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Dichlorvos Induced Oxidative and Neuronal Responses in Rats: Mitigative Efficacy of *Nigella sativa* (Black Cumin)

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Summary: Poisoning from Organophosphates (OPs), especially Dichlorvos (DDVP) has become endemic due to the increasing use in house hold and agricultural pests control, with most marked effects in the nervous system. However, it is evidenced that natural antioxidants are efficacious against OPs toxicity. Thus, this study investigated the possible antidotal efficacy of *Nigella sativa* oil (NSO) in Dichlovos (DDVP) induced oxidative and neuronal damages in Wistar rats. DDVP was administered at sub-chronic daily dosage of 8.8 mg/kg.bw for 7 days and a post-administration of NSO at 1 ml/kg.bw for the subsequent 7 days. The rats were euthanized on the 15thday, blood sample collected via cardiac puncture, centrifuged and the plasma used for biochemical analysis of total antioxidant capacity (TAC), reduced glutathione (GSH) and total reactive oxygen species (ROS), while the frontal, occipital and cerebellar cortices and the medulla were removed for histo-morphological examinations. The results showed significant ($P \leq 0.05$) decrease in plasma TAC and GSH, while a significant ($P \leq 0.05$) increase in ROS was recorded, and some vacuolation around the neurons especially in the frontal and cerebellar cortices following DDVP exposure. However, post treatment with NSO was observed to be efficacious in the recovery of the oxidative activities and the neuro-architectural integrities. Thus, it can be concluded that the antioxidant capacity of NSO could be efficacious against OPs induced oxidative damages, especially in dichlorvos accidents.
Keywords*:* Organophosphates, antioxidant capacity, antidote, *Nigella sativa* oil, neurotoxicity, poisoning.

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INTRODUCTION

Toxicity is an inevitable circumstance behind most human and animal diseases even more than the biological organisms, as toxic substances freely diffuse in air and water (Paliwal and Sharma, 2009). Many essential life supporting compounds that are necessary for human health and production are at the same time casualty to human wellbeing. An example of these compounds is the, irreversible acetyl cholinesterase inhibitors (ACHEIs) that are widely used in insect or pest control, meanwhile, their indiscriminate use and handling have resulted into high mortality in the developing world (Michael *et al*, 2008).

Dichlorvos (DDVP) is a common organophosphate (OP) used in diverged forms and applications in the tropical world (Uthman *et al*, 2013; Deka and Mahanta, 2015), mostly in the protection of domestic animals and livestock from parasite infestation, and in household or Agriculture insect and pests control, leaving residues in foods (Davies *et al*, 2016; Rashmikaa *et al*, 2016). Thus, the resulting accidental toxicity (Brown *et al*, 2015) is affecting the quality of life (Fariba *et al*, 2016) of the exposed individual and becoming a very important health concern (Farrukh *et al*, 2016).

Complicating the burden of OPs poisoning is the limitations of the available antidote (Yadav *et al*, 2012), thereby, requiring a search for alternative regimen. Phytomedicine is gaining high interest and almost becoming an alternative medicine, due to their perceived reduced side-effect, availability, and cost effectiveness. *Nigella sativa*, a phytonutrient antioxidant has been fairly reported to be efficacious in many diseases, and these are evidenced in its therapeutic efficacies as antioxidant (Ashraf *et al*, 2011), anti-inflammatory (Alemi *et al*, 2013), antineurotoxic (Beydilli *et al*, 2015), hepatoprotective (Ajao *et al.*, 2017a), anti-diabetic (Alli-oluwafuyi *et al.*, 2017), renal and hematoprotective (Ajao *et al.*, 2017b), efficacy in neurodegenerative diseases (Dariani *et al*, 2013) and memory enhancing effects (Imam *et al*, 2016a). Thus, the mitigative efficacy of NSO in DDVP induced oxidative stress and neuronal

toxicity was investigated due to its known antioxidant and/or anti-inflammatory properties.

MATERIALS AND METHODS

Chemicals and Drugs

Dichlorvos was purchased from the Sigma Chemicals (St. Louis, MO, USA), while the sunflower oil which was used to dissolve DDVP was purchased locally and of analytical grade. The *Nigella sativa* oil (100% pure natural oil) was obtained from Masra warda, Kingdom of Saudi Arabia.

