Original Article

Dichlorvos Induced AChE Inhibition in Discrete Brain Regions and the Neuro-Cognitive Implications: Ameliorative Effect of Nigella Sativa

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ABSTRACT

Background: There has been a rise in accidental poisoning cases resulting from the indiscriminate use and exposure to Dichlorvos (DDVP), especially in developing countries, and no antidote with satisfactory efficacy is currently available. Thus, we investigated the AChE reactivation potential of Nigella sativa oil (NSO) following DDVP induced AChE inhibition patterns in the brain and the associated cognitive implications.

Methods: Fourty Wistar rats were randomly divided into four groups of 10 each.; The controls were administered PBS (1 ml/kg); DDVP (8.8 mg/kg) was given to the experimental group I; while 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. All treatments lasted for 14 consecutive days. Morris Water Maze (MWM) paradigm was used to assess the working memory, then rats were euthanized, the brain excised, three brains were fixed for histological examination (Nissl staining), and the other seven brains were homogenized for AChE activity and Ca2+ concentrations. Data were analyzed statistically, using ANOVA method and P values of ≤0.05 was considered as significant.

Results: In this study, DDVP differentially inhibited AChE activities in various brain regions: cerebellum (86.1%), hippocampus (40.6%), frontal cortex (33.2%), medulla (21.5%), spinal cord (14.8%), and occipital cortex (8.9%). It reduced Ca2+ concentration, but had no effect on the delayed escape latency in the MWM, nor impaired the neuro-architectures. NSO caused increased AChE activities, Ca2+ concentration and reduced escape latency, and improved histologic architectures.

Conclusion: We concluded that NSO reactivated DDVP-induced AChE inhibition and improved memory indices, thus, it may serve as a potential treatment in the management of DDVP poisoning cases.

Keywords: Acetylcholine Esterase, Dichlorvos, Nigella Sativa, Organophosphate, Working Memory.

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INTRODUCTION

Unintentional or accidental poisoning is a common occurrence in developing countries, due to excessive exposures to toxic chemicals following indiscriminate use and handlings of organophosphate (OPs) compounds. Deaths resulting from OPs poisoning are estimated around 200,000 per year in these countries [1], where poisoning is a major occupational and public health concern [2].

In recent decades, the incidence of poisoning with OPs has doubled in developing nations, and has been associated with poor quality of life, depression, anxiety and stress [3], and impaired neuropsychological performances [4], among others. Most accidental poisoning with OPs in developing nations is highly associated with exposure to residues in food substances [5], resulting in neuropsychiatric conditions, and impaired personality even at low exposures.

A commonly used OP, Dichlorvos (C4H7Cl2O4P), is used for controlling a variety of insects and pests and is a major constituent of most insecticides used in developing countries, despite its reported and known toxicity [6]. It inhibits acetyl-cholinesterase (AChE) at the presynaptic cleft, causing the accumulation of acetylcholine, continuous triggering of postsynaptic neurons in target and non-target animals, and occasionally leading to death [7].

The treatment of OP induced toxicity, including DDVP's, has received high attention, and several antidotes have been developed and employed in the management and routine treatment of the poisoning, with atropine and pralidoxime chloride being the commonly used drugs, however, these drugs have certain limitations against their use [8].

The rising use of Ops plus the complications and limitations are the associated neurocognitive complications, which remain as major social and health

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burdens even after treatment [3]. Therefore, research for alternative management of OPs poisoning is crucial, with a great focus being on supplementary regimen with a more effective reactivation of the inhibited AChE.

Nigella sativa oil (NSO) is a valuable, traditionally used medical regimen in the management of various diseases. Its efficacy has been extensively studied and reported to provide protection against neurologic damages [9], inflammation [10], psychiatric conditions [11], seizures [12], memory and recall impairment [13], and blood disorders [14], among other activities.

Previous studies have shown the potency and protective efficacies of antioxidants and anti-inflammatory drugs and supplements against OPs toxicity, including DDVP, either by prevention of AChE inhibition and/or by facilitating the reactivation of inhibited cholinesterase [15, 16]. This study aimed to investigate the efficacy of NSO in DDVP induced neurotoxicity, due to its known antioxidant and anti-inflammatory properties.

MATERIALS AND METHODS

Chemicals and Drugs

Dichlorvos (PESTANAL®) analytical standard was purchased from Sigma (Sigma-Aldrich; St. Louis, MO, USA), while Phosphate Buffer Solution was prepared in our laboratory. The black seed oil (100% pure natural oil) was obtained from Masrawarda, Saudi Arabia.

