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Inhalation effect of insecticides on some Haematological parameters of rabbits

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Abstract

High malaria burden has led to the increase use of insecticides in the tropics and subtropics. This study thus aimed at assessing the effect of insecticides inhalation on some haematological parameters using experimental animal model.Sixteen adult male rabbits divided into four groups A, B, C and D. Group A,B and C were exposed to 20ml of pyrethroid insecticides containing 0.05% pralletrin and 0.15% cyfluthrin by inhalation for 10mins,20mins, and 30mins respectively. Group D serve as the control and was not exposed. Baseline study was done on all the animals before grouping for exposure. The pyrethroid insecticides was soaked in cotton wool (2.5g) in a container that was able to prevent the animal from ingesting it, which was placed inside the room A, B and C for 10mins,20mins and 30mins respectively. The rabbits were exposed for three weeks and sample were collected at the end of each week. Exposure was discontinued after day 21and Samples were collected again on day 28 and 35 respectively which is the fourth and fifth week. All the animals were monitored twice daily for clinical signs like jerky movement, skin scratching, licking of legs and other body parts. Data analysis revealed that there wassignificant effect of inhalation of insecticides on some haematological parameters of rabbits at 10min, 20mins and 30mins of exposure. There were significant increase in PCV, Eosinophil Platelets WBC and Neutrophil and decreased in ESR, Lymphocyte. The results from this study have shown that aerosol of these pyrethroid insecticides(pralletrin and cyfluthrin) has effect on haematological parameters. It is thus recommended that one should avoid exposure to the aerosol of these insecticides during domestic, veterinary,agricultural or industrial use.

Keywords: Inhalation, insecticides, haematological parameters, rabbits

Introduction

Excessive use of various chemicals including insecticides has become a public health concern. Use of insecticides and other organophosphates is one of the major ways through which manufacturing workers and farmers are exposed to toxicants and this has impact on the ecosystem and public health[1]. Pyrethroid insecticides are used extensively in agriculture, commercial facilities and in residential homes to control insect pests [2]. An insecticide is a natural or man-made preparation that is used to kill or control insects such as mosquitoes, cockroaches, bees, and wasp. The most common active ingredient in insecticide are synergist, carbamate, whose common name is propoxur, pyrethrin (or synthetic pyrethroids), D-trans-alletrin, permethrin, pralletrin, tetrametrin, deltametrin, cyfluthrinimiprothrinchlopyritos, Diaznon, Malathion, Silical gel, Boric acid, Arsenicals, paradichlorobenzene, Naphthalene, N,N-diethyl meta-toluamide (Deet), Dimethlphthalate[3]. Pyrethrins, pyrethroids and carbamate are effective insecticides that are often used in household sprays, insect repellent, pet shampoo, and lice treatment. They are often combined commercially with other chemicals called synergists, which enhance their insecticidal activities. Synergists are chemicals that activate some insecticides making them more poisonous to insects and thereby enhancing the effectiveness of the active ingredients. MGK 264 and piperonyl-butoxide are two commonly used synergist[4]. Synthetic pyrethroid insecticides are now used as substitutes for pest control[5] thus accounting for over 30% of insecticide used globally[6]. Cypermethrin, a pyrethroid has been documented to cause clinical sign such as increased urination, licking of legs, jerky movements ataxia, incoordination, staggering gait dizziness, altered blood chemistry, hepatoxicity[7-8] and neutoxicity[9].

Materials and Methods

Sixteen (16) adult male and female rabbits weighing 1.5 - 2kg were obtained from Animal Care and Use Research Ethics Committee (ACUREC) University of Ibadan Oyo State, which served as subjects for this experiment. They were housed in a clean, quiet, well ventilated and temperature controlled room (21 ±4^{o}C) in experimental animal house of University of Ibadan, Oyo State.

The rabbits were fed continuously with pelletized guinea feed containing 16.5% protein and water, they were provided with clean drinking water throughout the duration of the experiment and were allowed to acclimatized to their new environment for two (2) weeks in separate rooms and their weight was taken before and after each week.

Procedure for insecticides exposure

The rabbits were grouped into four (4) A B C and D before acclimatization for two weeks in a separate room.

Group A consists of four animals shaved at the spine for group identification and were exposed to 20ml of pyrethroid insecticides containing 0.05% imiprothrin, 0.05% pralletrin and 0.15% cyfluthrin by inhalation for 10mins.

