



EFFECT OF *AZADIRACHTA INDICA* (NEEM) SEEDS AND LEAVES EXTRACT ON THE HAEMATOLOGICAL PARAMETERS OF ALBINO RATS

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ABSTRACT

The effects of *Azadirachta indica* (Neem) seeds and leaves extracts on some haematological parameters of albino rats were investigated in this study. *Azadirachta indica* (Neem) leaves and seeds were extracted using a mixture of hexane, acetone and ethanol in a ratio of 1:1:1 as solvent. Twelve albino rats were shared into 4 groups of 3 rats per group. Group 1 (CNT) which was the control group was not treated with the plant extracts. Group 2 (SE) was treated with the seed extract; Group 3 (LE) was treated with the leaf extracts while Group 4 (SY) was treated with Dichlorvos (DDVP) as the positive control. The extracts were administered to the rats by inhalation for 28 days. Haematological parameters were determined using standard method. Results show that the test groups showed a non-significant ($p > 0.05$) increase in the PCV levels when compared to the positive control group (SY). There was a non-significant ($p > 0.05$) difference in WBC concentration across all groups. The test groups showed a significant ($p < 0.05$) increase in lymphocytes levels when compared to the positive control group (SY). This present study demonstrated that Neem seeds and leaves extracts exhibited some level of toxicity and therefore may be a potential and novel source of bio-insecticides.

Keywords: Neem, rat, pest, blood, extracts, test and group

INTRODUCTION

In developing countries, the losses of crops due to pest, plant disease and competition from weeds is great. In households, pest and insects such as mosquitoes, cockroaches, mice etc. pose risks such as the destruction of furniture, clothing and to the causation of various diseases, most seriously; malaria (Kwasi et al., 2011). Pesticides/insecticides produced to kill these pests in order to prevent these damages, also tend to have adverse effects on humans in various ways, most especially those produced from synthetic materials. These adverse effects of headache, dizziness, cataract and other factors make this topic worth investigating. The insecticides range from agricultural to household pesticides. Every category has its own effect, both on the targeted pest/insect and the environment in which it lives. Natural insecticides can be chemical, mineral, or biological. The common goal of all three is to kill, repel, or otherwise interfere with the damaging behavior of insect pests. Concerns about the negative impact of pesticide residues on human and animal health are based on many scientific studies that have indicated the harmful effects of some synthetic

active

substances of pesticides. For example, a number of epidemiological studies have been carried out to evaluate the association between exposure to pesticides and cancer. Botanicals may be considered organically-approved products depending on the extraction method and formulation (other ingredients included in the product). The advantage of using botanical insecticides is their short persistence in the environment due to rapid degradation. However, this short persistence can also be deemed a disadvantage since multiple applications may be needed to achieve adequate pest suppression. This research work therefore, will introduce and encourage the use of bioinsecticides/ biopesticides produced from organic source (especially plant extracts) in eradicating household and agro-pest. Studies have shown that organic products are economical, bio-degradable, eco-friendly and less toxic to the body system when compared to synthetically produced ones. The use of bio-insecticide will not only kill the pest; it is also safe and has no negative effect on the environment. The aim of this work therefore is to investigate the toxicity and effect of *Azadirachta indica* (Neem) seed and leaves extract on some haematological parameters of albino rats.

MATERIALS AND METHODS

Sampling

The neem leaves and seeds used for this analysis were collected from Student Park; Institute of Management and Technology, Enugu, Enugu state. The trade name of the commercially sold insecticide used in this analysis is "VIP (DDVP) insecticide for Agricultural and horticultural pest" a product of African Agro product limited. The commercial insecticide which contains dichlorvos as active ingredient was purchased from a road side hawker in "Ogbete" main market; beside Holy Ghost cathedral, Enugu, Enugu State, Nigeria. Twelve (12) Female albino rats weighing 100 and 120g used in this study were procured from the laboratory animal production unit of Brain phosphorylation (Brain P³) laboratory services, Ogui road, Enugu, Enugu state. Animals were housed in a clean plastic cage with free access to food (standard pellet diet, boiled yam and sweet potatoes; and fried fish) and clean water.

Extraction of Neem Extract

Leaves and seeds of Neem plant were left to dry under an airy shade for eight days then the dried leaves were ground into fine powder separately using mortar and pestle. Powder was soaked in a mixture of hexane, acetone and ethanol solvents in a ratio of 1:1:1 in a flask for about one week according to the method of Ghada et al., (2017). Finally, the flask was

shaken vigorously and its contents were filtered into a 1000ml reagent jar. The resultant crude extract was kept in cool place until use

STUDY DESIGN

The animals were acclimatized for one week prior to the experiment in the laboratory. The rats were randomly grouped into four of three each and labeled CNT, SE, LE and SY which denoted control, seed extract, leaf extract and synthetic respectively. SE, LE and SY groups were placed in a well-ventilated cage and exposed daily to the aerosol from neem seed extract, neem leaf extract and DDVP respectively by inhalation, for four (4) hours, for twenty-eight (28) days while the CNT group was not. Exposure was discontinued after day 28 and blood samples were collected after 24 hours for analysis.