Animals

We used forty adults male Wistar rats with an average weight of 200 ± 20 g in this study. The animals were housed under standard laboratory conditions in the animal holding facility of the Faculty of Basic Medical Sciences, University of Ilorin, Nigeria. All rats were allowed free access to water and food *ad libitum* and animals were assessed behaviorally. The brains of three rats were fixed for histology and those of seven rats were homogenized for biochemical analysis, respectively.

Treatments

The rats were randomly distributed into four groups (n = 10) as follows:
Control: Received PBS (1 ml/kg oral).
Exp. 1: Received DDVP (8.8 mg/kg/day oral) [14].
Exp. 2: Received DDVP (8.8 mg/kg/day orally) + NSO (1 ml/kg oal) 30 min. later.
Exp. 3: Received NSO (1 ml/kg orally) [13, 14].
All procedures were scheduled and carried out during the dark phase between 20:00 and 24:00 hours, and treatments were given for fourteen consecutive days.

Morris Water Maze (Mwm) Test

The Morris water maze (MWM) test is a common paradigm used for testing spatial learning and memory in rodents, and a modified procedure was used in the present study. The MWM consisted of a circular pool with a 1.6 m in diameter and 50 cm height and water filled to a 44 cm depth. The pool was colored with a black non-toxic dye, with the temperature set at 25°C ± 1°C. Four equally spaced points around the edge of the pool were designated as north (N), east (E), south (S) and west (W). A black-colored round platform, with a 9 cm diameter, was placed 1 cm below the water surface. The rats were trained to navigate the submerged platform, and were given a maximum time of 120 sec. (cut-off time) to find the hidden platform and were allowed to stay on it for 30 sec. The platform remained in the same position during the training days. Rats that failed to locate the platform within 120 sec. were placed on the platform only in the first session. The animals were given a daily session of five trials. The escape latency time to reach the platform was recorded for each trial. Three subsequent tests were conducted, 24 h after the last training session on 25th day of this study. The escape latencies from three consecutive tests were recorded, averaged, and then used as the index of the animals' working memory [13].

Acetylcholine Esterase (Ache) Assay

For the AChE assay, the various brain regions were remove and homogenized in 10 volumes of 0.1 mM potassium phosphate buffer at pH 7.5, and centrifuged for 10 min at $1,000 \times g$. The supernatants were used for the enzymatic AChE analyses. AChE activity was determined based on Ellman and colleagues (1961) and Scherer et al. (2010) methods. Hydrolysis rates were measured at ACh concentration of 0.8 mM in 300 μL assay solution with 30 mMol Neurobiol phosphate buffer, pH 7.5, and 1.0 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) at 25 °C. About 15 μL of cerebral cortex and hippocampus supernatant was added to the reaction mixture and preincubated for 3 min. The hydrolysis was monitored by the formation of thiolate dianion of DTNB at 412 nm for 2–3 min at 30 sec. intervals.

Histology

The rats were anaesthetized 24 hours after the behavioral tests, decapitated and their brains excised and stored in 4% paraformaldehyde solution. The brains were subsequently embedded in paraffin, refrigerated, coronally sectioned into 8 μm sections of the frontal cortices (from Bregma 2 mm to 4 mm), the hippocampus (from Bregma –2.5 mm to –4.5 mm), occipital cortices (from the occipital pole 2 mm to 4 mm), the cerebellar cortices (from Bregma –10 mm to –15 mm), the medulla and the spinal cord, using a rotary microtome (MK 1110). These sections were stained with Cresyl fast violet (CFV) for general neural architecture and Nissl granulation, following standard laboratory procedures. 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 MWM, AChE and Ca2+

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data were analyzed, using one-way analysis of variance (ANOVA). For post-hoc analyses, we used the Bonfferoni test [14]. A P value of ≤0.05 was considered statistically significant in all cases. The software package "*Graph Pad Prism*" was used to analyze and graphically present the data.

Ethical Approval

All experimental procedures were performed according to our institutional guidelines for animal care and use. The ethical approvals were received from the University of Ilorin's, Faculty of Basic Medical Sciences' Animal Research Ethics Committee.

RESULTS

Dichlorvos Induces Ache Deactivation in Discrete Brain Regions

In this study, an acute and continuous exposure to oral Dichlorvos (8.8 mg/kg) inhibited AChE activities in the

evaluated brain regions at varying degrees compared to those observed for the control group as follows: Cerebella cortices (86%), Hippocampus (40.6%), Frontal cortices (33.2%), Medulla (21.5%), Spinal cord (14.8%) and Occipital cortices (8.9%), respectively (Figure 1).

