Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Nigella sativa conserved hippocampal oxidative and neurogenic activities to salvage neuro-cognitive integrities in chlorpyrifos insult

Aminu Imam^{a,b,*}, Abideen Lawal^a, Lukuman Aboyeji Oyewole^c, Musa Iyiola Ajibola^d, Victoria Williams^b, Samson Chengetanai^e, Toyin Sheu-Tijani Shittu^f, Moyosore Saliu Ajao^a

^a Neuroscience Unit, Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, P.M.B 1515, Ilorin 240003, Nigeria

^b Comparative Neurobiology Unit, School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, 2193, Johannesburg, South Africa

^c Neuropgysiology Unit, Department of Physiology, College of Health Sciences, University of Ilorin, P.M.B 1515, Ilorin 240003, Nigeria ^d Institute of Neuroscience, National Yang-Ming University, Shih-Pai, Taipei 11221, Taiwan

^e Division of Basic Medical Sciences, National University of Science and Technology, Zimbabwe

^f Endocrinology and metabolism Research Unit, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

A R T I C L E I N F O

Article history: Received 14 August 2018 Revised 22 September 2018 Accepted 1 October 2018

Keywords: Nigella sativa oil Organophosphates Oxidative damage Acetylcholinesterase Cognition

ABSTRACT

Chlopyrifos (CPF) is an organophosphate, implicated in brain damage and cognitive deficits, whose consistence deposit in the environment has contributed to the toxicity burdens of xenobiotics. This study investigated the efficacy of Nigella sativa oil (NSO) against CPF insults on the hippocampus. Thirty-two rats were randomly divided into four groups of eight rats each, exposed to 1 ml/kg of Normal saline, 14.9 mg/kg of CPF, 14.9 mg/kg of CPF plus 1 ml/kg of NSO and 1 ml/kg of NSO respectively for 14 consecutive days. The rats were exposed to 3 trials each on the 11-13 days in the Morris water maze, and subsequently latency to hidden platform and time in the platform quadrant were recorded as measures of long term memory (LTM), short term memory (STM) and reference memory (RM) on the 14th day. The rats were euthanized on day 15, the brains excised, and the hippocampus of five brains removed, homogenized to analyze for total reactive oxygen species (ROS), nitric oxide (NO) levels and acetylcholinesterase (AChE) activities, while the other three were processed for histology and Ki67 immunohistochemistry. CPF caused a marked increase in hippocampal NO and ROS activities, depleted AChE activities and Ki67 expressions, delayed escape latency and reduced visit to the platform quadrant. Intervention with NSO depleted ROS/NO levels, improved neurogenic proteins, AChE activities and neuro-cognitive markers depletions in CPF exposure. Altogether, our findings showed that NSO is a potential

* Corresponding author at: Neuroscience Unit, Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, P.M.B 1515, Ilorin 240003, Nigeria.

E-mail address: imam.a@unilorin.edu.ng (A. Imam).

https://doi.org/10.1016/j.sciaf.2018.e00008

2468-2276/© 2018 The Authors. Published by Elsevier B.V. on behalf of African Institute of Mathematical Sciences / Next Einstein Initiative. This is an open access article under the CC BY license. (http://creativecommons.org/licenses/by/4.0/)





therapeutic drug for the treatment of CPF-induced cognitive deficit through its antioxidant property and adult neurogenesis in rats.

© 2018 The Authors. Published by Elsevier B.V. on behalf of African Institute of Mathematical Sciences / Next Einstein Initiative. This is an open access article under the CC BY license. (http://creativecommons.org/licenses/by/4.0/)

Introduction

Organophosphates (OPs) are widely used synthetic chemicals that are found in both the domestic and agricultural sectors to assist with pest control [62]. However, poisoning by these agents has been reported, particularly in developing nations, posing a serious public health problem with several cases reported each year [49]. Chlorpyrifos (CPF), [0,0-diethyl-o-(3,5,6trichloro-2-pyridyl) phosphorothionate] is among the most effective and widely used organophosphate pesticide (OP) pesticides in the world [35,65]. Acetylcholinesterase (AChE), an enzyme that hydrolyses acetylcholine (ACh) in the cholinergic synapses and in the neuromuscular junctions, is the primary target of CPF. It inhibits the enzyme, resulting in the accumulation of ACh in the synapses which results in hyperactivity in the cholinergic pathways [50,58]. The build-up of ACh in the synapses has been implicated in many conditions, including convulsions, cognitive deficits, movement disorder, respiratory diseases, metabolic deregulations, and neurologic disorders [38,42]. Nonetheless, it appears that AChE inhibition is not sufficient to result in severe, long-term cognitive deficits observed in CFP-treated rodents as other non-cholinergic pathways may be involved [14,60]. Understanding other non-cholinesterase pathways of CPF would provide us with a better understanding of the therapeutic strategies for pesticides-induced cognitive impairment. In addition, available evidence suggests that CPF-induced toxicity also involved reactive oxygen species (ROS) generation resulting in oxidative stress in affected tissues. After oral administration of CPF accumulates in the brain causing long-term brain damage, neurotoxicity [32,59], and in different organs eliciting reproductive toxicity [19,36], hematotoxicity [18], developmental toxicity [13], cardiotoxicity [10], hepatic dysfunction [21].

In the mammalian brain, the hippocampus is characterized as the primary structure for learning and memory formation [61]. The dentate gyrus (DG) is responsible receiving and integrating sensory inputs from the entorhinal cortex to the CA3 then to the CA1 in the classical tri synaptic pathway [7], which is clinical for learning and memory [56,64]. Each hippocampal subfield has specific cell types and plasticity contributing differentially towards the process of acquisition, storage and recall of learning and memory [46]. Disruption of synaptic and biochemical architecture of different hippocampal subfields as a result of chemical exposure has been extensively implicated in the pathophysiology of cognitive deficits and emotional behavior-related disorders [24,44,45]. Even though, there exist useful researches on the pathophysiology underlying the development of cognitive deficits, such as dementia, effective treatments remain elusive.

