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Subchronic dichlorvos-induced Cardiotoxicity in Wistar rats: Mitigative efficacy of *Nigella sativa* oil

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Abstract:

BACKGROUND: Accidental poisoning from indiscriminate use of organophosphates have become endemic in recent decades, most especially in developing nations, coupled with the limitations of the availability of satisfactory antidotes.

AIM OF THE STUDY: Thus, we investigated the cardioprotective efficacy of *Nigella sativa* oil (NSO) following dichlorvos dichlorvos (DDVP)-induced cardiotoxicity in Wistar rats.

MATERIALS AND METHODS: A total of 24 Wistar rats were randomly divided into four groups ($n = 6$); the control was administered sunflower oil (1 ml/kg), DDVP (8.8 mg/kg) to the experimental Group I, whereas DDVP + NSO (8.8 mg/kg +1 ml/kg) and NSO (1 ml/kg) was administered orally to the experimental Groups II and III, respectively. The animals were euthanized; blood was transcardially collected from the right atrium, centrifuged, and plasma extracted to analyze levels of total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol (LDL-C). While cardiac muscle tissue was collected from the left heart, processed and stained for general architecture (hematoxylin and eosin) and elastic morphology (orcein).

RESULTS: DDVP significantly ($P \leq 0.05$) increased the plasma levels of TC, LDL, atherogenic and atherosclerotic indices (TC/HDL-C and LDL-C/HDL-C ratios), but this was prevented by co-administration with NSO. Histological investigations showed that DDVP resulted in the pathological appearance of cardiac tissues, such as the lack of striations, myocardial hemorrhage, and necrosis-like features.

CONCLUSION: It can be concluded that NSO was able to attenuate DDVP-induced cardiotoxicity.

Keywords:

Cardiotoxicity, dichlorvos, *Nigella sativa* oil, organophosphate, poisoning

Introduction

Organophosphate (OP) pesticides are widely employed in the control of household and agricultural pests. However, their indiscriminate use has led to great environmental pollution, contaminating air, soil, water, and farm produce leading to unsuspected health burdens (Davies *et al*., 2016; Farrukh *et al*. 2016; Rashmikaa *et al*., 2016; Weidong *et al*., 2016).

The toxicity of most of these OPs, which are insecticidally active to animals, is based on their property to inhibit acetylcholinesterase, resulting in the accumulation of acetylcholine in the presynaptic space. This has been associated with several deleterious effects including muscle incoordination, tremors, myosis, chest discomfort, decreased heart irregularities, loss of reflexes, muscular paralysis, autonomic overstimulation, and cardiorespiratory failure among other potentially lethal syndrome (Roth *et al*.,

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1993; Mostafalou and Abdollahi, 2013; Rosman *et al*., 2014; Arthur *et al*., 2017).

Documented evidence have shown that OP poisonings are mostly accompanied by cardiovascular complications, with abnormalities on electrocardiography (ECG), conduction and ventricular arrhythmias (Karki *et al*., 2004; Anand *et al*., 2009; Mostafalou and Abdollahi, 2013).

Other complications of OPs exposure include mutagenicity (Bhinder and Chaudhry, 2013), neurotoxicity (Galal *et al*., 2014; Shrot *et al*., 2015), carcinogenicity (Greim *et al*., 2015), hepatotoxicity (Ogutcu *et al*., 2008), and nephrotoxicity (Hou *et al*., 2014).

Of all OPs, Dichlorvos is one of the most used in the developing countries (Deka and Mahanta, 2015), and has been reported to be the cause of severe poisoning and death associated with most OPs products used in these nations (Musa *et al*., 2010; Brown *et al*., 2015). It has been implicated to induce a more rapid onset of poisoning symptoms when compared with other OPs, although with rapid recovery (Erdman, 2004).

DDVP-induced toxicity has been linked to a number of mechanisms including cholinesterase and non-cholinesterase pathways, however, treatment with the available antidotes remains a challenge. Thus, finding novel alternative or supplementary regimen with potential efficacy against DDVP and OP poisoning is vital. Thus, research into an alternative regimen in the management of DDVP induced cardiotoxicity is crucial, with great focus on the supplementary regimen.