Reactivations of the Dichlorvos Induced Ache Inhibitions by Oral NSO And the Ca2+ Interactions

Dichlorvos significantly (P≤0.05) reduced AChE activities in the various brain regions when compared with the control (Table 1). While the treatment with NSO was able to reactivate the inhibited AChE activities in the brain regions by increasing the AChE activities, (P≤0.05) compared with the DDVP treated animals (Table 1). Ca2+ levels were significantly reduced by DDVP, but this was also regulated by the NSO treatment (Table 2).
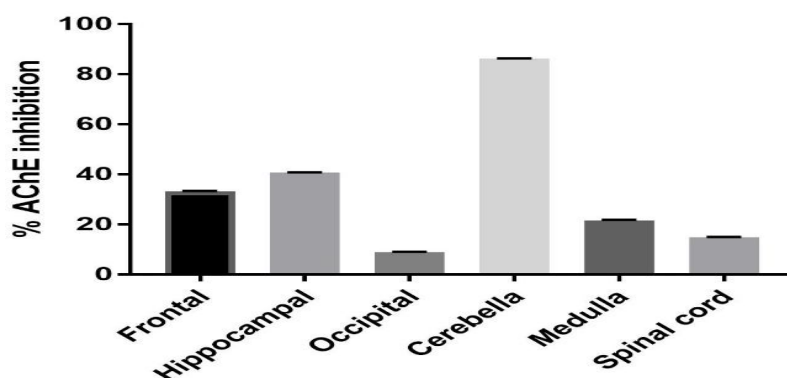

Figure 1. Inhibition of AChE activities in various rat brain regions after exposure to DDVP.

Table 1. Effects NSO treatment in DDVP-induced AChE inhibition in rat brain samples.

AChE activities in brain regions µmol ASSC h/h/mg protein				
Brain Region	**Control** [a]	**DDVP** [b]	**DDVP+NSO** [c]	**NSO** [d]
Frontal	0.76±0.15 (100%)	0.51±0.17 (66.8%)a*	0.61±0.04 (98.1%) b#	0.69±0.04 (90.8%)b#
Hippocampus	1.55±0.12 (100%)	0.92±0.02 (59.4%)a*	1.07±0.09 (69.0%)	1.36±0.06 (87.7%)b#
Occipital	0.75±0.22 (100%)	0.69±0.11 (91.1%)	0.68±0.05 (90.7%)	0.71±0.03 (94.7%)
Cerebellum	0.87±0.02 (100%)	0.12±0.07 (13.9%)a*	0.82±0.09 (94.8%)b,d#	0.54±0.02 (62.1%)b#
Medulla	1.10±0.16 (100%)	0.87±0.11 (78.5%)	1.04±0.11 (94.5%)	0.75±0.02 (68.1%)
Spinal cord	0.53±0.07 (100%)	0.45±0.04 (85.2%)	0.41±0.00 (77.3%)	0.42±0.03 (79.8%)

Data are presented as mean± S.E. (n = 6). Asterisk (*) indicates a significant reduction and hash (#) indicates significant increase at P≤0.05 between the groups. Numbers in parentheses denote the percent change compared to the control. Superscript letters indicate that the means differed significantly from the respective groups (a = control; b = DDVP; c = DDVP+NSO; d = NSO alone).

Table 2. Effect of NSO treatment on Ca2+ concentration in different regions of DDVP-exposed rat brain samples.

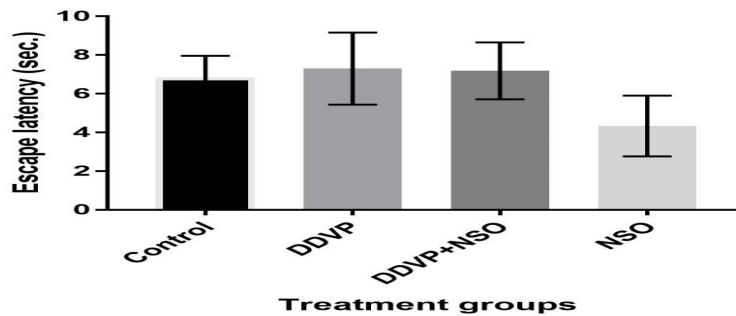| Calcium mg/dl				
Brain Regions	**Control** [a]	**DDVP** [b]	**DDVP+NSO** [c]	**NSO** [d]
Frontal	7.706±0.13	6.761±0.11a*	7.671±0.16b#	8.385±0.32b#
Hippocampus	7.792±0.06	8.091±0.08	7.73±0.17	8.182±0.29
Occipital	7.836±0.15	7.422±0.16	9.984±1.71b#	8.336±0.08a,b#
Cerebellum	7.497±0.13	7.72±0.41	9.894±1.71	8.336±0.08
Medulla	7.609±0.17	6.688±0.09a*	7.75±0.22b#	8.054±0.16b#
Spinal cord	7.408±0.08	6.556±0.19a*	7.384±0.19b#	8.172±0.20a,b,c#

