Protective Effect of *Nigella Sativa* (Black Caraway) Oil on Oral Dichlorvos Induced Hematological, Renal and Nonspecific Immune System Toxicity in Wistar Rats

Moyosore Salihu Ajao 1, Adebayo Babatunde Sansa 1, Aminu Imam* 1, Abdulmumin Ibrahim 1, Misturat Yetunde Adana 1, Abdulmusawwir Alli-Oluwafuyi 2, Suwebat Bidemi Kareem 1

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ABSTRACT

Background: Exposure to environmental toxins such as organophosphates poses a great threat to the health of the public. In this work, we investigated the effects of continuous exposure to dichlorvos (DDVP) on kidney function and hematological parameters, and the possible antidote activity of *Nigella sativa* oil (NSO).

Methods: This research was conducted in 2016, at The Animal Holding and Research Laboratory of Faculty Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria. Twenty-four Wistar rats were randomly divided into four groups, six rats each. The four groups received: 1. phosphate buffer solution as controls, 2. DDVP, 3. DDVP+NSO and 4. NSO alone. After 2 wk of treatment, blood samples were collected and hematological profile (RBC, Hb), erythrocyte indices (MCV, MCH, MCHC, and Plt), renal function parameters (albumin, urea, total protein, chloride, sodium, and potassium ions) and nonspecific immune response (WBC) were measured.

Results: Rat exposed to DDVP showed red blood cell count, hemoglobin, packed cell volume, albumin, and total protein levels was reduced from control, while white blood cell count and urea significantly increased as compared to controls, the change in K+ level was not significant. NSO maintained optimal levels of red blood cell count, hemoglobin, packed cell volume, albumin, white blood cell count, and urea, indicative of its protective effect against hemo-, immuno- and nephrotoxicity of DDVP.

Conclusion: *N. sativa* (Black Caraway) oil might be a potential antidote in hematoxicity, immunosuppression and renal dysfunction in organophosphate poisoning, especially dichlorvos. The protective effect of NSO against dichlorvos toxicity can be attributed to its antioxidant capacity.

Keywords: Anemia, Dichlorvos, Kidney Function Tests, *Nigella Sativa*, Organophosphates, Organophosphate Poisoning.

INTRODUCTION

Harmful chemicals in the environment and their burden on health have become an issue of great importance in recent decades [1], causing toxicity to a variety of biota and human life [2].

Organophosphates (OPs) are one of the most dangerous chemicals used in agriculture and household, and are highly toxic to liver and muscles, as well as, nervous, immune, urinary, reproductive and hematological systems [3-5].

A commonly used OP, dichlorvos or 2, 2-dichlorovinyl dimethyl phosphate (DDVP), is extensively used for pest and insect control [6], and despite the reported toxicity, it is a major constituent of most of the insecticides used in the developing world [7]. Although several antidotes are available in the management of OP toxicity, the available options have some limitations with unavoidable side effects [8]. Therefore, the search for new and novel therapeutic drugs for OP poisoning is crucial. Alternative medicine and supplementary substances with beneficial effects in...
immunological, hematological and renal systems
definitely have a place in the treatment of OP
poisoning.

In recent years, interest in the use of natural
products as alternative therapy in disease conditions
has increased, gaining wide acceptance from
the public and medical professionals. *Nigella sativa*
*Linn.* (Ranunculaceae) is a medicinal plant also
known as black caraway, black cumin, *Habbatul
Barakah* or and Kalonji seed, is widely used in the
treatment of various ailments such as bronchial
asthma, cough, diarrhoea, abdominal pain, and
dyslipidemia [9]. *N. sativa* oil (NSO) exhibits
pharmacological activities including the ability to
act as a chelating agent [10], antioxidant and anti-
inflammatory [11], immunomodulatory and
antitumor substances [12].

Blood, being the medium of intercellular
transport, plays an important role in the immune
system. It rapidly comes in direct contact with
various tissues in the body, therefore, the
physiological state of an organism is always
reflected in the contents of the blood [13], and thus,
the hematological and biochemical parameters can
indicate toxicity with great potential for
environmental monitoring of health [14].

Perturbations in immune functions are caused by
immunotoxic compounds, leading to immune
suppression, resulting in decreased resistance to
infections, hypersensitivity, and autoimmunity
caused by disorders in immune regulation [13].

This study investigated the antidotal efficacy of
*N. sativa* oil in mitigating dichlorvos induced
hematological, immunological and renal damage in
Wistar rats.

**MATERIALS AND METHODS**

**Chemicals and Drugs**

Dichlorvos was purchased from the Sigma
Chemicals (St. Louis, MO, USA), while the
phosphate buffer solution (PBS) was prepared in
our laboratory. The black caraway oil (100% pure
natural oil) was obtained from Masrawarda, Kingdom
of Saudi Arabia.

