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Experimental Studies on Locally Produced Insecticide (Ota-piapia)

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Authors' contributions

This work was carried out in collaboration among all authors. Author OGD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KAO and ANM managed the analyses of the study. Author ANM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Nigeria, like most countries worldwide find insect pest control a problem, unfortunately, the insecticides available are somewhat costly to the general populace, hence the dependence on the cheaply produced and widely available local insecticide famously called 'Ota piapia' literally translating to kill and dry. This study was carried out to determine the safety of 'Ota piapia' using Wistar rats as an animal model.
Materials and Methods: Fifteen rats were divided into five groups and exposed dermally to 0%, 25%, 50%, 75% and 100% of insecticide concentrations respectively. Evaluation of their behaviour, haematological indices, liver enzyme {alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST)} and gas chromatography mass spectrometry (GC-MS) analysis of the insecticide was used as experimentally indices in evaluating safety limits of the local insecticide.
Results: Behaviorally, the rats with higher concentrations of insecticide application showed acute signs of toxicity, with an increase in pack cell volume (PCV%) and lymphocyte and decrease in (total white blood cell count (TWBC) and neutrophils counts hematologically. There was a dose-

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dependent relationship between insecticide concentration and ALT and ALP activities, while this was not consistent with AST activity. The GC-MS analysis of the insecticide gave 43 components; mainly organic compounds with dichlorvos its main active component, having the highest percentage of 12.74%.

Conclusion: Increased PCV%, decreased TWBC and neutrophil counts established in this study and its significant adverse effect on the serum level of liver enzymes implied that the local insecticide ota-pia pia is toxic and must be used with caution.

Keywords: Ota-piapia; insecticide; haematological indices; liver enzymes; wistar rats.

1. INTRODUCTION

Insecticides are agents of chemical or biological origin that control insects, and often kill insects [1]. The efficacy of the insecticide depends on its mode of action as they sometimes work as nerve poisons, muscle poisons, desiccants, sterilants or pheromones; others exert their effects by physical means such as clogging air passages.

This mode of action describes how the insecticide kills or inactivates an insect and is also important in understanding its degree of toxicity to unrelated species, such as fish, birds, and mammals. The four main classes of insecticides namely: organochlorine, organophosphate, carbamate and pyrethroid insecticides (Ahmed, et al. (2000); Smith, [2] so classified as an indicator to their mechanism of action. Insecticide toxicity can result from ingestion, inhalation or dermal absorption. Therefore, people who use insecticides or regularly come in contact with them must understand the relative toxicity, potential health effects, and preventative measures to reduce exposure to the products they use [3]. Local insecticide makers in Nigeria emphasize the potency of their insecticides/rodenticides by the word "Ota piapia", (a coined name of Igbo origin locally translating to something that completely consumes or devours, Musa, et al. (2012) assuring its patronizers that their 'insect problem' will be effectively taken care of [4]. Its ready acceptance and widespread usage in Nigeria is due to its cheap production, efficacy, easy accessibility and affordability [5]. Its popular usage also stems from the fact that it has wide applicability as mostly or almost all or if not all household pests are controlled by these formulations. The product is still not registered with NAFDAC [6], but is in huge demand because of its efficacy against mosquitoes, food storage contaminants, insect infestation prevention in agricultural-based storage facilities [7]. The problem back here at home is that there is no 'one brand' of ota piapia, as there are so many formulations that even within a locality several formulations are existing that patronizing a particular 'brand' will be due to its efficacy reported amongst its users living in that area. This ota piapia is a combination of a registered insecticide and other chemicals are somewhat added to the extent that the concentration of dichlorvos in them varies from 1-10% [8,9,10]. Reports have shown that Ota piapia is an unspecified insecticide and its application regarded as a dangerous practice since its chemical constituent is unknown. Several authors [7,11,6,8] surveyed about 100 samples of this local insecticide in northeastern Nigeria and discovered that most of them had the active ingredient dichlorvos in their formulations. Some of these locally made insecticides had caused the death of so many Nigerian families in recent times [12] and worldwide [13,14] specifically through food contamination [6] and children are no exception to its harmful effects [15]. This study aimed to carry out experimental studies on the locally produced insecticide, ota piapia on Wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals

All reagents, chemicals and solvents used for the study were purchased from Kadlad Chemical Laboratory, Osogbo, Osun State, Nigeria.