Data are presented as mean± SE (n = 6). Asterisk (*) indicates a significant reduction and hash (#) indicates significant increase at P≤0.05 between the groups. Superscript letters indicate that the means differed significantly from the respective groups (a = control; b = DDVP; c = DDVP+NSO; d = NSO alone).

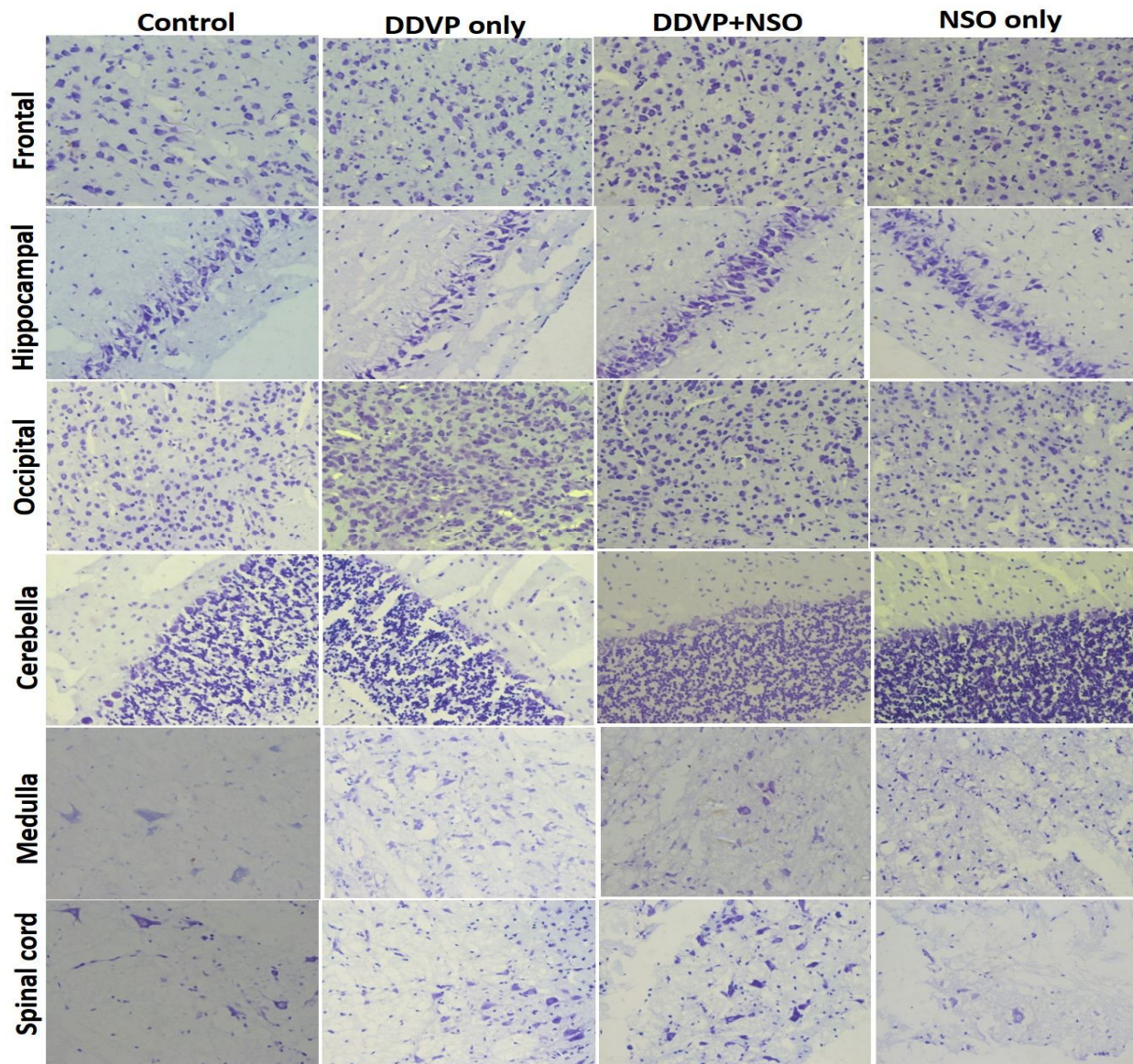### NSO Improved Working Memory and Acute Dichlorvos Exposure Did Not Impair Cognitive Functions

Although DDVP delayed escape latency in the treated animals, but this was not significant (P≤0.05). A post-treatment with NSO and in animals treated with NSO only, DDVP reduced the latency, but not to a statistically significant extent (Figure 2).