**Animals**

Twenty-four adult male Wistar rats with an
average weight of 200 ± 20 gr were used. The
animals were housed (six per cage) under standard
laboratory conditions in the animal holding of the
Faculty of Basic Medical Sciences, University of
Ilorin, Nigeria, in 2016. They were allowed free
access to water and food *ad libitum*.

**Treatments Schedule**

The rats were randomly divided into five groups
(n = 6) as follows:

- **Controls:** received phosphate buffer solution
  (PBS) (1 ml/kg oral)
- **Exp. 1:** received DDVP (8.8 mg/kg/day orally) [15]
- **Exp. 2:** received DDVP (8.8 mg/kg/day orally) +
  NSO (1 ml/kg orally) 30 min. post-treatment
- **Exp. 3:** received NSO (1 ml/kg orally) [16]

All administrations were scheduled and carried
out during the light phase between 7:00 and 9:00
am. All groups contained six rats each and
treatments continued for fourteen consecutive days.

**Ethical Approval**

All experimental procedures were performed in
accordance with our institutional guidelines for
animal care and use and ethical approvals were
received from the University of Ilorin's Ethics
Committee.

**Hematological, Immunological and
Erythrocytes Indices Study**

Animals were euthanized with intraperitoneal
ketamine injection, and after immobility was
confirmed, blood samples were collected. Freshly
collected blood samples were analyzed for
hematological analysis using an automatic
hematological assay analyzer (Beckman Coulter,
USA). Hematological parameters that were tested
were as follows: red blood cells count (RBC),
packed cell volume (PCV), white blood cells count
(WBC), mean cell volume (MCV), mean
corpuscular hemoglobin (MCH), mean corpuscular
hemoglobin concentration (MCHC), hemoglobin
(Hb), neutrophils and lymphocytes counts.

**Kidney Function Study**

Blood samples were centrifuged at 3000 rpm for
15 min. The plasma was collected and used for the
analysis of urea, albumin, total protein, Na⁺, K⁺,
and Cl⁻ using commercial kits supplied by Randox
Laboratories Limited, United Kingdom.

**Statistical Analysis**

Data recorded in this study were reported as
mean ± standard error of mean. They were analyzed
using one-way analysis of variance (ANOVA) and
for post-hoc analyses, we used the Bonferroni test.
A *P*-value of ≤ 0.05 was considered statistically
significant.
RESULTS

Effect of DDVP and NSO on Hematological Parameters

Dichlorvos (DDVP) caused a significant ($P \leq 0.05$) reduction in red blood cells count (RBC), hemoglobin (HGB) and packed cell volume (PCV), while post-treatment with NSO increased all these indices (Table 1).

Effects of DDVP and NSO on Erythrocytes Indices

DDVP resulted in reduction in the mean cell volume (MCV), mean cell hemoglobin (MCH), and increased mean cell hemoglobin concentration (MCHC), although not statistically significant. While NSO only and DDVP rats that were post-treated with NSO demonstrated fair levels of MCV, MCH and MCHC (Table 1).

Effects of DDVP and NSO on Kidney Functions

DDVP produced significant ($P \leq 0.05$) reduction in plasma concentrations of total protein and albumin. It also increased urea, $Na^{+}$, $K^{+}$, and $Cl^{-}$ levels in the treated animals (Table 2), and a pointer to renal dysfunction. However, post-treatment with NSO ameliorated these changes (Table 2).

Table 1. Showing levels of hematological parameters, Erythrocytes indices and Peripheral blood leucocytes in animals treated with PBS, DDVP, DDVP+NSO and NSO only.

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC (x10¹²/L)</th>
<th>HGB (g/dl)</th>
<th>PCV (%)</th>
<th>MCHC (g/dl)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>WBC (X10⁹/L)</th>
<th>NEUT (%)</th>
<th>LYMPH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>6.74±0.13</td>
<td>16.08±0.7</td>
<td>42.20±1.4</td>
<td>35.38±2.0</td>
<td>62.40±1.1</td>
<td>22.94±0.9</td>
<td>6.63±0.3</td>
<td>68.60±1.9</td>
<td>30.20±2.5</td>
</tr>
<tr>
<td>DDVP</td>
<td>3.54±0.24</td>
<td>7.86±0.52</td>
<td>23.80±2.0</td>
<td>36.80±1.2</td>
<td>57.60±3.1</td>
<td>22.16±0.0</td>
<td>11.67±1.0</td>
<td>68.60±3.4</td>
<td>31.40±1.8</td>
</tr>
<tr>
<td>DDVP+NSO</td>
<td>6.19±0.25</td>
<td>15.62±0.4</td>
<td>41.00±1.4</td>
<td>39.08±2.9</td>
<td>62.60±1.6</td>
<td>22.66±0.0</td>
<td>7.43±0.9</td>
<td>69.40±0.4</td>
<td>30.40±0.2</td>
</tr>
<tr>
<td>NSO</td>
<td>6.81±0.14</td>
<td>14.06±0.9</td>
<td>42.60±1.2</td>
<td>35.54±0.3</td>
<td>63.60±1.6</td>
<td>23.92±1.0</td>
<td>5.92±0.7</td>
<td>68.00±0.8</td>
<td>30.60±0.6</td>
</tr>
</tbody>
</table>

