INHALATION EFFECT OF INSECTICIDES ON HAEMORHEOLOGICAL PROFILES OF RABBITS

Abstract

Background: 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 haemorrheological parameters using experimental animal model.

Materials and methods: 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 21 and 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.

Results: Data analysis revealed that there was significant effect of inhalation of insecticides on some haemorrheological parameters of rabbits at 10min, 20mins and 30mins of exposure. There was decreased in Plasma viscosity. But there was no significant effect on whole blood viscosity and plasma fibrinogen concentration

Conclusion

The results from this study have shown that aerosol of these pyrethroid insecticides(pralletrin and cyfluthrin) has effect on haemorrheological 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, haemorheological profiles, 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,cyfluthrin imiprothrin chlopyritos, 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 $\pm 4^{o}$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] (Akhigbe *et al.*, 2011).

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](Kingsley *et al*., 2016).

The baseline parameters (Whole blood viscosity, plasma viscosity, packed cell volume) 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 Whole blood and plasma Viscosity. 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.

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.

Plasma Fibrinogen Concentration (PFC)

Procedure

ml of plasma was dispensed into the test tube and 1ml of prewarmed 0.025M calcium chloride was added. The content was thoroughly mixed, and an applicator stick was inserted, so that fibrin clot can be wound around it, an7d this was incubated for 30 minutes at 37^{0c} in a water bath. When all the fibrin has wound round the applicator stick, the adhered fibrin was removed from the the applicator stick after washing 3 – 4 times with distilled water and then blot dried, it was carefully removed with whatman filter paper no1. The adherent fibrin was carefully removed from the stick into a clean petridish for 3 – 4 days at room temperature to dry. The dried fibrin was weighed, and the plasma fibrinogen concentration was calculated using the formular:

Plasma Viscosity And Whole Blood Viscosity)

Procedure

A syringe (1ml) was clamped in a retort stand vertically. The blood /plasma was drawn up and care is taken to exclude all air bubbles into the vertical syringe until the plunger pass the 1.0ml mark. The plunger was then completely withdrawn and immediately the lower meniscus falls to the 1.0 mark, a stop watch was started. The time required for 1ml of blood /plasma to drain down the syringe was taken.This was repeated 3 times for each sample and average value was taken. The relative whole blood vicosity /plasma viscosity was then calculated from the formular.

Table 1: shows the comparison of the haemorrheological parameters of those exposed in Group A(10mins) with the control(Group D). There is a significant effect of inhalation of insecticides on plasma viscousity, packed cell volume, total white blood cell counts and platelets when compared with control, while (the whole blood viscosity and plasma fibrinogen concentration were not statistically significant.

Table 1: Comparison of the haemorrheological parameters of those exposed in Group A(10mins) with those not exposed in Group D (Controls) using paired sample t-test (n=8)

Haemorrheological Parameters	**Group A (n=4)**	**Controls (n=4)**	**t-test**	**p-value**
Plasma viscosity	3.36 ± 0.31	3.65 ± 0.15	-2.998	0.012*
Whole Blood viscosity	228.50 ± 2.31	229.58 ± 0.79	-1.384	0.194
Plasma Fibrinogen Concentration	1.92 ± 0.48	1.82 ± 0.24	0.583	0.571
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*
Platelet	520.75 ± 79.16	239.75 ± 39.68	6.583	0.000*

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

Table 2: shows comparison of the haemorrheological parameters of those exposed in Group B (20mins) with the control (Group D).There is significant effect of inhalation of insecticides on plasma viscousity (*P-value of 0.005*), packed cell volume, total white cell counts and platelet counts when compared with the control, But there is no significant effect on whole blood

viscousity and plasma fibrinogen/ concentration after 20mins of exposure when compare with the control.

Table 2: Comparison of the haemorrheological parameters of those exposed in Group B(20mins) with the controls(Group D) using paired sample t-test (n=8)

Haemorrheological Parameters	**Group B (n=4)**	**Controls (n=4)**	**t-test**	**p-value**
Plasma viscosity	3.30 ± 0.33	3.65 ± 0.15	-3.461	0.005*
Whole Blood viscosity	229.08 ± 1.56	229.58 ± 0.79	-0.897	0.389
Plasma Fibrinogen Concentration	1.71 ± 0.24	1.82 ± 0.24	-1.114	0.289
White blood cell	7616.66±1667.24	4825.0±525.19	3.229	0.006*
Platelet	386.41 ± 84.55	239.75 ± 39.68	3.292	0.005*
Packed cell volume (PCV)	32.58 ± 2.42	36.17 ± 1.11	-4.634	0.001*

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

RESULT AND DISCUSSION

This research was designed to look at the effect inhaling insectizide on short, midium and long term on haemorheological parameters using rabbits as model. The short term exposure was 10 minutes for seven days. Statistical significant difference decreased was observed in the packed cell volume and significant increase were observed in and Platelet count when compared with the control after inhalation. The relative increase seen in the total white blood cell count though not significant. coroborate the study done by Yousef et al. [13] in which changes in some haematological and biochemical indices of rabbits induced by cypermetin were evaluated, they reported 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. [14] 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 by Guyton and Hall, [15] 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.

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, [16] 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 [17]. The white blood cell counts, show significant increase when compared with the conrol. 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 [17].

All investigated haemorrheological factors at short term exposure (10mins),midterm exposure(20mins) and long term(30mins) exposure were statistically significant except whole blood viscousity and plasma fibrinogen concentration. which were statistically non significant when compared with control. Increased in plasma viscousity, packed cell volume,white blood cell counts and platelet counts weres observed in all the three phases which were in accordance with the work done previously where it was recorded that the increased in plasma viscousity and total white blood cell counts may be caused by infection or inflamatory disease

[18]. Plasma fibrinogen concentration and whole blood viscousity were statistically non significant.

CONCLUSION

The results from this study have shown that aerosol of these pyrethroid insecticides(pralletrin and cyfluthrin) has effect on haemorrheological 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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