Nigella sativa (NS), also called the black seed, belongs to the botanical family of Ranunculaceae. It is most found in Eastern Europe, the Middle East and Western Asia [20]. Several studies revealed that NS seeds and oil can be used to treat a variety of diseases, pointing to some of its pharmacological properties. Most of its therapeutic efficacies have been strongly linked to composite bioactive compounds, including thymoquinone, alkaloids, riboflavin, piridoksin, niacin, folic acid, minerals and proteins [33,51,52]. The reports of these pharmacological properties includes but not limited to the efficacy as antioxidant [2,31,32], anti-inflammatory [4], haematoprotective [3], neuroprotective [[27],[28,30]–[32],[34]], improvement of male infertility [40], efficacy in neurodegenerative diseases [5,16] and in memory enhancement [27,28,30].

Here, we set out to investigate the efficacy of NSO in ameliorating CPF-induced oxidative and neurogenic damages, with subsequent effects on learning and memory functions in rats.

Materials and methods

Chemicals and drugs

Chlorpyrifos (PubChem Substance ID 329756699) PESTANAL®, analytical standard was purchased from Sigma (Sigma-Aldrich)(St. Louis, MO, USA), while the Normal saline solution was prepared in our laboratory. The Nigella sativa oil (concentration; 100% black seed; HUSNA black seed oil, Fazhab Agency, Karachi, Pakistan) was purchased from a TIBB-medical store in llorin, Kwara state, Nigeria.

Animals and experimental design

Thirty-two adult male Wistar rats weighing between 150 g and 170 g were obtained from the University of Ilorin Biological garden, Ilorin. They were housed in cages and fed with standard laboratory diet and water ad libitum, in the animal holding unit of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin. The rats were exposed to a 12 h light/dark cycle at room temperature for 7 days before the commencement of the experiments. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

Treatment schedule

The rats were randomly divided into four groups (n=8) as follows:

- Group 1 (control)– were given Normal saline (1 ml/kg orally) daily
- Group 2- were given CPF (14.9 mg/kg orally) daily
- Group 3- were given CPF (14.9 mg/kg orally) plus NSO (1 ml/kg orally) daily [32]
- Group 4- were given NSO (1 ml/kg orally) daily [3,27,28,30-32].

The experiments were all conducted in the morning (between the 07:00 and 09:00 hours), and treatments with substances span a period of fourteen consecutive days.

Ethical approval

This research work was approved by the University of Ilorin ethical review committee (UERC) (UERC/ASN/2017/856), following the recommendation of the College of health sciences ethical review committee, in compliance with the Institutional Animal Care and Use Committee (IACUC).

Behavioral evaluations

The rats were subjected to behavioral evaluations on the 14th day of the treatment to assess short-term, long-term and reference memory in the Morris water maze (MWM) paradigm.

MWM procedure

The MWM apparatus in this study was used to assess the changes in memory indices following exposures to CPF and/or NSO in Wistar rats [43]. Each of the rats (n=32) were placed in a black, circular pool, which was filled with 23–24 °C water (pool dimensions: 60 cm deep × 136 cm diameter). The pool was divided into four quadrants, labeled north (N), east (E), south (S) and west (W). It contained a circular platform (10 cm diameter, 28 cm high) that was submerged (about 2 cm below water surface) in the central area of the SW quadrant of the pool. The rats swam until they found the platform (total time allowed on the platform = 15 s). If the subjects were unable to find the platform after 60 s of swimming, they were gently guided to the platform. The rats were then removed from the pool, dried and placed in their cage for 5 min. Each of the trials was recorded with the aid of a video system.

Animals received a training session (three trials per session), for three consecutive days (days 11, 12 and 13 of the experiment). Each of the trials was a maximum duration of 60 s. The time interval between trials was approximately 30 s in duration. 24 h after the acquisition phase, the time it took the subjects to find the hidden platform (referred to as 'escape latency') was recorded as long-term memory (LTM). An average of the escape latency of the two subsequent trials was recorded as short-term memory' (STM).A probe test was also conducted by removing the platform and allowing the rats to swim freely in the pool for 60 s; the time spent in the target quadrant, which had previously contained the hidden platform, was recorded as the reference memory on day 14 of the experiment. The time spent in the target quadrant indicated the degree of relative memory consolidation, which had taken place after learning [27–30].

Biochemical evaluation

Once the treatment period was completed, the rats were euthanized with an overdose of Ketamine (10 mg/kg ip) and their brains dissected and weighed. Blocks of hippocampal tissue (from Bregma –2.5 mm to –4.5 mm) were removed from the brains of five rats (from each of the four groups), dipped in 30% sucrose solution, homogenized and portions centrifuged at 2500 rpm for 10 min. The supernatant was then collected in tubes containing the compounds for NO metabolites and ROS analysis. ROS was measured by monitoring the increasing fluorescence of DCFH-DA following a previously described procedure using flow cytometry (Partec, Deutschland) equipped with a 488 nm argon ion laser and supplied with the Flomax software and the signals were obtained using a 530 nm band pass filter (FL-1 channel). Each determination was based on the mean fluorescence intensity of 10,000 counts [1].