Haematological Analysis

Blood was drawn by puncturing the retro-orbital plexus (venous plexus). The rat was restrained, the neck gently scuffed and the eye made to bulge. A capillary tube was then inserted dorsally to allow the blood flow into vials containing anti-coagulant, ethylene diamine tetra-acetic acid (EDTA). The parameters analyzed were packed cell volume (PCV), white blood cell count (WBC) and differential leukocyte count.

Statistical Analysis

The results of PCV and WBC analysis were expressed as the mean \pm Standard Deviation (SD). Data were analyzed statistically using student's t-test. The significance of the differences between control and the test groups were determined by the student's t-test and the values of $P < 0.05$ were taken to be statistical significant. However, the results of Differential leukocyte count were expressed in percentage means.

RESULTS

Toxicological Effects Of Blood Parameters

Table 1. Toxicological effects of insecticides on blood parameters of female albino rat.

GROUP	PCV (%)	WBC ($\times 10^3/L$)	Neutrophil (%)	Lymphocyte (%)	Monocytes (%)	Eosinophil (%)	Basophils (%)
CNT	44.33 \pm 0.47	18.3 \pm 0.45	32	53	5	8	2
SE	41.67 \pm 1.25	18.27 \pm 0.56	34	57	5	3	1
LE	42.0 \pm 1.63	19.17 \pm 0.29	32	54	7	6	1
SY	37.67 \pm 1.25	19.67 \pm 0.41	33	36	14	15	2

PCV Concentration (%)

FIG

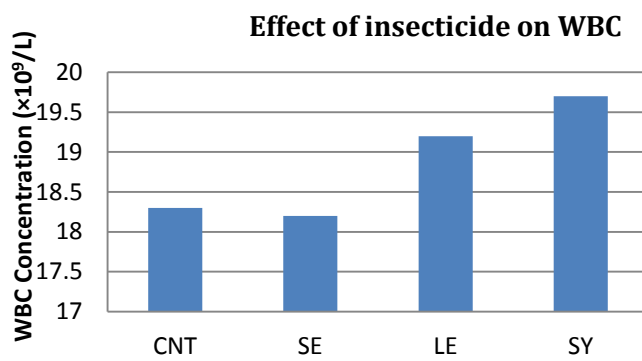


FIG. 2 Generally, ($P > 0.05$) across all groups, although all above normal range

Effect of insecticide on differential leukocyte count

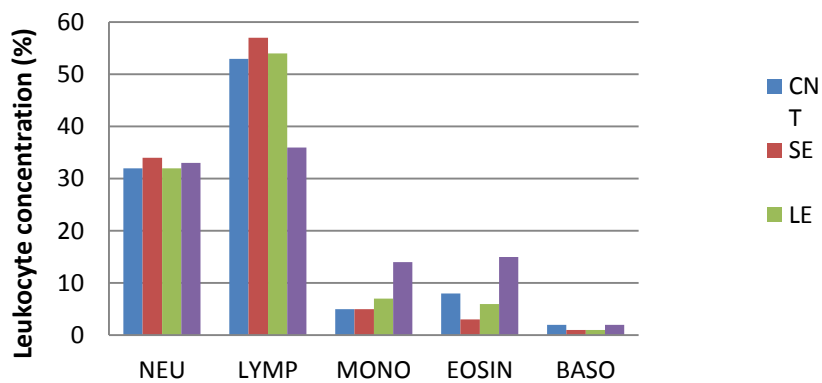


FIG. 3 No significant difference in the neutrophil count across all groups. The lymphocyte count of SY was significantly lower than CNT, SE and LE groups

DISCUSSION

In this study, a significant decrease in PCV levels of SY group is indicative of anaemia. This is in agreement with earlier findings of Holy et al., (2015) after intraperitoneal administration of dichlorvos. Akomas et al., (2014) reported the development of hypochromic anaemia due to a fall in the iron content of the body resulting from oxidative stress. Exposure to dichlorvos induces oxidative stress by generation of reactive oxygen species (ROS) (Wani et al., 2011). Oxidative stress occurs when the production of harmful molecules called free radicals is beyond the protective capability of the antioxidant defenses (Alia et al., 2014). Disruption of the activities of antioxidants suggests alteration of the oxidative state of blood cells by the pesticide formulation. Similar values of SE, LE and CNT rats indicate there was no anaemia and blood production was not affected. Thus the aerosol extracts produced from neem seeds and leaves had no acute toxic effect on the PCV of the rats. The results of the total white blood count revealed a mild leukocytosis in all groups compare to normal values. This is an indication of the likely presence of a mild infection of which the cause could be attributed to the rats' defense mechanism in response to the invading xenobiotic (Holy et al., 2015). However, there was no significant difference between the control and the test animals ($P > 0.05$). This research also shows that exposure to dichlorvos may lead to lymphopenia leucopenia which occurs as a result of abnormal low level of lymphocytes in the blood; thus affecting the immune system of the body. Earlier works have shown that pesticides are toxic to the cells of the immune system through the induction of necrosis and apoptosis. Immune systems are particularly sensitive to oxidative stress due to high percentage of polyunsaturated fatty acids (PUFAs) in their plasma membrane and the increased production of ROS.

CONCLUSION

This present study demonstrated neem extracts are less toxic to haematological parameters of rats as compared to those from the synthetic origin. However, this research is limited to only the effects of neem extracts on some hematological parameters.

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