Animal care and Ethics

This research work was approved by the University of Ilorin Ethical Review committee, following the recommendation of the College of Health Sciences Ethical Review Committee, University of Ilorin, Ilorin, Nigeria. The research was approved to be in compliance with the Institutional Animal Care and Use Committee (IACUC).

Twenty-four (24) adult male Wistar rats with an average weight of 200 ± 20 g were used in this study. The animals were housed (6 per cage) 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* and euthanized at the end of the experiment with intraperitoneal injection of Ketamine (10 mg/kg. ip).

Treatments schedule

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

Control: received sun flower oil (1 ml/kg by oral gavage), consecutively for 7 days

Experimental 1: received DDVP (8.8 mg/kg/day by oral gavage) (Sharma and Singh, 2012), consecutively for 7 days

Experimental 2: received DDVP (8.8 mg/kg/day by oral gavage) for 7 days (Day 1-7), then followed by NSO (1 ml/kg/day by oral gavage) consecutively for the next seven days (Day 8-14).

Experimental 3: received NSO (1 ml/kg/day by oral gavage) (Nahed and Bassant, 2011), for 7 days

Treatments of the control, experimental 1 and 3 were only commenced at the 8th day of the fourteen days experimental period.

Oxidative stress and Endogenous Antioxidant analysis

Twenty four hours after the completion of exposures, the animals were anaesthetized with Ketamine (10 mg/kg.ip), the thoracic cage was exposed, blood was collected from the heart via the right atria, then respective reagents were used to assay plasm levels of total antioxidant capacity (TAC), total reactive oxygen species (ROS), reduced glutathione (GSH) and C-Reactive protein (CRP) as markers of oxidative stress and inflammation.

Histopathology

After blood was collected for biochemical analysis, whole body transcardial perfusion fixation using 4% paraformaldehyde, the brains harvested after 30 mins and stored in 4% paraformaldehyde. 24 hours later, tissue blocks of the frontal cortices (from Bregma 2 mm to 4 mm), occipital cortices (from the occipital pole 2 mm to 4 mm), the cerebellar cortices (from Bregma –10 mm to –15 mm) and the medulla were separated. These later dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin block, and then sectioned in 5 µm thickness using a rotary microtome (MK 1110). The sections were stained with Cresyl fast violet (CFV) for general neural architecture and Nissl granulation following standard routine laboratory procedures (Bancroft & Gamble, 2008). Images of the general architectures were captured under 40X objective lens using the Zeiss Axiostar Plus Light microscope.

Statistical analysis

Data recorded in this study were reported as mean ± standard error of mean. The TAC, ROS, GSH and C-Reactive protein data were analyzed using one-way analysis of variance (ANOVA) and for post-hoc analyses, we used the Bonfferoni test. The software package Graph Pad Prism (version 6) was used to analyze and graphical presentation of the data.

RESULTS

Oxidative and inflammatory responses following DDVP and NSO exposures

DDVP significantly (P≤0.05) caused a reduction in plasma TAC and GSH levels in the DDVP only exposed rats, and increased total ROS levels with no significant effect on the levels of the C-reactive protein (Fig(s). 1, 2, 3 and 4).

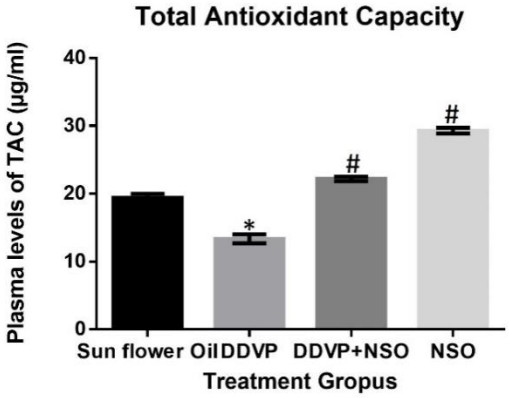

Fig 1: The effects of SFO, DDVP, DDVP+NSO, NSO on plasma TAC levels. ANOVA followed with Bonferroni. * indicates significant (P≤0.05) difference from DDVP+NSO and NSO while # indicates significant (P≤0.05) difference from DDVP only and SFO treated groups