The remaining tissue homogenate was added to the Griess reagents, sulfanilamide and naphthyl ethylene diamine solutions to measure nitrate/nitrite production (NO metabolites). Absorbance was measured with the aid of a microplate reader and the levels of NO metabolites were calculated from a standard curve [9]. The remaining portions of the homogenized hippocampal tissues were placed in phosphate buffer with 1% Triton-X 100 and centrifuged at 5000 rpm for 10 min. The following reagents were used; 35 μ L of 5 mM dithio-bisnitrobenzoic acid (also known as Ellman's reagent [DTNB]), 10 μ L of 75 mM acetylthiocholine (ATCh) and 50 mM phosphate buffer (pH 8.0). Protein concentration in brain homogenates was quantified using a Bradford assay. AChE activity was calculated in micromoles of ATCh, hydrolyzed per hour per milligram of protein and was expressed as percentage of control activity and measured values in micromole per hour per milligram of protein.



Fig. 1. Hippocampal NO levels in rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). Double asterisks (**) indicates significant ($p \le 0.01$) reduction when compared with the CPF only, while single asterisk (*) indicates significant ($p \le 0.05$) increase from all other groups.



Fig. 2. Hippocampal NO levels in rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). Double asterisks (**) indicates significant ($p \le 0.01$) reduction when compared with the CPF only, while single asterisk (*) indicates significant ($p \le 0.05$) increase from all other groups.



Fig. 3. Hippocampal AChE levels in rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). Double asterisk (**) indicates significant ($p \le 0.05$) reduction when compared with NS and NSO treated rats, while single asterisk (*) indicates significant ($p \le 0.05$) increase from control and other groups.



Fig. 4. Escape latency (LTM) of rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). Double asterisks (**) indicates significant ($p \le 0.05$) reduction when compared with the CPF only and CPF + NSO treated rats, while single asterisk (*) indicates significant ($p \le 0.05$) increase from control and NSO groups.



Fig. 5. Escape latency (STM) of rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). Double asterisk (**) indicates significant ($p \le 0.05$) reduction when compared with the CPF only treated rats, while single asterisk (*) indicates significant ($p \le 0.05$) increase from all groups.

Tissue processing and histopathology

After euthanasia and extraction of the three rat brains from each of the groups, the brains were fixed in 10% formalin for 24 h. The hippocampal blocks (from Bregma -2.5 mm to -4.5 mm) were removed, dehydrated through ascending grades of alcohol, cleared in xylene and embedded in paraffin blocks. Every second hippocampal tissue section (5 μ m in thickness) was stained with Cresyl fast violet (CFV) for Nissl substances or immuno-stained to reveal Ki67 protein containing nuclei in the tissues. The sections were finally examined under an AmScope 40X-2500X LED Lab Compound Microscope, and photographed using the AmScope 5.0 MP USB Still Photo & Live Video Microscope Imager Digital Camera 5MP, manufactured by iSCOPE corp., USA.

Immunohistochemistry for Ki-67

The Ki-67 is a chromosome-associated protein present during division (G_1 , S, G_2 , and M phases but absent from cells at rest, G_0). Paraffin embedded sections were incubated for epitope retrieval in citrate buffer, pH 6.0, at 90 °C for 40 min, followed by incubation in endogenous peroxidase blocking reagent, 0.6% H_2O_2 in Tris-buffered saline (TBS)-Triton (0.05% Triton X-100 in TBS, pH 7.4) for 30 min at room temperature. Thereafter, sections were pre-incubated in 2% serum (normal goat serum) + 0.1% bovine serum albumin (BSA) + 0.25% Triton in TBS for 60 min at room temperature. The sections were then incubated with polyclonal rabbit-anti-lyophilized-Ki-67p antibody (Novocastra, Newcastle, UK; 1:5000 in pre-incubation solution) overnight at 4 °C. Incubation with biotinylated goat anti-rabbit IgG (1:1000 + 2% normal goat serum + 0.1% BSA in TBS; Vector lab, CA, USA;1:250) was performed for 2 h at room temperature followed by incubation with streptavidin-biotin complex (Vecta stain Elite ABC kit) and stained with 3,3'-diaminobenzidine (DAB) as chromogen. Until incubation with primary antibody, all rinses in between incubations were made with TBS-Triton, afterwards with TBS alone.



Fig. 6. Time in quadrant location (reference memory) of rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). Double asterisk (**) indicates significant ($p \le 0.05$) reduction when compared with all other groups, while single asterisk (*) indicates significant ($p \le 0.05$) increase from control and or CPF only treated rats.



Fig. 7. Representative photomicrographs of cornu ammonis 1 sub-field of the hippocampus of rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). Red arrows indicates the sites of vacuolations around the pyramidal cells in the CPF only and CPF + NSO exposure hippocampal CA1 subfield. CFV 100X.



Fig. 8. Representative photomicrographs of cornu ammonis 2 sub-field of the hippocampus of rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). Red arrows indicates the sites of vacuolations around the pyramidal cells in the CPF only and CPF + NSO exposure hippocampal CA2 subfield. CFV 100X.

Statistical analysis

Data from the morphometry, behavior and biochemical were analyzed using one-way analysis of variance (ANOVA) and subjected to post hoc Bonferroni's multiple comparison tests. The results are expressed as mean \pm SEM. Statistical analyses were performed using Graphpad Prism software (version 5.0, La Jolla, CA). Values of $p \le 0.05$ were considered statistically significant.

Results

Effect of CPF and NSO on the nitrosative stress in hippocampus

Exposures of rat to CPF resulted in a significant increase in the level of hippocampal NO when compared to control rats that received Normal saline. However, simultaneous administration with NSO significantly reverses the NO outburst to a level comparable to control group. Administration of NSO alone significantly ($P \le 0.05$) decreased NO levels when compared with the control and the CPF alone treated rats (Fig. 1).

Effect of CPF and NSO on oxidative stress in hippocampus

CPF exposure caused a marked increase ($p \le 0.05$) in hippocampal ROS activities when compared to controls, however, a simultaneous treatment with NSO showed the depletion of ROS significantly, which was comparable with control group that received Normal saline. Rats given NSO only had total ROS level comparable to control group (Fig. 2).



