THE EFFECT OF SUBCHRONIC LOW DOSE OF DDVP AND SODIUM AZIDE ON THE HAEMATOLOGICAL INDICES OF ALBINO RATS

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ABSTRACT

Background: The frequent use of organophosphorous insecticides in our everyday life especially in our houses, offices and even against pests to preserve foods and agricultural products especially in the third world and sodium azide (a herbicide) extensive use in the farms have necessitated this study. Purpose: This research is designed to investigate the effect of low dose DDVP and sodium azide coadministration on hematological parameters of Albino rats. Method: Seven groups of five rats were used for the study. Group A was used as control and received no chemical treatment, groups B and C were injected with 1% and 3% LD50 of sodium azide respectively, while groups D and E received 1% and 3% LD50 of DDVP respectively. Equally, group F received combined doses of 1% DDVP and 1% Sodium azide while group G received 3% DDVP and 3% Sodium azide. The chemicals were administered at alternate days for twenty one days. At the end of twenty one days study period, the animals were anesthetized with chloroform and blood were aspirated through cardiac puncture into heparinized containers and then analyzed with hematology auto analyzer. Results: showed that the hemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), mean corpuscular haemoglobin concentration (MCHC) of rats in groups B and C were not statistically significantly affected.
compared to control while the total white cell count (TWCC) of group C, platelet, lymphocyte and neutrophil count for both groups B and C were statistically significantly reduced compared to control. For rats in groups D and E, the Hb, PCV, RBC count, TWCC, MCHC, platelet count, neutrophil and lymphocyte levels were significantly reduced compared to control. In both groups F and G, the values of TWCC, MCHC, neutrophil, lymphocyte and platelet count were significantly reduced compared to control. All the polymorphological leucocytes, red cell indices and platelets values in group F were dose dependently reduced compared to group G. **Conclusion:** Co-administration of DDVP and sodium azide produced severe reduction of polymorphological leucocytes, red cell indices and platelets.

**KEYWORDS:** DDVP, Sodium azide, Auto analyzer, Hematology parameters, Albino rats.

**INTRODUCTION**

Dichlovors (2,2–dichlorovinyl dimethyl phosphate) also called DDVP is an insecticide that is widely applied in homes, industries, offices and other public places, agriculture, and medicine against a wide, range of insect pests. In the developing world and in large area of sub-Saharan Africa, it is applied on smoked / dried fish, meat, agricultural cereals for preservation against pests. Human exposure ranges from occupational exposure to eating the residual content in DDVP preserved foods and other agric products, polluted air, drinking water etc.

DDVP is an organo-phosphorous compound which functions by inhibiting cholinesterase enzyme activity. Cholinesterase enzyme is an essential enzyme in the body that degrade/metabolizes acetylcholine. Acetylcholine itself is a neurotransmitter that is involved in transfer of impulse signals from nerve to nerve and to both smooth and skeletal muscle hence its primary site of action is peripheral and central nervous systems where it controls various organs and functions of the body. Inhibition of acetylcholinesterase enzyme results in increased activity/toxicity of acetylcholine which includes increased salivation, tremor, diarrhea and death may be caused by respiratory failure or cardiac arrest.

On the other hand, sodium azide is a colourless to white crystal powder/solid. Sodium azide is an ionic substance with two crystalline forms namely rhombohedral and hexagonal. Sodium azide is a very poisonous substance on contact with the skin and on being swallowed. It also readily explodes on heating. Sodium azide is also a well known herbicide that is...
widely applied in agriculture. It is also used in car air bags and air craft ejector systems.\textsuperscript{[9]} In industries, it is used in producing explosives and in the laboratories as preservatives in aqueous reagents.\textsuperscript{[10]} The chances of ingesting residual doses of both chemicals are very high hence this study is aimed at evaluating the toxicity effect of co-administration of low dose levels of both chemicals on hematological indices of Albino rat.

**MATERIALS AND METHODS**

**Study design**

**Animals**

Animals: Adult male albino rats weighing between 180 to 200g which were raised at the animal house of Anatomy Department of Imo State University Owerri Nigeria were used for the study. The animals were fed ad libitum with deionized water and standard rat chow manufactured by Pfizer Pharmaceuticals Plc. Ikeja, Lagos Nigeria.

**DDVP and sodium azide concentrations**

The DDVP used was manufactured by Nantong jiangshan Agrochemical and Chemical Ltd China, while sodium azide salt was made by Qualichems India. The LD\textsubscript{50} of DDVP is 56mg/kg\textsuperscript{[4]} and that of sodium azide is 27mg/kg.\textsuperscript{[11]} 1% and 3% concentrations of their respective LD\textsubscript{50} for both chemicals were got with sterile distilled water.