Nigella sativa oil (NSO) is a high-value traditionally used medical regimen in the management of various diseases. Its efficacy has been extensively studied and reported to be subserved with neuro-protective (Yaman and Balikci, 2010), antioxidant (Kanter *et al*., 2008), anti-inflammatory (Noor *et al*., 2015), anti-ischemic (Hobbenaghi *et al*., 2014), anti-seizure (Farzaneh *et al*., 2015), memory and recall (Imam *et al*., 2016) among other activities. Thus, the aim of this study was to investigate the attenuating efficacy of NSO in dichlorvos poisoning-induced cardiotoxicity in rats.

Materials and Methods

Chemicals and drugs

Dichlorvos (PESTANAL®), the analytical standard was purchased from Sigma (Sigma-Aldrich) (St. Louis, MO, USA), while an analytical grade of Sunflower oil (by Africa Sun oil Refineries) was purchased from a local pharmaceutical and supplement shop. The NSO (concentration; 100% black seed; HUSNA black seed oil, Fazhab Agency, Karachi, Pakistan) was purchased from a trado-medical store in Ilorin, Kwara state, Nigeria.

Animals

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

After the last treatment day, the rats were overdosed with appropriate doses of sodium pentobarbital. Once respiration had ceased and the animals were nonresponsive to vigorous tactile stimuli, blood was collected transcardially for biochemical estimation, and then whole-body intracardial perfusion was performed, initially, with a cold rinse of 0.9% saline solution, followed by 10% buffered formalin. Following fixation, the hearts were carefully removed from the thorax, and postfixed overnight in 10% buffered formalin, for further histopathological processing.

Treatments

The rats were randomly distributed into four groups ($n = 6$) as follows:

- Control: Received subfornical organ (1 ml/kg oral)
- Experimental 1: Received DDVP (8.8 mg/kg/day oral) (Sharma and Singh, 2012)
- Experimental 2: Received DDVP (8.8 mg/kg/day orally) + NSO (1 ml/kg oral) 30 min later
- Experimental 3: Received NSO (1 ml/kg orally) (Atef and Wafa'a, 2010; Nahed and Bassant, 2011).

All procedures were scheduled and carried out during the early light phase between 07:00 and 09:00 h, and treatments were given for 21 consecutive days.

Plasma lipid profile

Blood samples were centrifuged at 3000 rpm for 15 min, and plasma was collected. Plasma levels of high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), and total cholesterol (TC) were determined spectrophotometrically using commercially available diagnostic kits according to the standard procedures (Randox Laboratories Limited, United Kingdom). The atherogenic indices (AI) were determined using the Friedewald equation (Friedewald, 1972):

$$AI = (TC - HDL\text{-}C)/HDL\text{-}C$$

also calculated are: Atherosclerotic indices (TC/HDL-C and LDL-C/HDL-C ratios).

Histology

The heart tissues were subsequently embedded in paraffin, sectioned into 8 μm sections using a rotary

microtome (MK 1110). These sections were stained with hematoxylin and eosin for general cardiac architecture and Orcein staining for elastic fibers morphology following standard routine laboratory procedures. Six sections were prepared from each heart tissue and evaluated for the degree of injury. Images of the general architecture were captured under ×40 objective lens using the Zeiss AxiostarPlus Light microscope.

Statistical analysis

Data recorded in this study were reported as the mean ± standard error of the mean. The plasma levels of HDL-C, LDL-C, triglycerides, and TC were analyzed using one-way analysis of variance and for the *post hoc* analyses, we used the Bonferroni test. A value of $P \leq 0.05$ was considered statistically significant in all cases, using the software package Graphpad Prism software (version 5.0, La Jolla, CA) for analysis.

Results

Plasma lipid profile following DDVP exposure and *Nigella sativa* oil treatment

The changes in the level of plasma lipids in the control and experimental rats are shown in Table 1. Significant ($P \leq 0.05$) increase in the levels of TC, TG, and LDL-C were observed between control and DDVP-treated rats. While significant ($P \leq 0.05$) decrease in HDL-C level was observed in DDVP-treated when compared with the control. These variations were markedly improved following the posttreatment with NSO and in the NSO only treated rats.

Atherogenic and atherosclerotic indices following DDVP exposure and *Nigella sativa* oil treatment

Following DDVP exposure, the atherogenic and atherosclerotic indices (TC/HDL-C and LDL-C/HDL-C ratios) were markedly enhanced. However, posttreatment with NSO was able to mitigate the outburst induced by DDVP in the NSO co-treated rats [Figure 1].