Group B consists of four animals shaved at the left leg for group identification and were exposed for 20mins.

Group C consists of four animals shaved at the head for group identification and were exposed for 30mins daily respectively for three weeks[10].

Group D was used as control and was not shaved and exposed.

The rabbits in group A, B, C i.e the test group in poorly ventilated room for exposure, were exposed to 20mls of pyrethroid insecticides which was soaked in cotton wool (2.5g) and place inside the rooms (A, B, C) respectively, using a container that prevented the rabbits from ingesting them. The rabbits were exposed daily by inhalation for 10mins (group A) 20mins (group B) and 30mins (group C) for three weeks.

Exposure was discontinued after day 21 to day 35[11].

The baseline parameters (packed cell volume, white blood cell count, platelets count, red cell morphology and differential count) were carried out on the sixteen rabbits before exposure. The insecticides was purchased directly from the company at 13/14 Abimbola Street Isolo Industrial Estate Isolo Nigeria (Johnson Wax Nigeria)

All animals received humane care in compliance with the guidelines of the University of Ibadan Animal Care and Use Research Ethics Committee (ACUREC).

The post exposure samples were collected on day 28 and 35 respectively after three weeks of exposure i.e last sample was collected on the fourth and fifth weeks after the last exposure.

Collection of blood samples

Procedure

The hair at the ear vein of the rabbits was wiped with xylene and about 9ml of venous blood sample was collected from the ear vein of the rabbits in which 4.5ml of blood was dispensed into a 5ml bottle containing 0.5ml of sodium citrate and was mixed gently to avoid clotting, while the remaining 4.5ml of blood was dispensed into bottle containing EDTA and mixed to avoid clotting.

The EDTA blood was used for PCV, WBC, Platelet counts, ESR, Differential count/ Red cell morphology. Samples were labeled properly to avoid error. The analysis of blood samples was carried out at the Laboratory Unit, Jericho Specialist Hospital Jericho, and Ibadan.

Packed Cell Volume (PCV)[12].

Micro haematocrit method

Procedure: The anticoagulated blood was mixed carefully and the plain capillary tubes was filled with blood up to ¾ length of the capillary tube in which one end was sealed with plasticine, then spinned at 12000 rpm for 5 minutes. Therefore it was read with the aid of microhaematocrit reader[13].

Total White Blood Cell Count[12].

Procedure: 1 in 20 dilution of Turk's solution to blood was made by adding 0.02ml of blood to 0.38ml of Turk's fluid into a clean tube. The dilution was then charged into an improved Neubaur counting chamber with the use of pipette, the chamber was left undisturbed for 2 minutes to allow time for white cells to settle, and these cells were counted using x10 objective lens of microscope by counting the four outer square of the chamber.

Differential White Cell Count[12].

Procedure: A well-mixedsequestrated blood sample was dropped on a clean grease free glass slide at three quarter length of the slide and a thin film was made. After air drying, the slide was flooded with Leishman stain then left for 2 minutes so as to fix it properly; stain was diluted with twice its volume of buffered distilled water of PH 6.8 then left for 10 minutes for staining. It was allowed to air dry and examine under the microscope using x 100 objective

Red Cell Morphology

The red cell morphology was done towards the tail end of the film.

Platelets count[12].

Procedure: 1 in 20 dilution of blood to ammonium oxalate was made by adding 0.02ml of well mixed anticoagulated blood to 0.38ml of ammonium oxalate into clean tube. This solution was then charged into improved Neubaur counting chamber and was counted under X 40 objective lens of the Microscope by counting the inner five (5) squares.

Erythrocyte Sedimentation Rate[14].

Westergren Method

Procedure: 1.6ml of EDTA anticoagulated blood was added to 0.4ml of sodium citrate

anticoagulant in a small container and was mixed well. The cap of the container was removed and the sample was placed in the ESR stand. The westergren pipette was inserted, and ensured it is positioned vertically. The blood was drawn to the 0 mark of the westergren pipette avoiding air bubbles. The time was set up for 1hour. At exactly 1hour, the level at which the plasma meets the red cells in mm was read.