The local insecticide product (ota piapia) used for this study was purchased from a local market in Ibadan, Oyo State, Nigeria. The choice of the brand was based on its highest consumption rate among those available in the market.

2.2 Local Insecticide Formulation

The method of Ajiboso, et al. [16] was modified for this study. Approximately 0.5 ml of the product was dissolved in a proper amount of petroleum ether which was made up to 100 ml in a volumetric flask at room temperature to obtain a clear solution resulting to a stock concentration of 0.5 ml/100 ml.

2.3 Animal Handling and Experimentation

The research protocol was approved by the Animal Care Committee of Department of Science Laboratory Technology, Federal Polytechnic Ede, Osun State, while the animal usage itself 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).

2.4 Animal Treatments

Fifteen male Wistar rats weighing between 100g to 175 g were purchased from the Animal House of the Department of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun State. The animals were allowed to acclimatize for four weeks before treatment.

2.5 Animal Grouping and Insecticide Administration

Using a 25% scale in insecticide application, the animals were divided into 5 groups with group 1 the control group having 0% insecticide application.

2.6 Exposure of Animals to Insecticide

The method of Jeffcoat [17] which specified dermal means of insecticide administration was adapted for this study. 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, this application followed the animal groupings explained above.

2.7 Behavioural Evaluation

Behavioural 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 labelled using scales like slightly hazardous to indicate mild effect etc. Animals were observed daily with daily weight intake used as a form physical assessment criterion. [18,19].

2.8 Analytical Procedures

2.8.1 Toxicological studies

Blood samples were drawn from the rats under light chloroform anaesthesia and collected in glass tubes.

2.8.2 Determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities

Whole blood was collected immediately in plain tubes, spun at 3000 g for 5 min for serum separation after which it was stored at -20°C for marker enzyme analysis. AST and ALT enzyme activities were determined colorimetrically using the method of Reitman and Frankel [20] modified by Hammed, [21], carried out with commercially available Randox Kits, (Randox Laboratories Ltd. UK). Alkaline phosphatase activity was measured according to Schmidt, et al. [22], Bello, et al. (2014) using standard procedures as specified by Randox Laboratories Ltd. UK.

2.8.3 Haematological indices Determination

The methods of Uboh, et al. [23], Akpanabiatu, et al. [24] were used for the determination of haematological indices. The blood samples were collected into EDTA bottles for the determination of haematological parameters namely; packed cell volume PCV%, total white blood cell count TWBC, neutrophils and leukocyte count. These parameters were obtained using automated haematology Analyzer ERMA (model pce 210) made in Japan.

2.8.4 GC-MS analysis of local insecticide

Insecticide product solution obtained was transferred into 5ml glass vial and was taken to Obafemi Awolowo University Ile-Ife, Osun State, Nigeria for Gas Chromatography- Mass Spectroscopy (GC-MS) analysis. The method of Ofordile, et al. [25] was adopted.

The determination was carried out using an automated Shimadzu Gas chromatography (GC-17A) equipped with fused-silica capillary column. The analyte identification was confirmed on a second column of differing polarity from the analytical column, and using a different set of chromatographic conditions. The chromato-graphic conditions used for analysis consists The inlet head pressure = 16 psi, injector temperature = 270°C and the detector temperature = 290°C. The carrier gas was nitrogen (N_2) at a flow rate of 165 ml/min.

2.9 Statistical Analysis

The SPSS version 20 computer software package (SPSS Inc. Chicago, U.S.A) was used

for the computation of results obtained from this study. Data are presented as mean ± standard deviation (M±SD).

3. RESULTS

3.1 Behavioural and Physical Signs of Rats Administered Local Insecticide

Table 1 shows the behavioral signs observed in rats administered local insecticide ota piapia. Using a 100% measuring scale, it was observed that the higher the insecticide administration, the more adverse the toxicity signs showed behaviorally.