Fig. 9. Representative photomicrographs of cornu ammonis 3 sub-field of the hippocampus of rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). There is no marked damage in the CA3 subfield following CPF exposure. CFV 100X.

CPF induced AChE inhibition in the hippocampus: effects of NSO

CPF treated rats showed a significant ($p \le 0.05$) depletion in the hippocampal AChE activity compared to normal control rats and the NSO treated rats. Co-administration of NSO with CPF resulted in a considerable but non-significant reactivation of the depleted AChE activities in the hippocampus of the CPF exposed, while a significant ($p \le 0.05$) improvement was observed in the NSO only treated rats compared to the control and other groups (Fig. 3).

NSO preserved cognitive functions in CPF insult

CPF exposure significantly ($p \le 0.05$) caused a delay in the escape latency (STM and LTM), the time required to reach the submerged hidden platform in the Morris water maze (MWM) task, when compared with the saline treated control and the NSO only treated rats. Interventional treatment with NSO, alone or in concurrent exposure with CPF, significantly ($p \le 0.05$) shortened the escape latency in the treated rats. During the probe trial, CPF rats failed to retrieve the precise location of the hidden platform and spent significantly ($p \le 0.05$) less time in the target quadrant compared to the control group ($p \le 0.05$). However, those groups treated with NSO however, spent more time on average in the target quadrant searching for the hidden platform. (Figs. 4, 5 and 6)

Protective efficacy of NSO in CPF induced neurodegenerative like activities in the hippocampus

Exposure to CPF caused no marked deterioration in the general integrities of the hippocampal subfields (CA 1, 2, 3 and DG), but few necrotic like vacuolations in the cornuammonis pyramidal cells were noted, these effects were however in minimal in the CPF + NSO group. Similarly, a marked loss in density and denaturation were observed in the neurogenic



Fig. 10. Representative photomicrographs of dentate gyrus of rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). There is no marked damage in the dentate gyrus following CPF exposure. CFV 100X.

proteins (Ki67 +) cells in the hippocampal subfields, the dentate gyrus and the subventricular zone of the CPF exposed rats, but this was however prevented by NSO intervention (Fig. 13).

Discussion

Excessive exposure to CPF has been reported to result in cognitive deficit, depression and anxiety in rodents via oxidation and massive AChE inhibition [11,15,32,57,58]. To date, effective treatment of cognitive deficit induced by environmental toxic agents still remain a challenge to neuroscience community. Neuroprotective measures that can prevent and stop progression of neuronal damage cause by toxic agents would go a long way in improving cognitive performance and other neurological disorders observed in affected patients. In this study, we assessed the potential efficacy of NSO, a known antioxidant, in the prevention of CPF-induced cognitive impairment and its possible mechanism.

By combining biochemical, immune-histochemical and behavioral assay, we showed that NSO efficiently improved cognitive performance in CPF-induced cognitive deficit by increasing antioxidant capacity, anti-inflammatory activity and increased proliferation of adult-born granule cells via adult neurogenesis, evident by Ki-67 expression in the dentate gyrus of NSO treated rats. We recently demonstrated that CPF administration resulted in body and brain weight loss, which are considered as indirect markers of metabolic functions, and the recorded oxidative damage was implicated therein [32]. A similar phenomenon was also observed in the work of [54] and [67] where CPF altered body weight negatively, a change that we herein attribute to the oxidative damage, and confirmed as a possible trend from other OPs [23,55].

Based on previous reports that have implicated oxidative stress as the major underlying mechanism of CPF-induced toxicity [8,53], we assessed the effect of CPF on total hippocampal ROS. Administration of CPF significantly increased the ROS level in the CPF treated groups compared to the control. In our study, a marked increase in total ROS in the hippocampal tissues of the CPF exposed rats was recorded, suggesting damage to the cellular membrane of the major cells thus leading to the leak and subsequent oxidative damages in the hippocampus. This finding can be strengthened with previous reports



Fig. 11. Representative photomicrographs of the distribution of Ki67 immunoreactive cells in the Connus Amonis fields of hippocampus of rats exposed to Norm Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). The red arrows points to a Ki67 immunoreactive nuclei in the hippocampal subfields. A reduced number of the nuclei can be noted in the CPF exposed hippocampus, while a relative preserved density is observed with NSO intervention. Ki67 immunohistochemistry 100X.

that submitted that poisoning (inhalation or oral) from CPF exposure causes oxidative damages and more implicated is the high levels of ROS in the exposed animals [6,32,37,63].

But, in an interesting outcome, co-administration of NSO with CPF significantly prevented a rise in the level of ROS while the administration of NSO alone maintained an hippocampal ROS level that is comparable to the saline treated control. This result further confirmed oxidative stress as one of the mechanism of CPF cytotoxicity. In addition, it also showed the efficacy of NSO antioxidant property in combating environmental toxic agent-induced oxidative stress [31,32].

Furthermore, we evaluated the protective effect of NSO against CPF-induced enhancement of inflammatory marker, have been implicated in the pathophysiology of many neurodegenerative diseases and in neuro-inflammation during systemic inflammation, nitric oxide, NO [66,68], in the hippocampus. Animals treated with CPF alone showed a significant increase in the elevation of hippocampal NO, an indication of neuro-inflammatory activities which has been implicated in various neurological disorder [47]. It was recently reported that, increased neuro-inflammation in the hippocampus resulted in pronounced poor cognitive performance [25], and the induced increase in hippocampal NO by CPF in this study, further suggested it promotes neuro-inflammation.

Concomitant administration of NSO however, prevented elevation of hippocampal NO levels in the exposed rats, and consequently inferred to reduce neuro-inflammation. This data is suggestive of NSO a potent anti-inflammatory, and may be further explored against neurological insults.