**Ethical approval**

The research and ethics committee of our school, Federal University of Technology Owerri Nigeria approved the research methodology including the use and method of handling the animals.

**Sub chronic toxicity study**

Thirty five male albino rats were randomly allocated to seven groups of five (5) rats each in seven different cages labeled A to G. The rats in group A received no chemical treatment and were used as control. Group B and C received 1% and 3% LD\textsubscript{50} of sodium azide which is 0.27 mg/kg and 0.81 mg/kg respectively, while group D and E received doses of 1% and 3% LD\textsubscript{50} of DDVP which is also 0.56 mg/kg and 1.68 mg/kg respectively. The rats in groups F were co-administered with 1% (0.56 mg/kg) DDVP and 1% (0.27mg/kg) sodium azide, while group G received 3% (1.68mg/kg) DDVP and 3% (0.81mg/kg) sodium azide. The chemicals were given to the rats intraperitonealy at alternate days for twenty one days duration of the study. The rats were given free access to water and feeds. At the end of the three weeks study
period, the rats were anesthetized with chloroform before blood was collected through cardiac puncture. The blood samples were collected into heparinized containers before analyzing with haematology auto analyzer ERMA (model pce 210) made in Japan. The parameters analysed include haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), red blood cell count (RBC), total white cell count (TWCC), neutrophil, lymphocyte and platelets.

Statistical Analysis
The data were reported as mean ± SEM. Students t-test was used to analyze the differences between sample means while analyses of variance (ANOVA) was employed to analyze the data using computer software, standard package for social sciences (SPSS) version 16. P≤0.05 was considered significant.

RESULTS
The results as shown in table 1 indicated that 0.27/kg (group B) and 0.81mg/kg (group C) sodium azide had no effect in all red blood cell indices of the Albino rats which include haemoglobin concentration (Hb), mean corpuscular haemoglobin concentration (MCHC), red blood cell count (RBC), and packed cell volume (PCV). The neutrophil, lymphocyte and platelet count were all statistically significantly reduced compared to control while total white cell count (TWCC) did not show significant reduction compared to control. The 0.56mg/kg (group D) and 1.68mg/kg (group E) DDVP had statistically significant reduction in all the red and white blood cell indices which include haemoglobin concentration (Hb), mean corpuscular haemoglobin concentration (MCHC), red blood cell count (RBC), packed cell volume (PCV), neutrophil, lymphocyte and platelet count compared to control.

The co-administered doses, that is 0.56mg/kg DDVP and 0.27mg/kg Sodium azide (group F) and 1.68mg/kg DDVP and 0.81mg/kg Sodium azide (group G) as shown in table 1 showed that all the indices including platelets were all statistically significantly reduced compared to control except Hb concentration that presented no effect. The results as shown were dose dependently reduced in 1.68mg/kg DDVP and 0.81mg/kg Sodium azide co-administration (group G) when compared to 0.56mg/kg DDVP and 0.27mg/kg Sodium azide (group F).

DISCUSSION
DDVP (dichlorvos) an insecticide and sodium azide a herbicide are widely applied against pests and also in agricultural sector. This exposes them directly and indirectly to us and our
food chain which poses a threat to humans. This study therefore is designed to investigate the effect of low dose DDVP and sodium azide co-administration on hematological parameters of Albino rats. Previous works done to study toxicological effects of pesticide and herbicide co-administration scarcely exists in the literature. In the present study, we reported significantly decreased value for Hb, hematocrite, MCHC, neutrophil, lymphocyte and platelet count for DDVP sub chronic administration. Our findings were in agreement with the work earlier done by Elliger et al\cite{12} who reported increased values of Hb, PCV and MCHC with DDVP treatment in acute exposure but decreased values in sub chronic exposure. In acute DDVP exposure, there is hypoxia which stimulates red blood cell production\cite{12}, but as exposure increases to sub chronic and chronic conditions, the bone marrow is depressed with decreased blood cells production. Reduced red blood cell production limits the ability of red blood cell to carry oxygen to other tissues\cite{13} and in this way may increase the chances of toxicity.

Sodium azide administration did not affect red cell indices in our study and did not present anemic effect. The lymphocyte, neutrophil and platelet levels were however statistically significantly reduced compared to control. Neutrophils and lymphocytes are polymorphs which are essential in phagocytosis which helps our body systems to fight infections.\cite{14,15} Therefore the decreased level of the polymorphs renders the body ineffective in fighting infections.