Table 2 showed the 7 days weight change in the experimental animals, the control group rats had a daily increase in weight with weight change as high as 17.7 g, while group B animals had a steady increase in weight which plateaued out on the 4th day, indicating that dermal exposure of rats to 50% insecticide caused an increase in rat body weight (21.3 g) when compared to control. Physically for groups C and D animals, there was an initial decrease in weight gain which then

Table 1. Behavioural and physical evaluation i.e. signs of toxicity following insecticide application

Group	Toxicity	Aggressiveness (%)	Bristly hair (%)	Confusion (%)	Drowsiness (%)
Control	None	0	0	0	0
A	Slightly Hazardous	0	15	9	9
B	Moderately Hazardous	0	45	30	60
C	Moderately Hazardous	39	45	30	60
D	Highly Hazardous	50	54	60	66

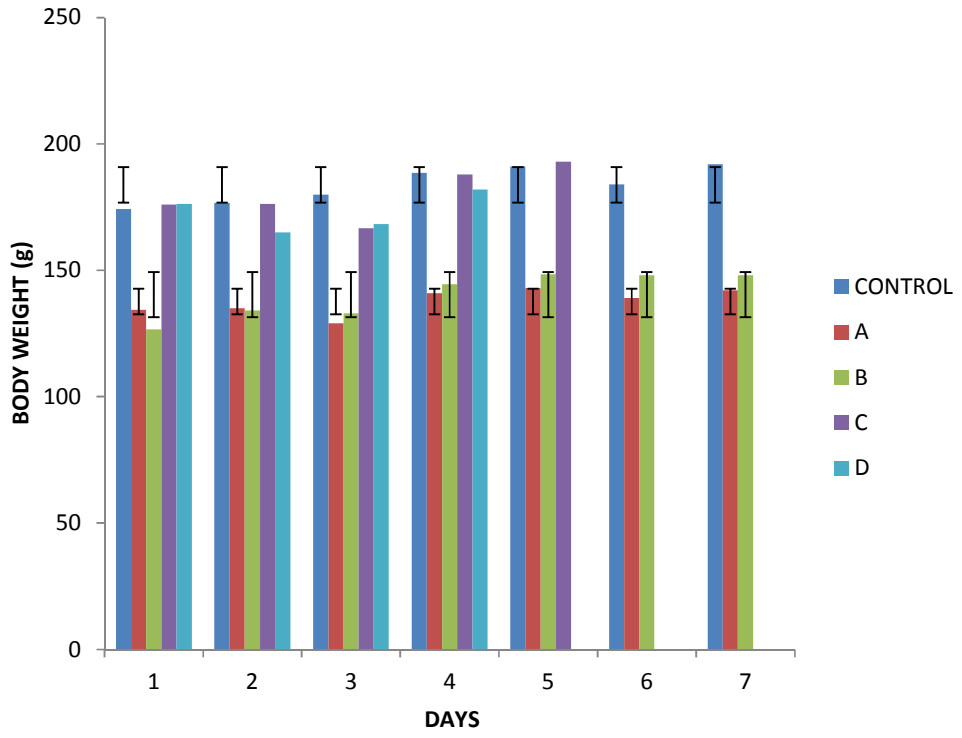

Fig. 1. Comparison between average weights of rats administered ota piapia for 7 days

Table 2. Mean body weight (g) of rats following insecticide application for 7 days

Day	Control	A	B	C	D
1	174.3	134.3	126.7	176.0	176.3
2	176.7	135.0	134.0	176.3	165.0
3	180.0	129.0	133.0	166.7	168.3
4	188.5	141.0	144.5	188.0	182.0
5	191.0	143.0	148.5	193.0	---
6	184.0	139.0	148.0	---	---
7	192.0	142.0	148.0	---	---
M ± S.D	183.8±7.03	137.6±5.06	140.4±8.96	180.6±10.48	172.9±7.70

--- = dead rats after insecticide administration

followed a drastic increase in weight before the eventual death of the animals on day 6 and 5 respectively.

3.2 Haematological Interpretation of Rats Administered Ota piapia

As shown in Table 3, there was an increase in PCV% of exposed groups A, B and D except for C and in lymphocytes counts of A to C, when compared with the control group. Although lymphocyte count was absent in group D. TWBC and neutrophils levels were significantly decreased in exposed group A, C, D and B, C, D respectively when compared with the control group.

