INHALATION EFFECT OF INSECTICIDES ON COAGULATION 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 coagulation profile using experimental animal model.

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 served 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 samples were collected at the end of each week. Exposure was discontinued after day 21(Space) 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 coagulation profile of rabbits at 10min, 20mins and 30mins of exposure. There ~~were~~wassignificant decrease~~d~~ in PTTK and INRvalues. But, there was no significant effect on prothrombin time.

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

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 wasps. The most common active ingredients in insecticide are synergist, carbamate, whose common name is propoxur, pyrethrin (or synthetic pyrethroids), D-trans-alletrin, permethrin, pralletrin, tetrametrin,deltametrin,(Space)cyfluthrinimiprothrinchlopyritos, diaznon, malathion, silical gel, boric acid, arsenicals, paradichlorobenzene, naphthalene, N,N-diethyl meta-toluamide (Deet), dimethlphthalate[3]. Pyrethrins, pyrethroids and carbamates are effective insecticides that are often used in household as 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 synergists[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 moves, 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 placed 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 (PT and PTTK [expansions required]) 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(ethylenediamine tetra acetic acid) and mixed to avoid clotting.

The sample collected into the citrate bottle was spun immediately and plasma was separated and kept for the analysis of PT, PTTK, and fibrinogen concentration. 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.

One Stage Prothrombin Time (PT) [12].

Procedure:

-Test plasma(0.1ml) and control was put into two 75 x 10mm glass test tube placed in water bath at 37^{0c}

- 0.1ml of brain thromboplastin was added to it and left for 2minutes.

- 0.1ml of pre warmed calcium chloride was added to it and the stopwatch was started.

- The stopwatch was stopped at the first sign of clot.

Normal range for PT = 10s – 14s

Partial Thromboplastin Time With Kaolin[12].

Procedure: 0.1ml of test plasma and control was put into two 75 x 10mm glass test tube respetively placed in water bath at 37^{0c}**.**

- 0.1ml of kaolin was added to it, 0.1ml of inocitin /cephalin was added and left in waterbath for 10minutes.

- 0.1ml of calcium chloride was also added and the stopwatch was started.

-The stopwatch was stopped at the first sign of clot.

Normal range 35 – 45secs, but this depends on the sensitivity of commercial reagents.

Plasma Fibrinogen Concentration (PFC)

Procedure

1ml 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, and 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:

$$\text{Plasma fibrinogen Concentration} = \frac{\text{Dry weight}}{\text{Volume of plasma}} \times 10\text{g/l}$$

Normal Ranges

Men - 1.5 – 3.5g /l

Non Pregnant women - 1.5 – 4.0g

Table 1: represent the comparison of the coagulation profiles of those exposed in Group A(10mins) and their baseline which shows significant effect of inhalation on PTTK (p -value0.045) and INR (p –value 0.044), while prothrombin time was statistically not significant.

Table 1: Comparison of the coagulation parameters of those exposed in Group A (10mins) with their Baseline using Independent sample t-test (n=4)

Coagulation Parameters	**Group A (n=4)**	**Baseline in Group A (n=4)**	**t-test**	**p-value**
Prothrombin time	13.33 ± 0.49	14.00 ± 0.81	-2.000	0.065
Partial Thromboplastin time with Kaolin	39.33 ± 0.49	40.75 ± 2.21	-2.199	0.045*
International normal ratio	1.13 ± 0.04	1.25 ± 0.17	-2.213	0.044*

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

Discussion

The short term exposure (10mins) of the coagulation profile show significant decrease in International Normalised Ratio(INR) and Partial Thromboplastin Time With Kaolin (Space) (PTTK) when compared with the baseline which is in agreement with the work done by Ahmed and Elkhalifa [13],in which effect of cigarette smoking on coagulation screening test and platelets count in a Sudanese male adult population was evaluated(How do this relate to insecticidesexposure?Explain in detail).It was reported that the decreased INR and PTTK is associated with the dose of aerosol that was inhaled which may hinder the function of clotting factors. The midterm (20mins) and longterm(30mins) exposure of INR and PTTK were

insignificantly reduced in the test group when compared with the control,which is in accordance with the work done by Ahmed and Elkalifa [13].(Explain in detail with repective values)

Conclusion

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