AChE being an enzyme involved in the hydrolysis of ACh at cholinergic synapses, is said to be essential for modulation of neurobehaviors such as learning, memory and movement [17]. AChE inhibition has been largely reported after CPF administration in rats and suggested as one of the main mechanisms of CPF-induced impaired cognitive performance [48]. Consistently, our data showed that CPF administration caused a significant reduction in the hippocampal AChE activity. Co-administration of NSO with CPF slightly increased hippocampal AChE activity but could not return to the level found in



Fig. 12. Representative photomicrographs of the distribution of Ki67 immunoreactive cells in the dentate gyrus of rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). The red arrows points to a Ki67 immunoreactive nuclei in the dentate gyrus. A reduced number of the nuclei can be noted in the CPF exposed dentate gyrus, while a relative preserved density is observed with NSO intervention, especially in the NSO only treated rats. Ki67 immunohistochemistry 100X.

control rats. This observation could be due to fact that NSO has less effect on AChE activity or CPF-induced AChE inhibition might be largely irreversible [15]. However, cholinergic pathway inhibition is said not to be sufficient to produce marked memory deficit observed in CPF treated rats, as other non-cholinergic pathways have been reported to be involved in the modulation of learning and memory. We hypothesized that CPF also altered the generation and proliferation of new born neurons in the sub-granular layer of the DG, and subsequently prevented integration of new neurons into learning and memory circuitry resulting in cognitive deficits. CFP administration substantially reduced Ki67, a proliferating cell marker and adult neurogenesis, expression in the SGZ which indicated a reduction in the proliferation of adult born neurons. Interestingly, rats treated with NSO and CPF showed high expression of Ki67 in the SGZ. It is worthy to note that rats given NSO only show significantly higher expression of Ki-67 compare to the controls, indicating NSO is capable of improving cell proliferation in the DG and consequently integration of the adult born new cells into memory and learning circuitry. The higher number of Ki-67-immunopositive cells suggests a larger population of dividing progenitor cells [41].

Learning and memory are the main function of the hippocampus, and these cognitive functions have been reported to be modulated by adult hippocampal neurogenesis in rats [22,39]. Good learner mice are reported to have high rate of neurogenesis in the adult DG [12]. Hippocampal neurogenesis has been observed throughout the adult lives of many mammals studied to date [12]. New neurons in the adult hippocampus originate in the progenitor cells located at the border between the hilus and GCL, which is a region called the sub-granular zone (SGZ) of the DG.

In conformity with the oxidative damages and ChE dysfunctions enumerated above, coupled with the loss in Ki67 immunoreactive nuclei in the hippocampus, the spatial memory assessed using MWM showed that CPF exposed rats had poor performance in spatial learning as they spent more time in locating hidden platform, and spent less time exploring the platform quadrant during the probe test. These activities were significantly mitigated by intervention with NSO, in the CPF and NSO co-administrated rats. Consistent with the Ki67 expression in the SGZ in the NSO treated rats, NSO only treated rats



Fig. 13. Representative photomicrographs of the distribution of Ki67 immunoreactive cells in the sub-ventricular zone of rats exposed to Normal saline (NS), chlopyrifos (CPF), chlopyrifos + Nigella sativa oil (CPF + NSO) and Nigella sativa oil (NSO). The red arrows points to a Ki67 immunoreactive nuclei in the subventricular zones. A reduced number of the nuclei can be noted in the CPF exposedrats, while a relative preserved density is observed with NSO intervention. Ki67 immunohistochemistry 100X.

showed improve cognitive performance in LTM, STM and reference memory. Altogether, our neurobehavioral assessment showed that NSO administration significantly improved the spatial learning and reference memory possibly via improved integration of adult born cells into functional hippocampal circuitry. These effects can be supported by its reported potency in enhancing indices of neurocognitive and psycho-cognitive functions in models of neurodegenerative disorders and neurotoxicity [26,30].

Conclusion

The data presented in this study showed that exposure to CPF caused impaired cognitive performance via multifaceted pathways including oxidative damages, AChE inhibition and reduced potential adult neurogenesis in the hippocampus of rats. Interestingly, interventional treatment with NSO shortly after CPF insult mitigated and improved all the neurocognitive indices which may possibly be via the preservation of adult neurogenesis and depletion of stressor markers in the hippocampus of the treated rats. Altogether, our findings revealed NSO supplementation as a potentially effective strategy in combating pesticide-induced cognitive deficits.

Acknowledgment

The authors appreciate the research incentives support from the Deanship Research support of the Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria. Also, the technical assistance of Ms Ali Hussain of the Immunohistochemistry and histology laboratory, School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, South Africa.

Conflict of interest

There is no conflict of interest, it was aimed at environmental problems.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2018. e00008.

