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BIOMARKER RESPONSE OF MALE ALBINO WISTAR RATS (RATTUS NORVEGICUS) EXPOSED TO A DAILY DOSE OF BAYGON INSECTICIDE

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ABSTRACT

The aim of the study was to evaluate the possible effect of an organophosphate insecticide (Baygon insecticide) on the liver function, sperm count hormonal assay and hematological parameters. 20 albino rats were separated into 4 groups; group A (3 mins daily exposure), group B (6 mins daily exposure), group C (9 mins daily exposure) and group D (control), the baygon insecticides was administered from the aerosol container to each group for 10 seconds in closed air-tight rat cages for a period of 3 weeks before blood samples were collected. The result obtained from the hematological parameters revealed that WBC for the test groups had the highest value of 4.7 and lowest value of 3.4, for the lymphocyte, the highest value was 6.75 and the lowest was 5.50, while for the PCV, the test group had the highest value of 36.0 and the lowest value was 33.0, showing that there was a significant difference ($p<0.05$). The total sperm count for the test groups had the highest value as 14.0 and the lowest value as 10.25 with a significant difference ($p<0.05$). ALT for the test groups had the highest value as 0.39 and lowest value as 0.26, AST had the highest value as 0.016 and the lowest value as 0.013 within the test groups while testosterone had the highest value as 2.60 and lowest value as 2.13. This shows that organophosphate insecticides (baygon insecticides) had a negative effect with a very high significant difference ($p<0.05$) on sperm count and testosterone in the test when compared with the control. These findings demonstrate that exposure to organophosphate insecticides may result in defects in hematological parameters and can also affect the sperm which may lead to infertility in males.

INTRODUCTION

Organophosphate pesticides are one of the most common causes of insecticide poisoning in developing countries which has led to high level of mortality in recent times. (Risal, 2019). Organophosphate insecticide was manufactured in the mid-1800s but was not in use until after the Second World War. During this period, people that came in contact with these insecticides became exposed to organophosphate. Eddleston et al. indicated that, self-poisoning by organophosphate pesticide kills an estimated of 200,000 people every year. Organophosphates compounds are mostly utilized for medications, insecticides, and nerve agents. Pesticides like organophosphate, organochlorine and carbamate compounds are generally applied to agricultural produce and for domestic purposes that has lead to a large case of toxic effects on organisms that inhale them especially mammalian(humans). The pesticide affects different organs like, peripheral and central nervous system, muscles, liver, pancreas and brain of animals. (Karami-Mohajeri, and Abdollahi, 2010).

Artificial pesticides have been massively discharged into the rural areas through agricultural activities that are used for pest and weed control in both developing and developed countries. Organophosphates (OPs) poisoning is a vital public health problem in developing countries. (Hung *et al*,2015). According to world health organization 1993, the agricultural sector are currently in use of almost 85% of pesticides in the world, 10% for the public health sector and the other 5% are used in specific areas such as building areas, resident areas etc.

The toxicity mechanism proceeds in the accumulation of neurotransmitter acetyl chlorine and continuous stimulation of acetyl chlorine receptors in the peripheral nervous system and central nervous system. Organophosphate produces acute and chronic syndromes, (Sinha, and Sharma, 2003). Acute cholinergic dysfunction, muscle weakness, seizures, coma, and respiratory failure can be caused by acute organophosphates poisoning. Nicotinic and muscarinic acetylcholine receptors are both stimulated by organophosphate pesticides, as well as adrenergic receptors through the inhibition of acetylcholinesterase which leads to the accumulation of acetylcholine and generating of severe functional damage within the peripheral nervous system and central nervous system. (Eddleston *et al*. 2008). The exposure of Organophosphate molecules can be engrossed through the skin, inhalation, or in the gastrointestinal tract (ingestion). (Jokanović 2018, Naughton and Terry 2018, Sikary 2019, Dardiotis 2019). In most developing countries like India, farmers that engage in spraying of crops with these pesticides gets exposed directly because they do not use personal protective equipments (PPE) for example gloves, nose masks etc and also lack of proper work practice. (Fareed *et al*, 2013). Agricultural workers could come in contact with some crops that may contain organophosphate such as potatoes, apples, celery, cucumbers, peaches, strawberries, grapes, lettuce, domestic blueberries, nectarines etc. (Robb and Baker, 2019). Symptoms often occur from the beginning of exposure, it could appear within a minute and also could take weeks to disappear. Symptoms that may occur to patients that are exposed to organophosphate insecticides include increased saliva and tear production, diarrhea, nausea, vomiting, small pupils, sweating, muscle tremors, and confusion. (Mendes *et al,* 2018, Dagg *et al,* 2019, Chen *et al,* 2019, Verheyen and Stoks 2019, Aroniadou-Anderjaska *et al,* 2020)