Table 4 shows the effect of ota-piapia on liver enzymes ALT, AST and ALP. When compared with controls the ALT values increased in groups B and C and decreased in A and D, while AST values were within range for all the groups. ALP values increased in groups A, C and D and decreased in group B alone when compared with controls.

3.3 Gas Chromatography-Mass Spectrophotometry (GC-MS) Peaks Interpretation

Forty-three (43) peaks representing forty-three (43) components were present in the local insecticide (Fig. 2). Each component was identified by the GC-MS library (Table 5). The GC-MS analysis of this insecticide product revealed the presence of Dichlorvos (12.74%) as its main active ingredient; other chemical constituents mostly aromatic compounds like ethylbenzene, p-cymene and alkanes like decane and tetradecane were also present in the local insecticide preparation.

Table 3. Hematological indices of rats administered of local insecticide

Group	PCV %	TWBC $X10^3$/L	Neutrophils	Lymphocytes
Control	37.0±11.50	7.96±2.55	73.7±6.66	25.0±4.36
A	38.3±12.40	6.90±3.23	74.3±6.51	24.7±6.66
B	44.7±2.90	9.03±5.00	70.7±3.79	26.7±2.89
C	35.3±11.50	6.73±2.07	70.7±2.31	28.7±1.16
D	40.7±9.50	6.20±0.36	72.3±0.58	0

Data are mean values of triplicate determinations and expressed as Mean± SD
Key: PCV%- Pack cell volume, TWBC-total white blood cell count

Table 4. Serum activities of ALT, AST and ALP of rats administered local insecticide ota piapia

Group	ALT $*10^{-2}$ (U/L)	AST $* 10^{-2}$ (U/L)	ALP (U/L)
Control	5.62±0.05	54.19±0.02	156.4±153.88
A	4.15±0.04	54.47±0.00	354.2±235.95
B	6.32±0.04	56.08±0.02	92.0±55.77
C	9.84±0.08	54.61±0.00	395.6±207.15
D	2.29±0.04	53.84±0.01	257.6±339.25

Data are represented as mean values of triplicate determinations and expressed as Mean± SD
Key: ALT-alanine aminotransferase, AST-aspartate aminotransferase, ALP-alkaline phosphatase