Results

Table 1: shows the comparison of the haematological parameters of exposed animals in Group A (10mins) after seven days of exposure with the control (Group D), At 10mins of exposure, there is significant effect of inhalation of insecticides on packed cell volume,(p-value 0.028),eosinophil (p-value 0.012) and platelet counts (p-value 0.024) of rabbits in the cases when compared with the control (Group D),While WBC, Neutrophil, lymphocyte, monocyte and Basophil were statistically non-significant

Table 1: Comparison of the haematological parameters of those exposed in Group A (10mins) after seven days of exposure with the control (Group D) using paired sample t-test (n=8)

Haematological Parameters	Group A (n=4)	Controls (n=4)	t-test	p-value
Packe d cell volume (PCV)	34.08 ± 2.61	36.17 ± 1.11	-2.538	0.028*
White blood cell	5050.00±582.31	4516.67±472.58	1.957	0.076
Neutrophil	58.08 ± 2.19	58.42 ± 2.31	-0.300	0.770
Lymphocyte	38.67 ± 3.02	39.83 ± 1.46	-1.370	0.198
Monocyte	0.83 ± 1.11	0.67 ± 0.77	0.352	0.732
Eosinophil	2.42 ± 1.24	1.00 ± 1.12	3.027	0.012*
Basophil	0.00 ± 0.00	0.08 ± 0.28	-1.000	0.339
Platelet	208.33 ± 29.52	184.58 ± 8.47	2.614	0.024*
Erythrocyte sedimentation rate	1.67 ± 0.49	1.33 ± 0.49	1.483	0.166

N=8, *p<0.05 (i.e. Significant).

Table 2: shows the comparison of the haematological parameters of those exposed in Group A(10mins) after seven days of exposure with their baseline. There is significant effect of inhalation of insecticides on Neutrophil (P- value 0.007)and Eosinophil (P –value 0.029) at 10mins when compared with their baseline, while lymphocyte, WBC, monocyte, Basophil, platelet and ESR were statistically not significant after 10mins of exposure when compared with their baseline.

Table 2: Comparison of the haematological parameters of those exposed in Group A (10mins) after seven days of exposure with their Baseline using Independent sample t-test (n=8)

Haematological Parameters	Group A (n=4)	Baseline in Group A (n=4)	t-test	p-value
Packed cell volume (PCV)	34.08 ± 2.60	32.00 ± 2.44	1.401	0.183
White blood cell	5050.0±582.31	4550.0±525.99	1.517	0.151
Neutrophil	58.08 ± 2.19	61.75 ± 1.25	-3.129	0.007*
Lymphocyte	38.66 ± 3.02	37.00 ± 2.44	0.991	0.338
Monocyte	0.83 ± 1.11	0.50 ± 0.57	0.564	0.581
Eosinophil	2.41 ± 1.24	0.75 ± 0.95	2.435	0.029*
Basophil	0.00 ± 0.00	0.00 ± 0.00	-	-
Platelet	208.33 ± 29.52	178.25 ± 20.30	1.873	0.082
Erythrocyte sedimentation rate	1.66 ± 0.49	1.50 ± 0.57	0.564	0.581

N=8, *p<0.05 (i.e. Significant).

Table 3: shows comparison of the haematological parameters of those exposed in Group B (20mins) after three weeks of exposure with the control(Group D).There is significant effect of inhalation of insecticides on PCV (P-value 0.010), WBC (P-value 0.000), Eosinophil (P –value 0.024),Platelet counts (P-value 0.000) and ESR(*P –value of 0.025*) while at 20mins of exposure Neutrophil, Lymphocyte and Basophil were statistically non-significant when compared with the control.

Table 3: Comparison of the haematological parameters of those exposed in Group B(20mins) after three weeks of exposure with the control (Group D) using paired sample t-test (n=8)

Haematological Parameters	Group B (n=4)	Controls (n=4)	t-test	p-value
Packed cell volume (PCV)	33.42 ± 2.93	36.17 ± 1.11	-3.094	0.010*
White blood cell	7616.67±1667.24	4516.67±472.58	6.090	0.000*
Neutrophil	62.33 ± 8.56	58.42 ± 2.31	1.830	0.095
Lymphocyte	34.67 ± 8.69	39.83 ± 1.46	-2.189	0.051
Monocyte	0.83 ± 0.83	0.67 ± 0.77	0.561	0.586
Eosinophil	2.25 ± 1.48	1.00 ± 1.12	2.611	0.024*
Basophil	0.00 ± 0.00	0.08 ± 0.28	-1.000	0.339
Platelet	364.83 ± 92.22	184.58 ± 8.47	6.744	0.000*
Erythrocyte sedimentation rate	2.00 ± 0.85	1.33 ± 0.49	2.602	0.025*

N=8, *p<0.05 (i.e. Significant).