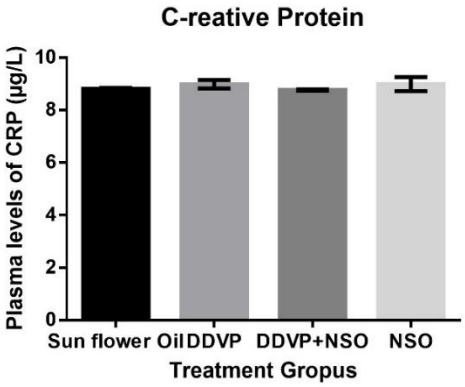

Fig 2: The effects of SFO, DDVP, DDVP+NSO, NSO on plasma C-reactive protein levels. There are no significant (P≤0.05) differences across the groups.

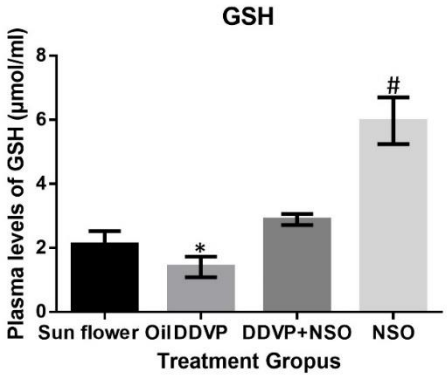

Fig 3: The effects of SFO, DDVP, DDVP+NSO, NSO on plasma GSH levels. * indicates significant (P≤0.05) difference from DDVP+NSO and NSO while # indicates significant (P≤0.05) difference from DDVP only and SFO treated groups

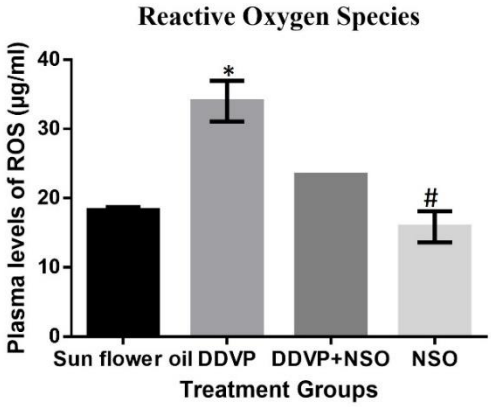

Figure 4: The effects of SFO, DDVP, DDVP+NSO, NSO on plasma ROS levels. * indicates significant (P≤0.05) difference from DDVP and NSO, while # indicate significant (P≤0.05) difference from the DDVP only and DDVP+NSO treated groups.

But NSO was observed to relieve these activities by effecting a significant (P≤0.05) increase in the levels of TAC and GSH, with a complementary reduction in ROS levels (Figs. 1, 2, 3 and 4) in the rats that received NSO only and those that received NSO after DDVP.

Neuronal responses to DDVP and NSO in various regions of the brain

Normal neuronal architectures were obvious in all the brain regions (frontal, occipital, cerebella and medulla) following SFO and NSO only treatments (Fig(s). 5-8). The brain regions of the DDVP treated animals, although not conspicuous appear to show some necrotic-like neurons with obvious vacuolations in the neuropils, especially in the cerebellar Purkinje cells and the frontal pyramidal neuron (Figures 5-8). However, the vacuolations were markedly reduced following a combined DDVP and NSO treatment (Fig(s) 5-8).