During the period of aerial spraying, pesticides which could leads to the entry of pesticides into the blood stream through the respiratory tract by means of inhalation which can adversely alter the mechanism of the respiratory system and can also affect the hematological alterations among farm workers. Researcher have reported earlier on (Wilkins *et al,* 1999, Kimbell-Dunn *et al,* 2001, Radon *et al,* 2001, Hoppin *et al,* 2006)the high risk of respiratory problems, such as asthma, wheeze and chronic bronchitis among agricultural workers. Pesticides on getting to the lungs through inhalation to the systemic circulation and absorption, generally affect the lung tissues (Dinsdale *et al,* 1992). Documentation has been made in a number of epidemiologic studies that there is a higher risk of respiratory symptoms on the health effects of pesticide inhalation through occupational exposure. (Hoppin *et al,* 2006, Zhang *et al,* 2002, Bener 1999). Studies has shown that pesticides have hematotoxic properties and may cause thrombopenia, neutropenia, agranulocytosis, and a plastic anemia (Parent, D. and Thouvenot, D. 1993). Both acute and chronic exposure to toxic doses of pesticides may to induce hematological, congenital abnormalities and thalassemia (Khristeva and Mirchev 1993). The continuous interaction of organophosphate pesticides (OP) with iron aims at lesser binding efficiency of Hemoglobin with iron leading to anaemic conditions. The most common causes of death in acute organophosphate pesticides poisoning patients are respiratory paralysis and cardiac arrest. (Fukushima *et al,* 2010, Aghabiklooei *et al,* 2013)

MATERIALS AND METHOD

Experimental setup: Male albino wistar rats weighing from a range of 200g to 400g were obtained from the Animal House Unit, Department of Human Physiology University of Port Harcourt and housed in a neat plastic cage with a relative temperature and humidity with natural light-dark cycle on a standard of laboratory chow of pelleted diet and water. The rats were grouped and acclimatize for a week in the cage.

The tested insecticides (Baygon insecticides) were administered from the aerosol container for 10 seconds in the rat cages which were enclosed air-tight to prevent escape of the insecticide gas.

Three treatment groups and a control group of five replicates each with treatments at different time duration of exposure group A(control group with no exposure), group B(3 minutes of exposure), group C(6m minutes of exposure), group D(9 minutes of exposure).

Biochemical analysis

The white blood cell (WBC) count was determined by the improved Neubauer haemocytometer method (Deep $^{1}/_{10}$ mm, LABART, munich, Germany). The packed cell volume (PCV) was determined by micro-haematocrit technique using capillary tube as described by Schlam *et al*. 1975. Schilling method of differential leukocyte count was used to determine the distribution of various white blood cell (Mitruka and Rawnsley, 1977). The plasma activity of alanine transminase and aspartate was determined using Reitman and Frankel method. Testosterone level was determined by using enzyme immune essay (EIA) technique while the epididymal sperm count was done using the light microscope at 40X magnifications and Neubauer haemocytometer method (Deep $^{1}/_{10}$ mm, LABART, munich, Germany).