NSO Improved, But DDVP Did Not Distort Histological Architectonics in All Brain Regions

There was no observable distortion in the brain regions (frontal, hippocampal, occipital, medulla and spinal cord), but some distortion was seen in the cerebellar cortex after DDVP treatment (Figure 3). Post-treatment with NSO restored the cerebellar integrity and the treatment showed no adverse effects on any of the brain regions (Figure 3).

Figure 2. Escape latencies in rats following exposures to PBS, DDVP, DDVP+NSO or NSO alone.

Figure 3. Neuronal architecture in various rat brain regions following exposures to PBS, DDVP, DDVP+NSO or NSO alone. Stain: Cresyl fast violet; magnification: X100.

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DISCUSSION

Dichlorvos in this study significantly inhibited AChE activities in various brain regions of the treated animals, in a decreasing order, as follows: cerebellum (86.1%), hippocampus (40.6%), frontal cortex (33.2%), medulla (21.5%), spinal cord (14.8%) and occipital cortex (8.9%), respectively. The observed inhibition was less than 50% in all regions but the cerebellum. Such regional differences in the AChE inhibition in the various brain regions was consistent with the deactivation patterns reported in a previous study following chlorpyrifos exposure [17]. The patterns of inhibition suggest the differences in the functional cholinesterase interaction within the brain regions. Also, it may indicate the differential effects of DDVP inhibition on the rat brain.

Although DDVP blocked the activities of AChE in various brain regions, the inhibition did not produce significant alteration in the working memory, consistent with the findings reported by similar study previously [18]. These observations support the established role of AChE has in learning and memory [19]. Further, a slight inhibition of AChE activities may not pose negative consequences for rat brain. It has been postulated that most clinical manifestations of OPs induced AChE inactivation cases are demonstrable only when AChE activity is inhibited below 10-20% [20]. In this study, the AChE inhibition by DDVP in all brain regions was below 50% of the level observed for the control group, and this could explain the lack of DDVP adverse effects in the rat samples. It may be argued that AChE inhibitions following moderate exposure to low or moderate doses of OPs may not cause observable neurological toxicity. Further, it has been suggested that adverse effects of low-level exposure to OPs are likely to occur through mechanisms other than AChE inhibition.

In this article, we have reported that acute DDVP did not have damaging effects on the neuronal integrity of various rat brain regions. This finding contradicts other reports that OPs caused pathological changes in the cytological architecture of various rat brain regions [21, 22]. The contradiction may be due to the duration, or method of exposure or the dosage of DDVP.

Our findings demonstrated that Nigella sativa moderately inhibited AChE activities, leading to improved working memory in the treated rats. The finding supports the suggestion of previous studies that ChE inhibition occurred following Nigella sativa administrations or its constituents [23, 24]. These cholinergic interactions can further strengthen the efficacy of NSO in the managements of neuro-cognitive, neuropsychiatric and neurodegenerative impairments as evidenced in the literature [13, 25, 26].

Also, we observed partial reactivation of AChE activities by NSO after DDVP exposure. The reactivation did not reach the level demonstrated in the control group in all brain regions. Such cholinergic interactions by NSO can be supported by the previously reported protective and ameliorative effects against OPs induced damages to functional and biochemical activities of the rat brain and other body organs [27, 28, 29].

The ChE reactivation we observed the after NSO treatment also improved the memory index in the treated animals. This effect supports it's the memory enhancing property of NSO by all indices of neurocognitive and psycho-cognitive functions in animal models of neurodegenerative and neurotoxicity disorders [11, 12, 28]. The positive cholinergic effect of NSO complements the previously reported protective activity on the neuronal integrity of all brain regions. Such positive effects support other properties of NSO, such as its reported effects on various brain regions following brain exposures to neurotoxic substances [9, 30, 31].

CONCLUSION

Based on the findings of this study, it can be concluded that concurrent exposure to low dosage of oral DDVP differentially inhibits AChE activities in various brain regions; however, it may not impair the cognitive activities nor it damages the neuronal architecture. We also demonstrated that NSO was able to reverse the inhibited AChE activities by DDVP. NSO improved cognitive function and neuronal architectures, thus it may be effective as a treatment in cases of OPs poisoning, especially with DDVP.

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