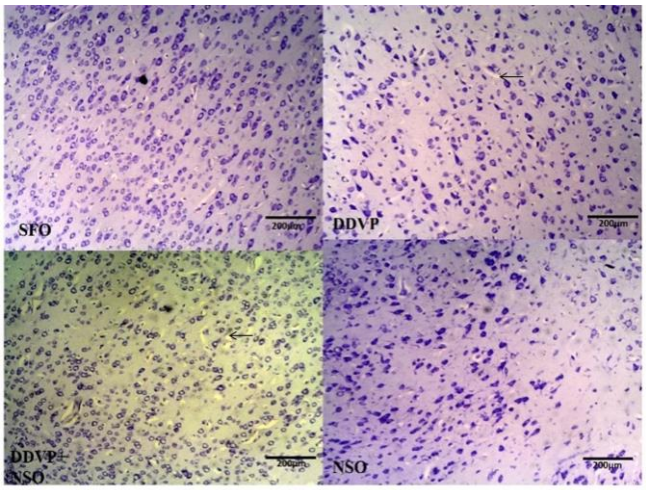

Figure 5: Representative photomicrographs of the frontal cortices of rats following administration of SFO, DDVP, DDVP+NSO, NSO. Arrow showing the vacuolation around a medium sized pyramidal neuron. (CFV 100X; Scale bar 200µm)

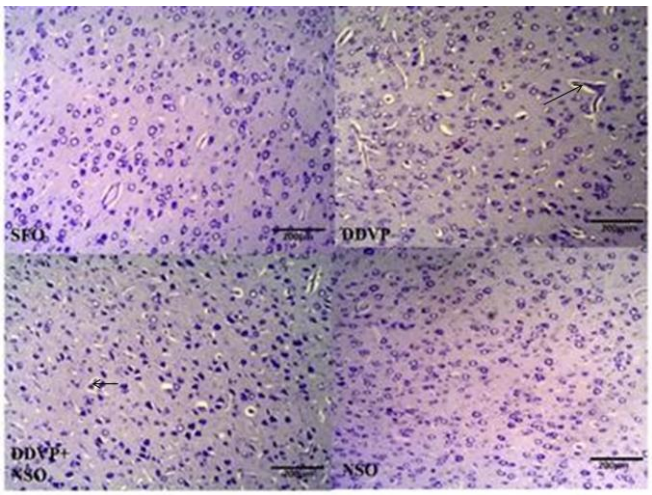

Figure 6: Representative photomicrographs of occipital cortices of rats following administration of SFO, DDVP, DDVP+NSO, NSO. Arrow showing the vacuolation around a small sized pyramidal neuron. (CFV 100X; Scale bar 200µm)

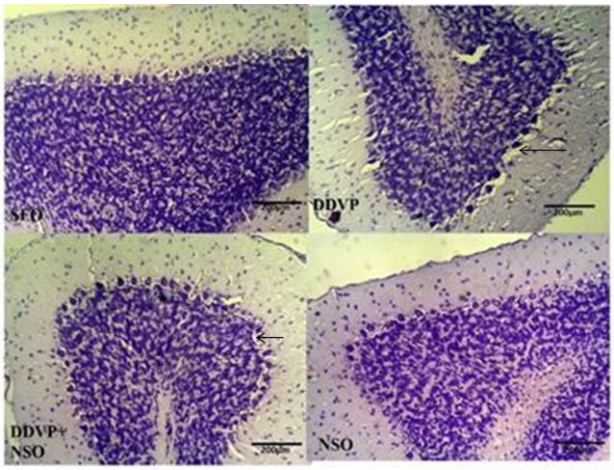

Figure 7: Representative photomicrographs of cerebella cortices of rats following administration of SFO, DDVP, DDVP+NSO, NSO. Arrow showing the vacuolation around purkinje cells in the purkinje cell layer. (CFV 100X; Scale bar 200µm).

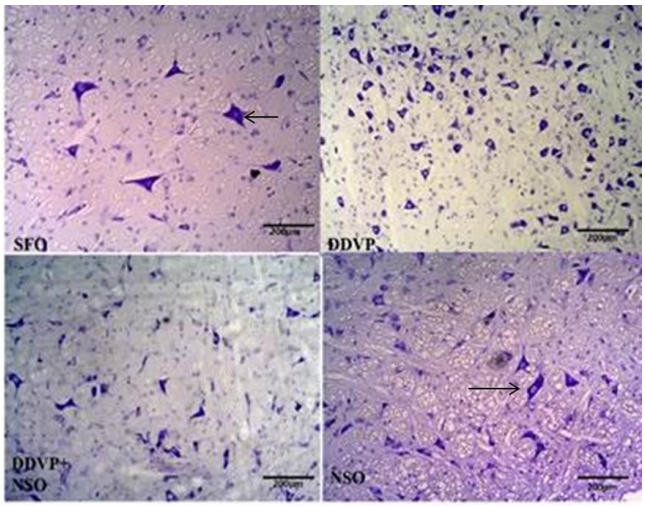

Figure 8: Representative photomicrographs of medulla oblongata of rats following administration of SFO, DDVP, DDVP+NSO, NSO. Arrow showing the large pyramidal cells in the rostral medulla. (CFV 100X; Scale bar 200µm)