Method of data analysis

Data were analyzed using Tukey test at a level of 5% probability. Using Assitat Software Version 7.7en (2017)

RESULTS

The results of hematological analysis on the effect of insecticides on albino rats are presented in table 1; the WBC count test group was 4.7, 3.4 and 4.3 for group A, B and C, the control group had 4.5. There was significant difference ($p<0.05$). The neutrophils test groups showed 21.75, 32.0 and 20.0 for group A, B and C, the control group had 25.5. There was significant difference ($p<0.05$) when compared to the control. The lymphocyte test groups had 47.5, 36.0 and 45.8 for group A, B and C, the control group had 44.5. There was significant difference ($p<0.05$) only for test group B when compared to the control. For monocyte, the test groups had 5.50, 5.75 and 6.75 for group A, B and C, the control group had 4.50. There was significant difference ($p<0.05$). The Eosinophils test groups had 0.75, 1.25 and 2.5 for group A, B and C, the control group had 0.5. Test group B and C had a significant difference ($p<0.05$) when compared to the control group. PVC had 36.0, 33.3 and 33.0 for group A, B and C respectively, the control group had 36.0. The test group B and C had a significant difference ($p<0.05$).

The results of semen in male albino rats are presented in table 2. For variable, the test groups had values of 96.76, 97.50 and 96.25 for group A, B and C respectively while the control group had 96.0, there was no significant difference ($p>0.05$). The non variable test groups had 3.25, 2.50 and 3.75 for group A, B and C, the control group had 4.0, there was significant difference ($p<0.05$). For the total sperm count, the test groups had 10.25, 14.0 and 12.5 for group A, B and C, the control group had 19.0 there was significant difference ($p<0.05$) . The motile cell, the test groups had 36.25, 68.75 and 66.25 for group A, B and C, the control group had 87.5, there was significant difference ($p<0.05$) only for test group A and B when compared to the control group. The active cell test groups had 18.75, 51.25 and 45.0 for group A, B and C, the control group had 76.25, there was significant difference ($p<0.05$). The sluggish cell test groups had 17.5, 17.5 and 21.25 for group A, B and C, while the control group had 11.25, there was significant difference ($p<0.05$) only for test group A and C when compared to the control. For dead cell, the test groups had 63.75, 30.0 and 33.75 for group A, B and C while the control group had 12.5, there was significant difference ($p<0.05$) for group A and B when compared with the control.

The results on the effect of insecticide on the liver and hormones of male albino rats are presented in table 3. For alanine aminotransferase, the test groups had a value of 0.26, 0.32 and 0.39 for group A, B and C, while the control group had a value of 0.31, there was no significant difference ($p>0.05$). For aspartate aminotransferase, the test groups had a values of 0.013, 0.016 and 0.016 for group A, B and C while the control group had a value of 0.09, there was no significant difference ($p>0.05$). The testosterone test groups had 2.56, 2.60 and 2.13 for group A, B and C, while the control group had 3.23. There was significant difference ($p<0.05$) only for test group B and test group C when compared to the control group.

Table 1; Result showing the effect of insecticides on hematological parameters

	WBC ($X10^9$)	N (%)	L (%)	M (%)	E (%)	PCV (%)
TEST GROUP A	4.7 ± 1.6^b	21.75 ± 7.4^a	47.5 ± 15.9^b	5.50 ± 3.0^b	0.75 ± 0.5^a	36.0 ± 12.0^b
TEST GROUP B	3.4 ± 1.7^a	32.0 ± 10.7^c	36.0 ± 12.1^a	5.75 ± 3.3^b	1.25 ± 0.5^b	33.3 ± 11.2^a
TEST GROUP C	4.3 ± 1.9^b	20.0 ± 8.2^a	45.8 ± 15.8^b	6.75 ± 2.4^c	2.5 ± 1.4^c	33.0 ± 11.3^a
CONTROL	4.5 ± 1.5^b	25.5 ± 8.8^b	44.5 ± 15.5^b	4.50 ± 3.5^a	0.5 ± 0.5^a	36.0 ± 12.0^b

$^{a\text{-}d}$ Different letters in the same column indicate significant different ($p<0.05$) within the week

Key: WBC = White blood cell, N = Neutrophils, L = Lymphocytes, M = Monocytes, E = Eosinophils, PCV = Packed cell volume