Table 4: Comparison of haematological parameters of those in Group B after seven days of withdrawal from exposure i.e day 28 with their baseline.The comparison shows significant effect of inhalation of insecticides on PCV (*P-value* of 0.010), WBC (P –value 0.000), Neutropphil (*P-value of 0.000*), Lymphocyte (*P –value of 0.000)*, Eosinophil *(P –value of 0.011*), ESR (*P –valueof 0.014)* while platelet counts only was statistically non-significant.

Table 4: Comparison of the haematological parameters of those in Group B after seven days of withdrawal from exposure i.e day 28 with their Baseline using Independent sample t-test (n=4)

Haematological Parameters	Group B (n=4)	Baseline in Group B (n=4)	t-test	p-value
Packed cell volume (PCV)	37.0 ± 2.13	32.25 ± 2.98	3.200	0.010*
White blood cell	10512.5±1066.96	4825.0±525.19	9.903	0.000*
Neutrophil	76.5 ± 3.70	49.75 ± 7.32	8.621	0.000*
Lymphocyte	18.75 ± 3.65	48.75 ± 6.60	-10.345	0.000*
Monocyte	1.0 ± 0.75	0.5 ± 1.0	0.976	0.352
Eosinophil	3.87 ± 1.24	1.0 ± 2.0	3.104	0.011*
Basophil	0.00 ± 0.00	0.00 ± 0.00	-	-
Platelet	543.62 ± 94.34	492.0 ± 525.00	0.283	0.783
Erythrocyte sedimentation rate	2.5 ± 0.75	1.25 ± 0.5	2.962	0.014*

N=4, *p<0.05 (i.e. Significant).

Table 5: shows the comparison of the haematological parameters of those in Group C(30mins) after three weeks of exposure with the control(Group D).There is significant effect of inhalation of insecticides on PCV (P-value of 0.001),WBC (*P-value of 0.002*),Eosinophil(*P – value 0.001*), and platelet count (*P-value of 0.000*) after 30mins of exposure when compared with the control.while Neutrophil,Lymphocyte,Monocyte, Basophil and ESR were statistically non-significant when compared with the control after 30mins of exposure.

Table 5: Comparison of the haematological parameters of those in Group C (30 mins) after three weeks of exposure with the Group D (Controls) using paired sample t-test (n=8)

Haematological Parameters	Group C (n=4)	Controls (n=4)	t-test	p-value
Packed cell volume (PCV)	32.58 ± 2.42	36.17 ± 1.11	-4.634	0.001*
White blood cell	10050.00±4900.37	4516.67±472.58	3.953	0.002*
Neutrophil	58.92 ± 6.64	58.42 ± 2.31	0.257	0.802
Lymphocyte	37.33 ± 5.80	39.83 ± 1.46	-1.487	0.165
Monocyte	0.83 ± 0.83	0.67 ± 0.77	0.518	0.615
Eosinophil	2.92 ± 1.24	1.00 ± 1.12	4.412	0.001*
Basophil	0.00 ± 0.00	0.08 ± 0.28	-1.000	0.339
Platelet	386.42 ± 84.55	184.58 ± 8.47	8.349	0.000*
Erythrocyte sedimentation rate	1.67 ± 0.88	1.33 ± 0.49	1.483	0.166

N=8, *p<0.05 (i.e. Significant).

Table 6: represent the comparison of haematological parameters of those in Group C after 14 days of withdrawal from exposure i.e. day 35 with their baseline, show significant effect on PCV ,WBC, Eosinophil, Platelets counts and ESR while Neutrophil, lymphocyte and monocyte were statiscallynon-significant when compared with their baseline.

Table 6: Comparison of the haematological parameters of those in Group C after 14 days of withdrawal from exposure i.e day 35 with their baseline using Independent sample t-test (n=4)

Haematological Parameters	Group C (n=4)	Baseline in Group C (n=4)	t-test	p-value
Packed cell volume (PCV)	35.0 ± 1.51	30.5 ± 1.73	4.648	0.001*
White blood cell	13387.5±5846.47	5225.0±206.15	2.724	0.021*
Neutrophil	61.25 ± 14.48	61.5 ± 3.10	-0.033	0.974
Lymphocyte	32.5 ± 12.83	37.0 ± 2.44	-0.679	0.513
Monocyte	1.87 ± 1.35	0.5 ± 1.0	1.782	0.105
Eosinophil	4.37 ± 0.91	1.00 ± 1.15	5.546	0.000*
Basophil	0.00 ± 0.00	0.00 ± 0.00	-	-
Platelet	520.75 ± 79.16	239.75 ± 39.68	6.583	0.000*
Erythrocyte sedimentation rate	0.87 ± 0.64	1.75 ± 0.5	-2.373	0.039*

N=4, *p<0.05 (i.e. Significant).