References

- [1] M. Ahadpour, M.R. Eskandari, V. Mashayekhi, Mitochondrial oxidativestress and dysfunction induced by isoniazid: study on isolated rat liver and brain mitochondria, Drug Chem. Toxicol. 39 (2) (2016) 224–232.
- [2] S. Ahmad, Z.H. Beg, 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. 12 (1) (2013) 86.
- [3] M.S. Ajao, S. Adebayo, A. Imam, A. Ibrahim, M.Y. Adana, A. Alli-Oluwafuyi, S.B. Kareem, Protective Effect of Nigella sativa (Black Caraway) oil on oral dichlorrvos induced hematological, renal and nonspecific immune system toxicity in Wistar rats, Iran. J. Toxicol. 11 (6) (2017) 1–5.
- [4] M. Alemi, F. Sabouni, F. Sanjarian, K. Haghbeen, S. Ansari, Anti-inflammatory effect of seeds and callus of Nigella Sativa L. extracts on mixglial cells with regard to their thymoquinone content, AAPS PharmSciTech 14 (1) (2013) 160–167.
- [5] A.H. Alhebshi, M. Gotoh, I. Suzuki, Thymoquinone protects cultured rat primary neurons against amyloid β -induced neurotoxicity, Biochem. Biophys. Res. Commun. 433 (4) (2013) 362–367 19.
- [6] N. Aly, K. El-Gendy, F. Mahmoud, A.K. El-Sebae, Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice, Pestic. Biochem. Phys. 97 (2010) 7–12.
- [7] D.G. Amaral, H.E. Scharfman, P Lavenex, The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies), Prog. Brain Res. 163 (2007) 3–22.
- [8] B.D. Banerjee, V. Seth, R.S. Ahmed, Pesticide-induced oxidative stress: perspectives and trends, Rev. Environ. Health 16 (2001) 1-40.
- [9] R. Bargi, F. Asgharzadeh, F. Beheshti, M. Hosseini, H.R. Sadeghnia, M. Khazaei, The effects of thymoquinone on hippocampal cytokine level, brain oxidative stress status and memory deficits induced by lipopolysaccharide in rats, Cytokine 96 (2017) 173–184.
- [10] H. Basand, Y. Kalender, Chlorpyrifos induced cardiotoxicity in rats and the protective role of quercetin and catechin, GUJS 24 (3) (2011) 387–395 Biochemistry 36, 539–546.
- [11] B.K. Binukumar, A. Bal, R.J. Kandimalla, K.D. Gill, Nigrostriatal neuronal death following chronic dichlorvos exposure: crosstalk between mitochondrial impairments, alpha synuclein aggregation, oxidative damage and behavioral changes, Mol. Brain 3 (2010) 35.
- [12] M.A. Bonaguidi, J. Song, G.L. Ming, H.A. Song, A unifying hypothesis on mammalian neural stem cell properties in the adult hippocampus, Curr. Opin. Neurobiol. 22 (2012) 754–761.
- [13] W.J. Breslin, A.B. Liberacki, D.A. Dittenber, J.F. Quast, Evaluation of the developmental and reproductive toxicity of chlorpyrifos in the rat, Fund. Appl. Toxicol. 29 (1996) 119–130.
- [14] J.E. Casida, G.B. Quistad, Organophosphate toxicology: safety aspects of nonacetylcholinesterase secondary targets, Chem. Res. Toxicol. 17 (2004) 983–998.
- [15] W.Q. Chen, L. Yuan, R. Xue, Y.F. Li, R.B. Su, Y.Z. Zhang, J. Li, Repeated exposure to chlorpyrifos alters the performance of adolescent male rats in animal models of depression and anxiety, Neurotoxicology 32 (2011) 355–361.
- [16] S. Dariani, T. Baluchnejadmojarad, M. Roghani, Thymoquinone attenuates astrogliosis, neurodegeneration, mossy fiber sprouting, and oxidative stress in a model of temporal lobe epilepsy, J. Mol. Neurosci. 51 (3) (2013) 679–686.
- [17] S. Das, A review of dichlorvos toxicity in fish, Curr. World Environ. 8 (1) (2013) 143-149.
- [18] F. Demir, F.G. Uzun, D. Durak, Y. Kalender, Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and guercetin, Pestic. Biochem. Phys. 99 (2011) 77–81.
- [19] A.T. Farag, A.H. Radwan, F. Sorour, A. El Okazy, E. El-Agamy, A.E. El-Sebae, Chlorpyrifos induced reproductive toxicity in male rats, Reprod. Toxicol. 29 (2010) 80-85.
- [20] A. Gilani, Q. Jabeen, M. Ullah khan, A review of medicinal uses and pharmacological activities of Nigella sativa, Pak. J. Biol. Sci. 7 (2004) 441-451.