Table 2: Result on the effect of insecticide on semen parameters

	VARIABLE	NON VARIABLE	TOTAL SPERM COUNT	MOTILE SPERM	ACTIVE CELL	SLUGGISH CELL	DEAD CELL
TEST A	96.75 ± 0.85[a]	3.25 ± 0.9[b]	10.25 ± 4.4[a]	36.25 ± 12.5[a]	18.75 ± 7.7[a]	17.5 ± 4.8[b]	63.75 ±12.5[c]
TEST B	97.50 ± 0.29[a]	2.50 ± 0.3[a]	14.0 ± 5.4[c]	68.75 ± 8.3[b]	51.25 ± 12.5[c]	17.5 ± 5.2[b]	30.0 ± 9.4[b]
TEST C	96.25 ± 0.75[a]	3.75 ± 0.8[b]	12.5 ± 4.6[b]	66.25 ± 13.1[b]	45.0 ± 13.2[b]	21.25 ± 1.3[c]	33.75 ± 13.1[b]
CONTROL	96.0 ± 1.1[a]	4.0 ± 1.1[c]	19.0 ± 6.7[d]	87.5 ± 3.2[c]	76.25 ± 5.5[d]	11.25 ± 3.8[a]	12.5 ± 3.2[a]

[a-d] Different letters in the same column indicate significant different ($p<0.05$) within the week

Table 3: result on the effect of insecticide on hormonal and liver parameters

	ALT (UI/L)	AST (UI/L)	TESTOSTERONE
TEST GROUP A	0.26 ± 0.08^{a}	0.013 ± 0.017^{a}	2.56 ± 0.03^{b}
TEST GROUP B	0.32 ± 0.66^{a}	0.016 ± 0.016^{a}	2.60 ± 0.10^{b}
TEST GROUP C	0.39 ± 0.89^{a}	0.016 ± 0.06^{a}	2.13 ± 0.06^{a}
CONTROL	0.31 ± 0.13^{a}	0.09 ± 0.05^{a}	3.23 ± 0.58^{c}

$^{a\text{-}d}$ Different letters in the same column indicate significant different ($p<0.05$) within the week

DISCUSSION

The varied effect of treatment in the White blood count on the rats, were test group B and C having a decrease in WBC group and test group A with an increase when compared to the control. The increase in WBC indicates that the immune system is working to destroy an infection while viral infections that temporarily disrupt the work of the bone marrow can be due to a decrease in WBC, this is because the variations in the values are used as markers of probable infection and also trigger other evaluation, the increase and decrease are frequently used as laboratory markers portending possible underlying infectious processes (Bone *et al,* 1992). The decrease in test group A and C in Neutrophils count shows that there is a significant difference ($p<0.05$) when compared to the group while the test group B had an increased in Neutrophils count. Apparently the increased level in the body of the rat indicates a condition of normal immune responses to an event, such as infection, injury inflammation known as neutrophilic leukocytosis (Bone *et al,* 1992), while the decrease was caused by neutropenia. The lymphocyte count had a decreased level which revealed that there is a significant difference ($p<0.05$) between the control group and test group B that could thereby lead to various disorders and conditions as reported by Territo, 2018. The increase in monocyte for test group C having a significant difference ($p<0.05$) which may be caused by a chronic inflammatory disease, a parasitic or viral disease etc. (Ross and Jewell, 2016). The eosinophil count increased significantly between the control and the test group C. These findings is in conformity with Ross and Jewell, 2016 report on an increased percentage of eosinophils in the blood can indicate an allergic reaction skin inflammation etc. the result on the PCV count decreased significantly when test group B and C was compared with the control. This explains that the red blood cell loss from any variety of cell destruction, blood loss, a failure of bone marrow production. The significant decrease in the total sperm count within group A, B and C when compared with the control indicated that the insecticides had an adverse effect on the fertility of male albino rats. The result on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) had no significant difference ($p>0.05$) when compared to the control group. This indicates that insecticides may have little or no effect on the tested rats. Therefore, testosterone significantly decreased in test group C when compared to the control. This may be caused by delayed puberty, testicular damage, hypothalamic disease, pituitary disease or a non-cancerous pituitary tumor. (Martel 2017).

CONCLUSION

The result generally indicates that insecticide exposure was highly detrimental to the sperm count. It is also detrimental to the liver and blood. Therefore, insecticide effect was observed to be concentration and time dependent.

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