Discussion

This research was designed to look at the effect of inhaling insectizide on short, midium and long term on haematological parameters using rabbits as model. The short term exposure was 10minutes for seven days. Statistical significant difference decreased was observed in the packed cell volume and significant increase were observed in eosinophils and Platelet count when compared with the control after inhalation. Significant increase was observed in eosinophils and significant decrease in Neutrophil when the baseline results were compared with the post exposure result. The relative increase seen in the total white blood cell count though not significant.coroborate the study done by Yousef et al.[15] in which changes in some haematological and biochemical indices of rabbits induced by cypermetin were evaluated,theyreported that the increased in WBC may be indicative of activation of defense and immune system of the body but contrary to the results from work done by Kamal et al.[8] in which effect of cypermetrin on clinico haematological parameters in rabbit were evaluated, they reported that. decrease in WBC may be due to viral infection that temporarily distrupt the work of bonemarrow.,However, the exposed animal total white blood cell counts did not return from the effect of the exposure after 7days of withdrawal from exposure, this was in accordance with the work done by Adhikari et al.[16] in which effect of cypermetrin and carbonfuran on certain haematological parameters and prediction of recovery in a fresh water teleost, was evaluated and stated that increase in WBCs count occurred as a pathological response since these WBCs play a great role during infestation by stimulating the haemopoietic tissues and the immune system by producing antibodies and chemical substances working as defense against infection.

The packed cell volume of the treated rabbits was significantly increased when compared to that of the control group,which is contrary to the work done byGuyton and Hall,[17] stated that decease in PCV of the treated rabbits could have resulted from a likely bonemarrow aplasia reported to have been caused by aerosols of some chemicals such as insecticides, Significant increased

in Neuthrophil was observed when comparerd with the baseline this was is in line with the workdone by Kamal et al.[8]) reported that the increase in percentage of neutrophil could be as a reult of sudden invasion by pathogens or as a result of possible inflamation caused by active mast cells. but contrary to the work done by Yousef et al.[15].

The midterm exposure (20mins) show significant increased in packed cell volume when compared with the control, which differ from the work done by Sembulingam and Sembulingam,[18]reported that decrease in PCV was time dependent that longer treatment could result to the animals developing aplastic anaemia.but inline with the work done by Iteire who reported that the increase in PCV may be caused by dehydration [19]. The white blood cell counts, show significant increase when compared with the conro. Eosinophillia was observed in this study which coroborate the study done by Sangha et al.,and they reported that there is general immune response by the animals to the insecticides [5]. Reduction in ESR was observed to be statistically significant which is in accordance with the work done by Iteire [19]), he reported that the increased in PCV could lead to the decreased in ESR, which may be caused by dehydration. Increased in platelets count was also observed which is the same with the work done by Iteire where he reported that the increase in platelets may be due to infection or inflamatory disease [19].

Also the long term exposure(30mins) reveal significant increase in Eosinophil which is the same with th work done bySangha etal.[5] reported that the increase in eosinophil may be due to enviromental factors e.g allergic reaction,parasitic infection and asthma Packed cell volume remain higher at the longterm exposure when compared with the control which is the same with the previous work done by Iteire et al., and reported that it could be as a result of doses and time used in the experiment [19]. White blood cell counts show significant increase during long term exposure (30mins) which is in line with work done by Holy et al.[20] and reported that it may be caused by benigh conditions such as infection, significant increase in platelet counts was observed at 30 mins i.e longterm exposure which is also in agreement with the findings of Holy etal.[20] reported that increase in platelet counts suggest secondary thrombocytosis,and that Leucytosis and secondary thrombocytosis may be caused by benign conditions such as infections,inflamation necrosis,stress and haemolytic anaemia.

Conclusion

The results from this study have shown that aerosol of these pyrethroid insecticides(pralletrin and cyfluthrin) has effect on haematological parameters. It is thus recommended that one should avoid exposure to the aerosol of these insecticides during domestic, veterinary, agricultural or industrial use.

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