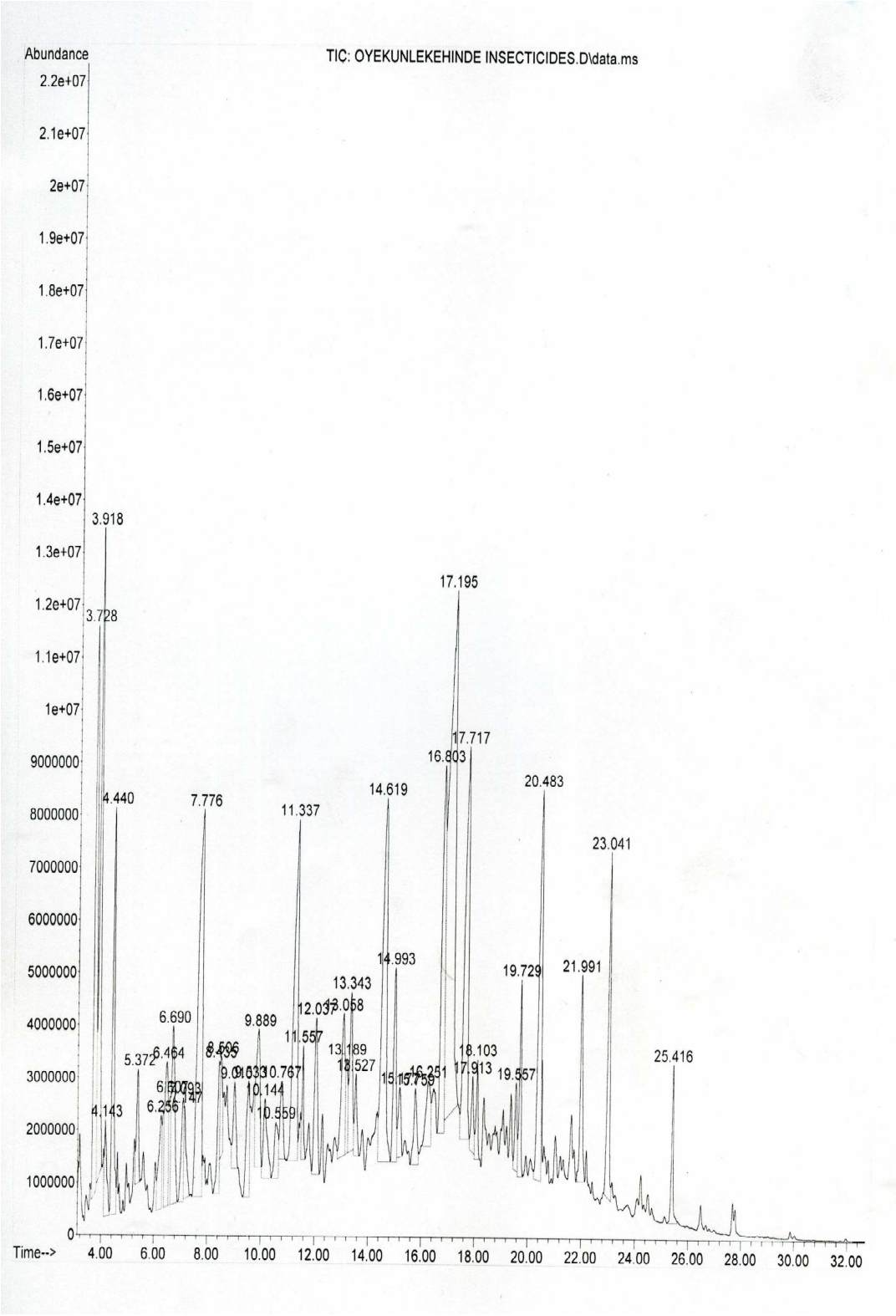

Fig. 2. Gas chromatogram of the local insecticide (Ota-piapia)

Table 5. GC-MS profile of the local insecticide (Ota-Piapia)