- [21] A. Goel, V. Dani, D.K. Dhawan, Chlorpyrifos-induced alterations in the activities of carbohydrate metabolizing enzymes in rat liver: the role of zinc, Toxicol. Lett. 163 (2006) 235-241.
- [22] J.T. Gonçalves, S.T. Schafer, F.H. Gage, Adult neurogenesis in the hippocampus: from stem cells to behavior, Cell 167 (2016) 897-914.
- [23] A.T. Hariri, S.A. Moallem, M. Mahmoudi, H. Hosseinzadeh, The effect of crocin and safranal, constituents of saffron, against subacute effect of diazinon on hematologicaland genotoxicity indices in rats, Phytomedicine 18 (2011) 499–504.
- [24] F.L. Hitti, S.A. Siegelbaum, The hippocampal CA2 region is essential for social memory, Nature 508 (2014) 88–92.
- [25] M. Hosseini, S.S. Dastghaib, H. Rafatpanah, M.A. Hadjzadeh, H. Nahrevanian, I. Farrokhi, Nitric oxide contributes to learning and memory deficits observed in hypothyroid rats during neonatal and juvenile growth, Clinics (Sao Paulo) 65 (2010) 1175–1181.
- [26] M. Hosseini, T. Mohammad pour, R. Karami, Z. Rajaei, H.R. Sadeghnia, Effects of the hydro-alcoholic extract of Nigella Sativa on scopolamine-induced spatial memory impairment in rats and its possible mechanism, Chin. J. Integ. Med. 21 (2015) 438–444.
- [27] A. Imam, M. Adeboye, W.I. Abdulmajeed, A. Alli-Oluwafuyi, A. Amin, A. Ibrahim, S. Gwadabe, N.G. Popoola, Dichlorvos induced AChE inhibition in discrete brain regions and the neuro-cognitive implications: ameliorative effect of Nigella sativa, Iran. J. Toxicol. 12 (5) (2018) 11–16.
- [28] A. Imam, M.S. Ajao, M.I. Ajibola, A. Amin, W.I. Abdulmajeed, A.Z. Lawal, A. Alli-Oluwafuyi, O.B. Akinola, A.O. Oyewopo, O.J. Olajide, M.Y. Adana, Black seed oil reversed scopolamine-induced Alzheimer and corticohippocampal neural alterations in male Wistar rats, Bull. Fac. Pharm. Cairo Univ. 54 (1) (2016) 1–106.
- [29] A. Imam, M.S. Ajao, O.B. Akinola, M.I. Ajibola, A. Ibrahim, A. Amin, W.I. Abdulmajeed, Z.A. Lawal, A. Ali-Oluwafuyi, Repeated acute oral exposure to Cannabis sativa impaired neurocognitive behaviours and cortico-hippocampal architectonics in Wistar rats, Niger. J. Physiol. Sci. 31 (2017) 153–159.
- [30] A. Imam, M.S. Ajao, A. Amin, W.I. Abdulmajeed, M.I. Ajibola, A. Ibrahim, O.J. Olajide, W.G. Balogun, Cannabis induced moto-cognitive dysfunctions in Wistar rats: ameliorative efficacy of Nigella sativa, Malays. J. Med. Sci. 23 (5) (2016) 17–28.
- [31] A. Imam, A. Ogunniyi, A. Ibrahim, W.I. Abdulmajeed, L.A. Oyewole, A.H. Lawan, F.A. Sulaimon, M.Y. Adana, M.S. Ajao, Dichlorvos induced oxidative and neuronal responses in rats: mitigative efficacy of Nigella sativa (black cumin), Niger. J. Physiol. Sci. 33 (2018) 83–88.
- [32] A. Imam, N.A. Sulaiman, A.L. Oyewole, A. Amin, S-T.T. Shittu, M.S. Ajao, Pro-neurogenic and antioxidant efficacy of Nigella sativa oil reduced vulnerability to cholinesterase dysfunction and disruption in amygdala dependent behaviors in CPF exposure, JKIMSU 7 (3) (2018) 1–12.
- [33] M. Jalali, M. Tehranipour, Effect of alcoholic extract of Nigella sativa seed on alpha motor neurons density of spinal cord following sciatic nerve compression in rats, J. Gorgan Univ. Med. Sci. 15 (4) (2013) 29–34. http://goums.Ac.Ir/journal/article-1-866-en.Html. (article in Farsi).
 [34] J. Javanbakht, R. Hobbenaghi, E. Hosseini, A.M. Bahrami, F. Khadivar, S. Fathi, M.A. Hassan, Histopathological investigation of neurons anterior horn
- [34] J. Javanbakht, R. Hobbenaghi, E. Hosseini, A.M. Bahrami, F. Khadivar, S. Fathi, M.A. Hassan, Histopathological investigation of neurons anterior horn spinal cord after sciatic nerve crush in rats, Pathol. Biol. 61 (2013) 250–253.
- [35] S.C. Joshi, R. Mathur, N. Gulati, Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rat, Toxicol. Ind. Health 23 (2007) 439-444.

