Original Article

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

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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 hematotoxicity, 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.

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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

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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 oral) [15]

Exp. 2: received DDVP (8.8 mg/kg/day orally) + NSO (1 ml/kg oral) 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^{2+}, 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 Peripheral Blood Leucocytes (Immunology)

DDVP induced significant ($P \leq 0.05$) increases in total white blood cells (WBC), but neutrophils (NEUT) and lymphocytes (LYMPH) did not change. Post-treatment with NSO improved white blood cell counts (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^{2+}, 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.

	Hematological Parameters			Erythrocytes Indices			Peripheral Blood Leucocytes		
Groups	RBC ($x10^{12}$/L)	HGB (g/dl)	PCV (%)	MCHC (g/dl)	MCV (fl)	MCH (pg)	WBC ($X10^{9}$/L)	NEUT (%)	LYMPH (%)
PBS	6.74±0.13	16.08±0.76	42.20±1.46	35.38±2.09	62.40±1.17	22.94±0.41	6.63±0.3	66.80±1.98	30.20±2.54
DDVP	3.54±0.24*	7.86±0.52*	23.80±2.08*	36.80±1.25	57.60±3.17	22.16±0.93	11.67±1.3*	68.60±3.44	31.40±1.86
DDVP+NSO	6.19±0.25	15.62±0.41	41.00±1.48	39.08±2.91	62.60±1.60	22.66±0.62	7.43±0.9	69.40±0.40	30.40±0.24
NSO	6.81±0.14	14.06±0.90	42.60±1.29	35.54±0.33	63.60±1.66	23.92±1.02	5.92±0.7*	68.00±0.89	30.60±0.60

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.

Groups	Alb (g/dl)	Urea (mmol/l)	Total Prot. (g/dl)	Na^{2+} (mg/dl)	K^{+} (mg/dl)	Cl^{-} (mg/dl)
PBS	1.27±0.06	11.68±0.34	5.57±0.47	29.64±4.33	3.94±0.49*	67.15±15.67
DDVP	0.75±0.63*	16.33±0.38*	5.40±0.59	34.34±5.69	8.76±1.61*	69.54±2.37
DDVP+NSO	1.22±0.08	13.58±0.61	5.48±0.34	30.64±6.04	8.40±1.18	62.42±5.98
NSO	1.12±0.07	9.79±0.50*	6.08±0.26	23.12±2.78	6.64±0.43	55.63±6.14*

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 where 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.

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REFERENCES

1. Lokke H, Ragas AM, Holmstrup M. Tools and perspectives for assessing chemical mixtures and multiple stressors. Toxicol 2013;313(2):73-82.
2. Zaluski R, Kadri SM, Alonso DP, Martins Ribolla PE, de Oliveira Orsi R. Fipronil promotes motor and behavioral changes in honey bees (Apis mellifera) and affects the development of colonies exposed to sublethal doses. Environ Toxicol Chem 2015;34(5):1062-9.
3. Abdel-Daim MM. Synergistic protective role of ceftriaxone and ascorbic acid against subacute diazinon-induced nephrotoxicity in rats. Cytotechnology 2016;68(2):279-89.
4. Li S, Cao C, Shi H, Yang S, Qi L, Zhao X, et al. Effect of quercetin against mixture of four organophosphate pesticides induced nephrotoxicity in rats. Xenobiotica 2016;46(3):225-33.
5. Mehri N, Felehgari H, Harchegani A, Behrooj H, Kheiripour N, Ghasemibasir H, et al. Hepatoprotective effect of the root extract of green tea against malathion-induced oxidative stress in rats. J Herb Med Pharmacol 2016;5:116-9.
6. Deka S, Mahanta R. Dichlorvos toxicity on fish-a review. Eur J Bio Res 2015;5(3):78-85.
7. Brown H, Oruambo F, Kenanagha B. Poor anted effects of copper and manganese on ratsexpossed to acute dose of dichlorvos. Ejpmr 2015; 2(1):290-303.
8. Yadav P, Jadhav S, Kumar V, Kaul K, Pant S, Flora S. Protective efficacy of 2-PAMCl, atropine and curcumin against dichlorvos induced toxicity in rats. InterdiscToxicol 2012;5(1):1-8.
9. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Tropic Biomed 2013;3(5):337-52.
10. Ahmad S, Beg ZH. Elucidation of mechanisms of actions of thymoquinone-enriched methanolic and volatile oil extracts from *Nigella sativa* against cardiovascular risk parameters in experimental hyperlipidemia. Lipids Health Dis 2013;12(1):86-7.
11. Ahmed MA, Hassanein KM. Cardio protective effects of *Nigella sativa* oil on lead induced cardio toxicity: Anti inflammatory and antioxidant mechanism. J Physiol Path 2013;4(5):72-80.