ANOVA* = $P \leq 0.05$

Table 2. Showing plasma levels of kidney function parameters in rats treated with PBS, DDVP, DDVP+NSO and NSO only.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alb (g/dl)</th>
<th>Urea (mmol/l)</th>
<th>Total Prot. (g/dl)</th>
<th>Na⁺ (mg/dl)</th>
<th>K⁺ (mg/dl)</th>
<th>Cl⁻ (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>1.27±0.06</td>
<td>11.68±0.34</td>
<td>5.57±0.47</td>
<td>29.64±4.33</td>
<td>3.94±0.49</td>
<td>67.15±15.67</td>
</tr>
<tr>
<td>DDVP</td>
<td>0.75±0.63</td>
<td>16.33±0.38</td>
<td>5.40±0.59</td>
<td>34.34±5.69</td>
<td>8.76±1.61</td>
<td>69.54±2.37</td>
</tr>
<tr>
<td>DDVP+NSO</td>
<td>1.22±0.08</td>
<td>13.58±0.61</td>
<td>5.48±0.34</td>
<td>30.64±6.04</td>
<td>8.40±1.18</td>
<td>62.42±5.98</td>
</tr>
<tr>
<td>NSO</td>
<td>1.12±0.07</td>
<td>9.79±0.50</td>
<td>6.08±0.26</td>
<td>23.12±2.78</td>
<td>6.64±0.43</td>
<td>55.63±6.14</td>
</tr>
</tbody>
</table>

ANOVA* = $P \leq 0.05$

DISCUSSION

Indiscriminate and uncontrolled use of pesticides and insecticides have resulted in severe environmental pollution, acute and chronic human poisoning. The bioaccumulation of these substances is associated with metabolic, immunological, oxidative and antioxidative changes, and can reduce human lifespan [17].

Blood is a pathological and physiological indicator [18] and chemicals, enzymes or ion concentrations in the blood or its derivatives are validated markers of health. The reduced levels of RBC, HGB, and PCV reported in this study, following DDVP exposure, suggested the hematotoxic effects of DDVP. This was in accordance with a previous study [19] and was strengthened by the reports concerning exposure to other OPs [13, 20, 21].

Red blood cell indices reflect the size (MCV) and hemoglobin content (MCH and MCHC) of red
blood cells and aid in the diagnosis of possible anemia inducing properties of different substances. The reduction in MCV and MCH with an increase in MCHC that we observed, were similar to previous researches [13, 19], but it was in disagreement with the report that claimed an undisturbed erythrocytes indices after exposure to OP [22]. Also, the changes in total WBC, neutrophils, and lymphocytes documented in this study were in line strengthened with the reports of previous studies [13, 14], with the exception of a reduction in lymphocyte count [13].

In the present study, levels of serum albumin, urea, total protein, chloride, sodium and potassium ions were also measured. Some impaired levels of these parameters following DDVP exposure suggested renal dysfunctions in the treated animals, and this was similar to what has been extensively reported in the scientific literature following exposures to most insecticides [3, 4, 23]. The elevation of urea concentrations may be attributed to a reduction in the glomerular filtration rate in the kidney [24], and the reduction in the albumin and total protein levels are significant indications of renal dysfunctions, as reported recently with OPs [13, 25].

Post-treatment with NSO neutralized DDVP associated hematological, immunological and renal toxicity. These effects can be attributed to NSO's efficacy against OP-induced toxicity in various body systems including hormonal, reproductive, liver and kidneys [26-29].

The kidney is the major target organ for exogenous toxicants, and it is important that any substance with possible therapeutic use against DDVP poisoning should protect renal function too. As observed in this study, NSO reduced serum urea concentration, thereby normalizing impaired urine filtration induced by DDVP. This finding was supported by a recent study employing NSO as a protective agent against cisplatin-induced nephrotoxicity in male rats [30].

The protective effect of *N. sativa* oil against dichlorvos toxicity can be attributed to its antioxidant capacity, as previous works have reported the beneficial effects of antioxidants drugs and supplements against OPs poisonings, including DDVP [31-33].

**CONCLUSION**

*N. sativa* (Black caraway) oil might be a potential antidote in hematotoxicity, immunosuppression and renal dysfunction in organophosphate poisoning, especially dichlorvos.

**ACKNOWLEDGEMENTS**

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