- [36] Y. Kalender, S. Kaya, D. Durak, F.G. Uzun, F. Demir, Protective effects of catechin and quercetin on antioxidant status, lipid peroxidation and testishistoarchitecture induced by chlorpyrifos in male rats, Environ. Toxicol. Pathol. 33 (2012) 141–148.
- [37] S.M. Khan, G. Kour, Subacute oral toxicity of chlorpyriphos and protective effect of green tea extract, Pestic. Biochem. Phys. 89 (2007) 118-123.
- [38] J.Y. Khokhar, R.F. Tyndale, Rat brain CYP2B-enzymatic activation of chlorpyrifos to the oxon mediates cholinergic neurotoxicity, Toxicol. Sci. 126 (2012) 325–335.
- [39] J.Y. Kim, X. Duan, C.Y. Liu, M.H. Jang, J.U. Guo, N. Pow-anpongkul, E. Kang, H. Song, G.L. Ming, DISC1 regulates new neuron development in the adult brain via modulation of AKT-mTOR signaling through KIAA1212, Neuron 63 (6) (2009) 761–773.
- [40] M. Kolahdooz, S. Nasri, S.Z. Modarres, S. Kianbakht, H.F. Huseini, Effects of Nigella sativa L. seed oil on abnormal semen quality in infertile men: a randomized, doubleblind, placebo controlled clinical trial, Phytomedicine 21 (2014) 901–905.
- [41] D.K. Sjoukje, E.S. Joern, T. Andrea, Changes in hippocampal neurogenesis throughout early development, Neurobiol. Aging 36 (2015) 365-379.
- [42] A. Malhotra, P. Nair, D.K. Dhawan, Efficacy of zinc as a nutritional supplement in ameliorating chlorpyrifos-induced neurotoxicity in rats, J. Environ. Pathol. Toxicol. Oncol. Off. Organ Int. Soc. Environ. Toxicol. Cancer 30 (2011) 225–233.
- [43] R. Morris, Developments of a water-maze procedure for studying spatial learning in the rat, J. Neurosci. Methods 11 (1984) 47–60.
- [44] T. Nakashiba, J.D. Cushman, K.A. Pelkey, S. Renaudineau, D.L. Buhl, T.J. McHugh, V. Rodriguez Barrera, R. Chittajallu, K.S. Iwamoto, C.J. McBain, et al., Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion, Cell 149 (2012) 188–201.
- [45] T. Nakashiba, J.Z. Young, T.J. McHugh, D.L. Buhl, S. Tonegawa, Transgenic inhibition of synaptic transmission reveals role of CA3 output in hippocampal learning, Science (New York, NL) 319 (2008) 1260–1264.
- [46] K. Nakazawa, T.J. McHugh, M.A. Wilson, S. Tonegawa, NMDA receptors, place cells and hippocampal spatial memory, Nat. Rev. Neurosci. 5 (5) (2004) 361-372.
- [47] M.A. Ortiz-Ortiz, J.M. Moran, R.A. Gonzalez-Polo, M. Niso-Santano, G. Soler, J.M. Bravo-San Pedro, J.M. Fuentes, Nitric oxide-mediated toxicity in paraquat-exposed SH-SY5Y cells: a protective role of 7-nitroindazole, Neurotox. Res. 16 (2009) 160–173.
- [48] Y. Oulhote, M.F. Bouchard, Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children, Environ. Health Perspect 121 (11-2) (2013) 1378-1384.
- [49] V. Pandit, S. Seshadri, S.N. Rao, C. Samarasinghe, A. Kumar, R. Valsalan, A case of organophosphate poisoning presenting with seizure and unavailable history of parenteral suicide attempt, J. Emerg. Trauma Shock 4 (2011) 132–134.
- [50] T. Rush, X.Q. Liu, J. Hjelmhaug, D. Lobner, Mechanisms of chlorpyrifos and diazinon induced neurotoxicity in cortical culture, Neuroscience 166 (2010) 899–906.
- [51] M.L. Salem, Immunomodulatory and therapeutic properties of the Nigella sativa L. seed, Int. Immunopharmacol. 5 (13) (2005) 1749-1770.
- [52] A. Sarwar, Z. Latif, GC-MS characterization and antibacterial activity evaluation of Nigella sativa oil against diverse strains of Salmonella, Nat. Prod. Res. 29 (5) (2015) 447-451.
- [53] P. Sharma, R. Singh, Dichlorvos and lindane induced oxidative stress in rat brain: protective effects of ginger, Pharmacogn. Res. 4 (1) (2012) 27-32.
- [54] S. Singh, A. Prakash, S. Kaur, L.C. Ming, V. Mani, A.B. Majeed, The role of multifunctional drug therapy as an antidote to combat experimental subacute neurotoxicity induced by organophosphate pesticides, Environ. Toxicol. 31 (2016) 1017–1026.
- [55] V. Singh, P. Rupali, In vivo antioxidative and neuroprotective effect of 4-Allyl-2-methoxyphenol against chlorpyrifos-induced neurotoxicity in rat brain, Mol. Cell. Biochem. (2013), doi:10.1007/s11010-013-1899-9.
- [56] P. Somogyi, Hippocampus: intrinsic organisation, in: P Shepherd, S Grillner (Eds.), Hand of Brain Microcircuits, Oxford University Press, New York, 2010, pp. 148–164.
- [57] F.A. Suleiman, O.M. Annas, S. Muftau, A.A. Stephen, O.M. Favour, Alleviating effect of phyllanthusniruri on sensorimotor and cognitive changes induced by subacute chlorpyrifos exposure in Wistar rats, Am. J. Med. Med. Sci. 2 (3) (2012) 50–58.
- [58] J. Tang, Y. Cao, R.L. Rose, A.A. Brimfield, D. Dai, J.A. Goldstein, E. Hodgson, Metabolism of chlorpyrifos by human cytochrome P450 isoforms and human, mouse, and rat liver microsomes, Drug Metab. Dispos. Biol. Fate Chem. 29 (2001) 1201–1204.
- [59] E.M. Tanvir, R. Afroz, M. Chowdhury, S.H. Gan, N. Karim, M.N. Islam, M.I. Khalil, A model of chlorpyrifos distribution and its biochemical effects on the liver and kidneys of rats, Hum. Exp. Toxicol. 35 (2016) 991–1004.
- [60] A.V. Terry Jr., Functional consequences of repeated organophosphate exposure: potential non-cholinergic mechanisms, Pharmacol. Ther. 134 (2012) 355–365.
- [61] D. Tranel, Higher brain functions, in: PM Conn (Ed.), Neuroscience in Medicine, JB Lippincott Company, Philadelphia, 1995, pp. 555–580.
- [62] F.G. Uzun, Y. Kalender, Chlorpyrifos induced hepatotoxic and hematologic changes in rats: the role of quercetin and catechin, Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 55 (2013) 549–556.
- [63] R.S. Verma, A. Mehta, N. Srivastava, Comparative studies on chlorpyrifos and methyl parathion induced oxidative stress in different parts of rat brain: attenuation by antioxidant vitamins, Pestic. Biochem. Phys. 95 (2009) 152–158.
- [64] C. Vivar, H. VanPraag, Functional circuits of new neurons in the dentate gyrus, Front. Neural Circuits 7 (2013) 15, doi:10.3389/fncir.2013.00015.
- [65] Y. Wang, L. Lv, Y. Yu, G. Yang, Z. Xu, Q. Wang, L. Cai, Single and joint toxic effects of five selected pesticides on the early life stages of zebrafish (Denio rerio), Chemosphere 170 (2017) 61–67.
- [66] M.L. Wong, V. Rettori, A. al-Shekhlee, P.B. Bongiorno, G. Canteros, S.M. McCann, P.W. Gold, J. Licinio, Inducible nitric oxide synthase gene expression in the brain during systemic inflammation, Nat. Med. 2 (5) (1996) 581–584.
- [67] M.Y. Xu, P. Wang, Y.J. Sun, Y.J. Wu, Metabolomic analysis for combined hepatotoxicity of chlorpyrifos and cadmium in rats, Toxicology 384 (2017) 50-58.
- [68] J.E. Yuste, E. Tarragon, C.M. Campuzano, F. Ros-Bernal, Implications of glial nitric oxide in neurodegenerative diseases, Front. Cell. Neurosci. 9 (2015) 322, doi:10.3389/fncel.2015.00322.