In our findings, co-administration of sodium azide and DDVP caused severe reduction of the polymorphs and platelets including the red blood cell indices. Sodium azide generally is not toxic to the red cell component of the blood. However sodium azide generates nitrous oxide via catalase reaction which is a potent vasodilator.\cite{14} In all the treatment groups of DDVP and sodium azide, platelet count were statistically significantly reduced with platelet levels in the co-administered groups showing further reduction. Platelets are not cells but small fragments of marrow cells called megakaryocytes. The platelets or thrombocytes functions by aiding blood coagulation and also performs other functions by internalizing bacteria and destroying them. They also secrete growth factor that stimulate mitosis in fibroblasts and smooth muscles and also serves as vasoconstrictors.\cite{13} In view of all these important functions of platelets, sodium azide and DDVP are therefore detrimental to normal physiological functions by directly depressing platelets production. However, platelets have also been implicated in ischemic heart disease where they aid in coronary artery occlusion in which case reduced level of platelets as presented in this study may be beneficial. Atropine is
a natural antidote to DDVP but sodium azide is not known to have one. Ahmed and co-workers\textsuperscript{[9]} have demonstrated that L-carnitine prevented degenerative effects of sodium azide in rat brain. Further studies will be needed to determine a possible antidote to toxicity associated with sodium azide and DDVP co-administration.

In conclusion, sodium azide co-administration with DDVP produced severe reduction of polymorphological leucocytes, red cell indices and platelets.

Table 1 Effect of Sodium azide and DDVP on haematological parameters of Albino Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb (g/dl)</th>
<th>TWCC($\text{mm}^3$)</th>
<th>MCHC(pg)</th>
<th>RBC ($\times 10^6$)</th>
<th>PCV (%)</th>
<th>Neutrophil</th>
<th>Lymphocyte</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A Control</td>
<td>16.12±0.056</td>
<td>4710±33.2</td>
<td>32.64±0.31</td>
<td>7.6±0.03</td>
<td>48.8±0.4</td>
<td>18.5±0.2</td>
<td>89.8±1.6</td>
<td>695,200±1392</td>
</tr>
<tr>
<td>Group B (0.27mg/kg sodium azide)</td>
<td>16.02±0.76</td>
<td>4650±50</td>
<td>31.96±0.08</td>
<td>7.44±0.10</td>
<td>48.7±0.4</td>
<td>16.46±0.14*</td>
<td>84.0±0.7*</td>
<td>416,200±1392*</td>
</tr>
<tr>
<td>Group C (0.81mg/kg sodium azide)</td>
<td>16.12±0.93</td>
<td>4100±35</td>
<td>30.80±0.05</td>
<td>7.38±0.05</td>
<td>48.7±0.84</td>
<td>14.8±0.03*</td>
<td>75.8±0.5*</td>
<td>410000±1345*</td>
</tr>
<tr>
<td>Group D (0.56mg/kg DDVP)</td>
<td>12.14±0.07*</td>
<td>2540±67</td>
<td>31.5±0.17*</td>
<td>6.31±0.13*</td>
<td>46.6±0.25*</td>
<td>16.0±0.03*</td>
<td>75.6±1.03*</td>
<td>457,200±8266*</td>
</tr>
<tr>
<td>Group E (1.68mg/kg DDVP)</td>
<td>12.4±0.12*</td>
<td>3930±37.5*</td>
<td>30.4±0.45*</td>
<td>6.42±0.06</td>
<td>41.3±0.8*</td>
<td>15.14±0.57*</td>
<td>72.1±0.79*</td>
<td>415,750±6536*</td>
</tr>
<tr>
<td>Group F (0.56mg/kg DDVP &amp; 0.27mg/kg sodium azide)</td>
<td>16.02±0.17</td>
<td>2100±0.3*</td>
<td>31.46±0.32</td>
<td>6.0±0.16</td>
<td>47.2±0.66</td>
<td>8.96±0.11*</td>
<td>71.4±0.75*</td>
<td>398,200±4923*</td>
</tr>
<tr>
<td>Group G (1.68mg/kg DDVP &amp; 0.81mg/kg sodium azide)</td>
<td>16.02±0.17</td>
<td>1120±37.42*</td>
<td>30.80±0.27*</td>
<td>4.83±0.027*</td>
<td>40.24±0.19*</td>
<td>18.54±0.2*</td>
<td>68.8±0.58*</td>
<td>398200±4923.41*</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SEM

*= Statistically significant compared to control

P ≤ 0.05

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CONFLICT INTEREST: Non declared.

GRANT SUPPORT: Non

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