Table 1: Lipid profile (total cholesterol, triglyceride, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol) in plasma of control (sunflower oil), Dichlovos exposed, combined Dichlovos + *Nigella sativa* oil treated and *Nigella sativa* oil treated rats respectively. Analysis of variance + Bonferroni, *$P \leq 0.05$

Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Sunflower oil	29.64±4.33	188.57±12.02	23.39±5.88	0.68±0.19
DDVP	31.74±6.89	224.92±25.89	10.59±0.38	1.51±0.33
DDVP + NSO	30.64±6.04	220.82±13.89	20.18±2.78	1.23±0.43
NSO	23.12±2.78	145.78±14.89	34.89±4.47	0.54±0.17

+ Nigella sativa oil treated and Nigella sativa oil treated rats respectively. Analysis of variance+Bonferroni, *$P \leq 0.05$

Cardiac histoarchitecture following DDVP exposure and *Nigella sativa* oil treatment

Histological assessment showed a normal muscle and elastic fiber morphology in the heart tissue in control rats [Figures 2 and 3]. Exposure to DDVP-induced structural changes in this tissue, characterized by cytoplasmic vacuolization of cardiac muscle cells [Figures 2 and 3]. The latter was significantly decreased when NSO was administered to the DDVP combined treated rats when compared with those exposed to DDVP without treatment [Figures 2 and 3]. In rats treated with NSO alone, heart histoarchitecture was normal [Figures 2 and 3].

Discussion

In the recent decades, we have witnessed a drastic increase in the use of pesticides worldwide; due to growing effort to increase food production and control vector-borne diseases, and this have consequentially become harmful to the environment and to the human health (Hou and Wu, 2010). Biochemical changes associated with myocardial necrosis and toxic myocarditis have been associated with OPs poisoning, accounting for the death of exposed patients even after apparent clinical recovery (Anand *et al.*, 2009; Vijayakumar *et al.*, 2011; Wahab *et al.*, 2016).

Unfortunately, very little is known about the cardiovascular complications from OPs poisoning due to limited studies, whereas cardiotoxic effects have been documented in other insecticides (Papaefthimiou and Theophilidis, 2001; Won *et al.*, 2012; Mariem *et al.*, 2016).

The present study showed that DDVP (8.8 mg/kg) exhibits cardiac toxicity in subchronic exposure (21 days) by inducing morphological damages as well as elevated lipid profiles, atherogenic, and atherosclerotic indices. The results implicated DDVP in the deterioration of

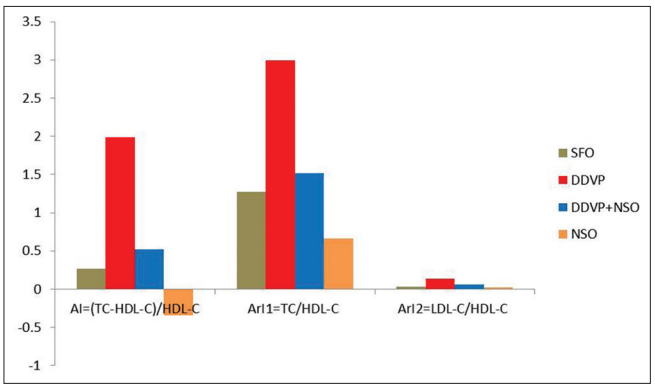

Figure 1: Atherogenic index ([TC-HDL-C]/HDL-C) and atherosclerotic indices (TC/HDL-C and LDL-C/HDL-C) of control (SFO), DDVP exposed, combined DDVP + NSO treated and NSO treated rats, respectively. SFO - Subfornical organ, NSO - *Nigella sativa* oil, HDL-C - High-density lipoprotein-cholesterol, TC - Total cholesterol, LDL-C - Low-density lipoprotein-cholesterol, DDVP - Dichlorvos