12. Bai T, Lian L-H, Wu Y-L, Wan Y, Nan J-X. Thymoquinone attenuates liver fibrosis via PI3K and TLR4 signaling pathways in activated hepatic stellate cells. Int Immunopharmacol 2013;15(2):275-81.
13. Narra MR. Haematological and immune upshots in *Clarias batrachus* exposed to dimethoate and defying response of dietary ascorbic acid. Chemosphere 2017;168:988-95.
14. Narra MR. Single and cartel effect of pesticides on biochemical and haematological status of *Clarias batrachus*: A long-term monitoring. Chemosphere 2016;144:966-74.
15. Sharma P, Singh R. Dichlorvos and lindane induced oxidative stress in rat brain: Protective effects of ginger. Pharm Res 2012;4(1):27-8.
16. Korany NS, Ezzat BA. Prophylactic effect of green tea and *Nigella sativa* extracts against fenitrothion-induced toxicity in rat parotid gland. Arch Oral Biology 2011;56(11):1339-46.
17. Jordaan MS, Reinecke SA, Reinecke AJ. Biomarker responses and morphological effects in juvenile tilapia *Oreochromis mossambicus* following sequential exposure to the organophosphate azinphos-methyl. AquatToxicol 2013;144:133-40.
18. Jorum O, Piero N, Machocho A. Haematological Effects of Dichloromethane-Methanolic Leaf Extracts of *Carissa edulis* (Forssk.) Vahl in Normal Rat Models. J Hematol Thrombo Dis 2016;4(232):2-3.
19. Kanu KC, Ijioma SN, Atiata O. Haematological, Biochemical and Antioxidant Changes in Wistar Rats Exposed to Dichlorvos Based Insecticide Formulation Used in Southeast Nigeria. Toxics 2016;4(4):28-9.
20. Narra MR. Single and cartel effect of pesticides on biochemical and haematological status of *Clarias batrachus*: A long-term monitoring. Chemosphere 2016;144:966-74.
21. Rehman H, Aziz AT, Saggu S, VanWert AL, Zidan N, Saggu S. Additive toxic effect of deltamethrin and cadmium on hepatic, hematological, and immunological parameters in mice. Toxicol Ind Health 2017;33(6):495-502.
22. Chakroun S, Ezzi L, Grissa I, Kerkeni E, Neffati F, Bhouri R, et al. Hematological, biochemical, and toxicopathic effects of subchronic acetamiprid toxicity in Wistar rats. Environ Sci Pollut Res 2016;23(24):25191-9.
23. Gh Farag A, M Gamila A, Kotb, Hamza A, Hussein R, Elhalwagy M. Impact of Organophosphorus insecticide Triazophos on Liver, Kidneys and Thyroid in Albino Rats. Int J Adv Res Biol Sci 2016; 3:199–208.
24. Nasr HM, El-Demerdash FM, El-Nagar WA. Neuro and renal toxicity induced by chlorpyrifos and abamectin in rats. Environ Sci Pollut Res 2016;23(2):1852-9.
25. Kanbur M, Siliğ Y, Eraslan G, Karabacak M, Sarıca ZS, Şahin S. The toxic effect of cypermethrin, amitraz and combinations of cypermethrin-amitraz in rats. Environ Sci Pollut Res 2016;23(6):5232-42.
26. Farooqui Z, Afsar M, Rizwan S, Khan AA, Khan F. Oral administration of *Nigella sativa* oil ameliorates the effect of cisplatin on membrane enzymes, carbohydrate metabolism and oxidative damage in rat liver. Toxicol Rep 2016;3:328-35.
27. Mohtashami A, Entezari MH. Effects of *Nigella sativa* supplementation on blood parameters and anthropometric indices in adults: A systematic review on clinical trials. Journal of research in medical sciences: the official Isfahan J Res Med Sci 2016;21:3-4.
28. Mosbah R, Yousef MI, Maranghi F, Mantovani A. Protective role of *Nigella sativa* oil against reproductive toxicity, hormonal alterations, and oxidative damage induced by chlorpyrifos in male rats. Toxicol Ind Health 2016;32(7):1266-77.
29. Farooqui Z, Ahmed F, Rizwan S, Shahid F, Khan AA, Khan F. Protective effect of *Nigella sativa* oil on cisplatin induced nephrotoxicity and oxidative damage in rat kidney. Biomed Pharmacother 2017;85:7-15.
30. Hosseinian S, Rad AK, Mousa-Al-Reza Hadjzadeh NM, Roshan SH, Shafiee S. The protective effect of *Nigella sativa* against cisplatin-induced nephrotoxicity in rats. Avicenna J Phytomed 2016;6(1):44-5.
31. Dhouib IE-B. Anti-inflammatory Effects of N-acetylcysteine against Carbosulfan-induced Hepatic Impairment in Male Rats. Recent Adv Biol med 2015; 1:29-40.
32. Nurulain SM, Ojha S, Tekes K, Shafiullah M, Kalasz H, Adem A. Efficacy of N-acetylcysteine, glutathione, and ascorbic acid in acute toxicity of paraoxon to Wistar rats: survival study. Oxid Med Cell Longevity 2015;2015.
33. Manal EA, Elhalwagy AA, Nahas RMZ, Hoda EAF. Potential Effect of Vitamin C and Curcumin on Oxidative Stress and Skin Lesion Induced by Dermal Intoxication with Cypermethrin. Amer Chem Sci J 2015; 8(1):1-12.