Peak no	RT time	Area %	Formula	Compound name	Type of organic vompound
1	3.728	5.80	C_8H_{10}	Ethylbenzene	Aromatic
2	3.918	5.02	C_8H_{10}	P- Xylene	Aromatic
3	4.143	0.86	C_9H_{18}	Ethyl 4-methylcyclohexane	Cycloalkane
4	4.440	3.35	C_8H_{10}	Benzene 1,3-dimethyl-o-xylene	Arene
5	5.372	0.91	$C_{10}H_{22}$	Octane 2,6-dimethyl	Alkane
6	6.256	1.11	$C_8H_{14}O$	3,4,4-trimethyl-1-pentyn-3-ol	Alcohol
7	6.464	1.87	C_9H_{12}	Benzene 1-ethyl-2-methyl	Arene
8	6.607	0.71	C_9H_{12}	Benzene 1,2,3-trimethyl	Aromatic
9	6.690	2.23	C_9H_{12}	Benzene 1,2,3-trimethyl	Aromatic
10	7.093	1.01	C_8H_{16}	Cyclohexane 1,4-dimethyl trans	Cycloalkane
11	7.147	0.79	C_8H_{16}	Cyclopentane 1,1,3-trimethyl	Cycloalkane
12	7.776	6.56	$C_{10}H_{22}$	Decane	Alkane
13	8.435	1.17	$C_{11}H_{24}$	Decane 4-methyl	Alkane
14	8.506	0.86	$C_{11}H_{24}$	Decane 4-methyl	Alkane
15	9.010	0.92	C_8H_{16}	Cyclooctane	Cycloalkane
16	9.533	1.43	$C_{10}H_{14}$	Benzene 1-methyl-3-propyl	Arene
17	9.889	2.03	$C_{10}H_{14}$	P-cymene	Aromatic
18	10.144	1.15	$C_{11}H_{24}$	Decane 3-methyl	Alkane
19	10.559	0.78	$C_{10}H_{14}$	Benzene 2-ethyl 1,4-dimethyl	Arene
20	10.767	0.90	$C_{10}H_{12}$	Benzene 2-butenyl	Arene
21	11.337	5.08	$C_{11}H_{24}$	Undecane	Alkane
22	11.557	0.65	$C_{11}H_{20}$	Trans Decalin 2-methyl	Bicyclic
23	12.037	1.80	$C_{10}H_{18}O$	Cyclohexanone 5-methyl 2-(1-methylethyldiene)	Aldehyde
24	13.058	2.02	$C_9H_{10}O$	Ethanone 1(4-methyl phenyl)	Aldehyde
25	13.189	0.70	$C_{10}H_{14}$	Tetracyclo[3.3.1.1.(1,8).0(2,4)] decane	Cycloalkane
26	13.343	1.76	$C_{27}H_{55}Cl$	Heptacosane 1-chloro-	Aliphatic
27	13.527	0.71	$C_{11}H_{24}$	Undecane 3-methyl	Alkane
28	14.619	6.18	$C_{12}H_{26}$	Dodecane	Alkane
29	14.993	1.98	$C_{13}H_{28}$	Undecane 2,6-dimethyl	Alkane
30	15.177	0.75	$C_{18}H_{36}$	5-Octadecene (E)	Alkene
31	15.759	0.92	$C_{13}H_{24}$	Z-1,6-tridecadiene	Alkadiene
32	16.251	1.16	$C_{19}H_{38}$	1-Nonadecene	Alkene
33	16.803	4.95	$C_4H_7Cl_2O_4P$	Dichlorvos	Phosphate ester
34	17.195	12.74	$C_4H_7Cl_2O_4P$	Dichlorvos	Phosphate ester
35	17.717	5.61	$C_{13}H_{28}$	Tridecane	Alkane
36	17.913	0.65	$C_{11}H_{10}$	Bicyclo[4.4.1] Undeca-1,3,5,7,9-pentaene	Bicyclic
37	18.103	0.70	$C_{43}H_{88}$	Tetracontane 3,5,24-trimethyl	Aliphatic
38	19.557	0.73	$C_{54}H_{108}Br_2$	Tetrapentacontane 1,54-dibromo-	Aliphatic
39	19.729	1.34	$C_{15}H_{32}$	Dodecane 2,6,10-trimethyl	Alkane
40	20.483	4.51	$C_{14}H_{30}$	Tetradecane	Alkane
41	21.991	1.80	$C_{15}H_{32}$	Dodecane 2,6,11-trimethyl	Alkane
42	23.041	2.78	$C_{15}H_{32}$	Pentadecane	Alkane
43	25.416	1.03	$C_{16}H_{34}$	Hexadecane	Alkane

4. DISCUSSION

The toxic effects of local insecticides particularly organophosphates are predominantly produced through the inhibition of acetylcholinesterase, causing accumulation of acetylcholine at peripheral and central cholinergic receptors, resulting in overstimulation of the cholinergic system [26,27,28,29,30,31] which often leads to oxidative stress induction an often consequence of hepatotoxicity. It has also been reported that organophosphorus insecticide self-poisoning is a crucial clinical problem mostly associated with the rural populace of developing worlds in which Nigeria is a part of [14].

The behavioural signs of the insecticide preparation observed dermally on the animals

indicated that the more the insecticide preparation the more adverse its effect. This was seen in the death of the animals administered the highest concentration.

As a physical parameter, weight gain or loss showed its efficacy as a possible way of evaluating toxicity which was in agreement with the work of Aldana, et al. [32] in their assessment of pyrethroid insecticides. This study revealed that concentration is a good indicator of weight measurement.

Blood analysis is crucial in many fields of toxicology research and environmental monitoring as a possible indicator of physiological or pathological changes in disease investigation [12]. In warm-blooded animals, changes in the blood parameters occur because of injuries or infections of some tissues or organs, leading to the dysfunction or injuries of organs or tissues [33]. According to Gu, [34], dehydration is an important factor that increases PVC levels while seizure leads to neutrophilia (increased neutrophil). Leukopenia (decreased TWBC) recorded in this present study may be attributed to the destruction of WBC [34] due to continuous and prolonged exposure to the insecticide. This was seen in the increment in PCV% and lymphocyte count and the decreased value of TWBC and neutrophils.