DISCUSSION

The incidences of OPs poisoning in developing nations, have become endemic and threat to the quality of life in recent time, associated with high levels of depression, anxiety and stress (Fariba *et al*, 2016).

In this study, DDVP induced oxidative stress in the treated animals and markedly impaired anti-oxidant capacities, a report that is similar to what was reported in another OP (chlorpyrifos) which increased malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) activities, complicated by a reduced cytosolic glutathione S-transferase (GST) levels (Asma *et al*, 2016), and the impaired antioxidant capacity, observed in the reduced TAC and GSH levels is in agreement with Owoeye *et al.* (2014). Ezeji and colleagues have also reported depletion in GSH level in response to OPs poisoning (Ezeji *et al*, 2012).

These deleterious activities or damaging effects of DDVP in the exposed rats, strengthens its previous report and of other OPs in impaired personality (Weidong *et al*, 2016), impaired neurocognitive behaviors, psych cognitive derangements (Alessandra *et al*, 2016; Farrukh *et al*, 2016).

NSO was able to cushion the oxidative damages caused by DDVP, this can be associated with its previously reported anti-oxidant capacities, and more so its reported therapeutic efficacies in OPs induced biochemical damages (Atef *et al*, 2010; Mohamadin *et al*, 2010; Nahed *et al*, 2011; Hashem, 2012; Halil *et al*, 2015). These activities against OPs induced oxidative stress or poisoning, can be validated by the facts that natural or phytonutrient antioxidants have been largely proven to be efficacious in OPs toxicity (Colovic *et al*, 2015; Beydilli *et al*, 2015; Lari *et al*, 2015; Elsaid *et al*, 2015; El-Demerdash and Nasr, 2014). These can also be strengthened by our previous reports using the same dosage of NSO as employed in this study on its neuroprotective efficacy against cannabis and scopolamine modelled amnesia, (Imam *et al*., 2016a; 2016b; Ajao et al., 2016)

Although, the activities of DDVP on oxidative stress and endogenous antioxidants in this work were damaging, the effects on the neuronal integrity of the various brain regions were not pronounced in this study, and thus, may be too exaggerating to report any damage. Such minimal effect may be due to the period of exposures and the dosage employed in the study (Alessandra *et al*, 2016), even though, such conclusion may contradict other reports with marked deleterious changes in the cyto-architectonic of different brain regions following OPs exposures (Du *et al*, 2014; Olatunde *et al*, 2014; Ojo *et al*, 2014; Omar *et al*, 2016), but partially supported with some marked distortion characteristics in the frontal and cerebella cortices.

Complementing the effects of NSO on re-installing oxidative activities and strengthening anti-oxidant capacities in the treated rats, was the improved neuronal integrities when given alone and its protective effects when co-administered with DDVP. These reports can be supported by the previous reports on its prophylactic, ameliorative and protective efficacies in the frontal cortical pyramidal neurons, dentate gyrus granule cells, hippocampal CA pyramidal neurons, cerebellar cortices, brain stem and spinal cord following degenerative exposures to scopolamine, toluidine, autoimmune encephalomyelitis, lead induced neuronal degeneration and axonal demyelination and spinal cord injury respectively (Kanter *et al*, 2008; Heba *et al*, 2015; Farimah *et al*, 2016; Imam *et al*, 2016; Norsharina *et al*, 2008; Khaled *et al*, 2014).

Following the results of this study, it can be concluded that NSO due to its antioxidant efficacy and effects on neuronal integrities, may be a potent

supplementary remedy in OP poisoning, especially the Dichlorvos.

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