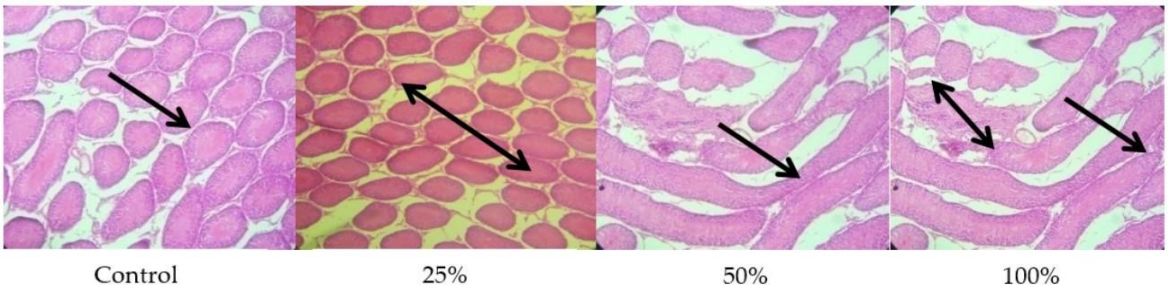

Plate 9. Photomicrograph of cross sections of rat testis via dermal route (H & E, X 100).

Control: Sections shows testicular tissue with normal histo-architecture. The seminiferous tubules and interstitial legdig cells are intact while within the tubules are spermatogenic cells of varying maturation stages.

25%: Section shows testicular tissue. The histo-architecture appears normal. The seminiferous tubules are of varying sizes while the spermatogenic cells are in varying stages of maturation. The capsule is also intact, except for areas of edema.

50%: Section shows testicular tissues with varying sizes of seminiferous tubules. The interstitial legdig cells appear eroded.

100%: Section shows testicular, histo-architecture. The testicular morphology is distorted while the seminiferous tubules have varying levels of necrosis and edema. The interstices are eroded while the capsule appears unremarkable and atrophied.

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diclorvos to be a major component of the insecticide. Benjamin et al. [40] and Angina et al. [41] observed the sub-lethal doses of *Ota-piapia* and found it somewhat toxic at that level when administered to Wistar rats.

Via oral and dermal routes of administration, the degree of damage was observed at 25% administration and progressed with an increase in concentration. Common damages observed were bronchioles being slightly distorted or congested. The lungs are one of the most sensitive organs in the body and early signs of lung toxicity or distress are distortion around the bronchioles [40].

A common feature of testicular damage is edema and distortion of testicular morphology. Similar to the present findings, Tiemann and Cigankova et al. [42-43] observed degeneration and depletion of spermatocytes and spermatids after insecticide exposure. Creasy and Foster [44] suggested that necrosis and loss of germ cells are the most frequent manifestations of testicular injuries.

5. Conclusions

This study showed that the best means of local insecticide (*Ota-piapia*) administration is inhalation and therefore it is suggested to use in households. Histological results from the study revealed that local insecticides could cause damage to the liver, lungs, and hence, prolonged exposure should be avoided.

For safe handling, hand gloves and nose masks should be worn during the preparation of diluted solutions. Hands should be washed and disinfected after the application of insecticides. The insecticide should be kept in a cool, dry place because of its volatile nature. When not in use, it should be kept in a tight, sealed, and non-perforated vessel. For household usage, the dilution factor should be 25% for insecticide and 75% for water.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, Copilot, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conceptualization, OD, MA; methodology, OD, KK; formal analyses investigation and writing-original draft preparation, K.K.; resources O.D., M.A.; Writing-review and editing and supervision, O.D.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare that there are no conflicts of interest as regards the publication of this article.

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