The liver has a variety of transaminases to synthesize and break down amino acids and to interconvert energy storage molecules. The concentrations of these in the serum (the non-cellular portion of blood) are normally low. However, if the liver is damaged, the hepatocytic cell membrane becomes more permeable and some of the enzymes leak out into the bloodstream. The two transaminases commonly measured are alanine transaminase (ALT) and aspartate transaminase (AST). Another transaminase measured is alkaline phosphatase (ALP). In general, very high elevations of the transaminases suggest severe liver damage, such as viral hepatitis, liver injury from lack of blood flow, or injury from drugs or toxins [16].

The apparent decrease and increase in activity of serum ALT observed with increased insecticide concentration in this study suggest toxic and harmful effects of insecticide on the serum. This effect was less pronounced on the groups that were exposed to higher insecticide concentrations indicating that ALT activity in exposed groups was not dose-dependent. This finding agrees with the earlier investigation of Atef, [35], on the effects of a class of organophosphate insecticide on physiological and toxicological investigations in rats.

Serum total ALP levels provide a useful but nonspecific indication of liver or bone disease with biliary tract obstruction which leads to its diffusion into the blood. The serum ALP level increased significantly and was consistent with the findings of Brown, et al. [36]. The measurement of the activities of markers or diagnostic enzymes in the serum plays a significant role in the diagnosis of diseases and the assessment of drugs or plant extract for safety or toxicity risk. The enzymes considered in this study (AST, ALT and ALP) are useful marker enzymes of the liver cells [22], as they are often used in the measurement of liver functionality.

The GCMS analysis showed that there are forty-three peaks which represented forty-three components present in the local insecticide. Eighty percent of the compounds identified were aliphatic and aromatic hydrocarbons which are main constituents of kerosene oil and fuel oil and when present in the environment at a substantial amount has no or little environmental impact [25]. These chemicals are not likely to persist in the environment, as they largely partition to the air where they will degrade via photo-oxidation. Half-lives in the air (during daytime) are calculated to be 9.2 hours for n-decane, 10.2 hours for n-undecane and 11.5 hours for n-dodecane [37]. As these chemicals have shown the ability to biodegrade, the small portion that partitions to soil or sediment should not persist. Being insoluble in water and less dense than water, any releases to water of these n-alkanes should separate and volatilize to the air, hence their presence in such formulations are not environmentally hazardous since they are generally biodegradable, unlike their organochlorine counterpart. Their easy biodegradability lies in their unstable short half-lives hence are somewhat safe in terms of biodegradation in the environment [38], As far as their biodegradability in the environment, they are somewhat harmless, but regarding unprecedented exposure to human beings or even food crops, their safety limits are questionable as there have been continuous reports of their danger especially with children [39,12].

5. CONCLUSION

The results of this study have shown that daily exposure of experimental rats to the local insecticide for seven days have significantly affected the body weight, haematological indices and level of serum liver enzymes. According to the serum liver enzyme's results in this study, a local insecticide may cause liver injury and as such, they are toxic to the liver with continuous or prolonged exposure.

The analysis has demonstrated the presence of many other compounds that are not insecticide active ingredients in the analyzed product and research has shown that the constituents identified alongside with the active ingredient have no or little environmental impact at a substantial amount.

6. RECOMMENDATIONS

The following recommendations are hereby made:

The local insecticide should be adequately and accurately diluted by mixing 25 ml insecticide with 75 ml water, when used as a household insecticide. When not in use it should always be kept in a tight, sealed and non-perforated container. It should be kept in a safe place that is out of reach of children. It should be kept in a cool and not hot place to avoid explosion being a volatile compound. Hand gloves should be worn during the preparation of diluted solutions. Hands should be washed and disinfected after insecticide application.

DISCLAIMER

This manuscript was presented in a Conference. Availablelink:https://www.researchgate.net/publication/330289373_Experimental_Studies_on_Locally_Produced_Insecticide_Ota_pia_pia

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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