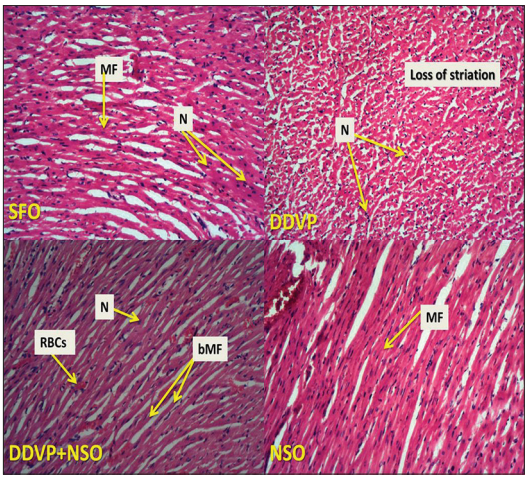

Figure 2: Photomicrograph of the cardiac tissue section following treatment with SFO, DDVP (H and E, ×100). DDVP + NSO and NSO. MF - Muscle fibers, N - Nuclei, bMF - Branching muscle fibers, RBCs - Red blood cells, SFO - Subfornical organ, NSO - *Nigella sativa* oil, DDVP - Dichlorvos

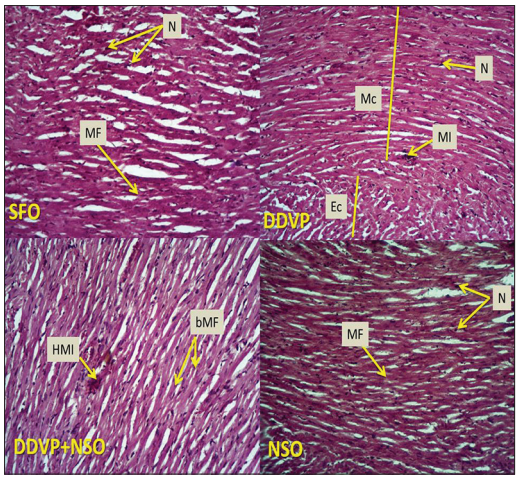

Figure 3: Photomicrograph of the cardiac tissue section following treatment with SFO, DDVP (Orcein, ×100). DDVP + NSO and NSO. Ec - Endocardium, Mc - Myocardium, MI - Myocardial infarct, HMI - Healing myocardial infarct, MF - Muscle fibers, bMF - Branching muscle fibers, N - Nucleus, NSO - *Nigella sativa* oil, SFO - Subfornical organ, DDVP - Dichlorvos

plasma lipid profiles where it markedly increased the plasma concentrations of levels of TC, TG, and LDL-C and depleted HDL-C levels.

The damaging effects of DDVP on cardiovascular functions was confirmed with the outturn of the AI as well as the atherosclerotic indices (LDLC/HDL-C and TC/HDL-C ratios) which are the important central indicators of cardiovascular complications (Meriem *et al*., 2016). These alterations in lipid profile, atherogenic, and atherosclerotic indices are indicators that DDVP might impair lipid metabolism and cause cardiotoxicity, a characteristic reported of some other OPs (Cetin *et al*., 2007; Hariri *et al*., 2010).

There were also some damages in the myocardial architecture, marked with vacuolations and disrupted general morphology of the tissue and these strengthen the extent of alterations in the underlined biochemical profiles. These observed cytoarchitectural changes are not unusual of an OP, as several OPs have been implicated in damaging of cardiovascular functions (electrical and mechanical) and structural architectures (Allon *et al*., 2005; Yavuz *et al*., 2005; Ogutcu *et al*., 2006; Calore *et al*., 2007; Zamzila *et al*., 2011).

NSO administration 30 minutes after exposure to DDVP in this study was able to rescue and prevent further perturbation of plasma lipid profiles, atherogenic and atherosclerotic indices induced by DDVP. The prophylactic efficacy of NSO in DDVP-induced cardiotoxicity as observed in this study could be attributed to its antioxidant efficacy (Kanter *et al*., 2008), and previously reported protection against OPs induced damages to functional and biochemical activities in various body organs (Atef and Wafa'a, 2010; Mohamadin *et al*., 2010; Nahed and Bassant, 2011; Hashem, 2012; Halil *et al*., 2015).

Various research works have also reported the therapeutic effect of *N. sativa* on lipid profile disturbance, atherogenesis, endothelial dysfunction, cardiac mass and contractility abnormality, platelet aggregation, heart rate, blood pressure disorder, and cardiotoxicity (Dehkordi and Kamkhah, 2008; Shabana *et al*., 2013; Zahra *et al*., 2016).

Conclusion

Based on the results of this study, it can be inferred that NSO has potential therapeutic efficacy against subchronic DDVP impaired lipid profile, atherogenic index, atherosclerotic indices and myocardia architecture in Wistar rats.

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Conflicts of interest

There are no conflicts of interest.

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