Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

SHORT COMMUNICATION

Ashwagandha Reverses the Dieldrin-induced Cognitive Impairment by Modulation of Oxidative Stress in Rat Brain

Tandra Ghosh, Sanvidhan G. Suke[1], Chandrashekhar Yadav[2], Rafat Ahmed[3], Basu Deb Banerjee[3]

Department of Physiology, Tripura Medical College, Agartala, Tripura, [1]Department of Biotechnology, Priyadarshini Institute of Engineering and Technology, Nagpur, Maharashtra, [2]Department of Zoology, Environmental Toxicology Lab, University of Rajasthan, Jaipur, Rajasthan, [3]Department of Biochemistry, University College of Medical Science, University of Delhi, New Delhi, India

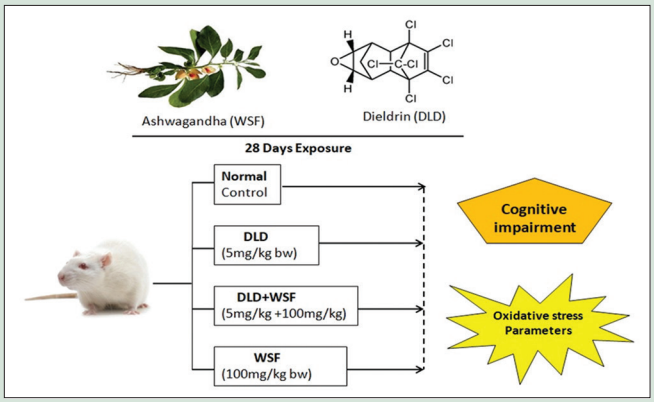
ABSTRACT

Dieldrin (DLD) is an organochlorine pesticide heavily used in agriculture to control pests. Widespread exposures of DLD to human population are likely to contribute in neurological disorders. *Withania somnifera* (WSF), commonly known as "ashwagandha," is used for its broad spectrum of pharmacological activity. The present study was designed to investigate the effect of WSF (100 mg/kg) on DLD (5 mg/kg)-induced modulation of cognitive function and oxidative stress in male Wistar rats. Cognitive function was measured using step-down latency (SDL) on a continuous avoidance apparatus and transfer latency (TL) on an elevated plus maze. Oxidative stress was estimated by measuring brain malondialdehyde (MDA) level, protein carbonyl (PC), and reduced glutathione (GSH) activity. Significant reduction in both acquisition and retention in SDL was found for the DLD-treated group at the end of the exposure study as compared to the control ($P < 0.001$). DLD caused a significant prolongation in both acquisition and retention in TL after 28 days of the treatment as compared to the control ($P < 0.001$). Four-week treatment of WSF antagonized the effect of DLD on SDL and TL at the 29th day. DLD produced a statistically significant increase in the brain MDA and PC levels ($P < 0.001$), and a significant decrease in the brain GSH activity ($P < 0.001$). Treatment with WSF attenuated the effect of DLD on MDA, PC, and GSH activities. Thus, the finding of this study suggests that WSF has potential in reversing cognitive dysfunction and oxidative stress induced by toxicants such as DLD in the brain.

Key words: Ashwagandha, dieldrin, glutathione, malondialdehyde, protein carbonyl

SUMMARY

- Studied the effect of ashwagandha on dieldrin-induced oxdative stress and cognitive dysfunction in rats. The results suggest that the treatment of ashwagandha attenuates the effect of dieldrin on oxidative stress parameters and reversing cognitive dysfunction in pesticide-exposed animals.



Abbreviations Used: DLD: Dieldrin; GSH: Reduced glutathione; LD: Lethal dose; LPO: Lipid peroxidation; MDA: Malondialdehyde; OCP: Organochlorine pesticide; PC: Protein carbonyl; SDL: Step-down latency; SFZ: Shock-free zone; TL: Transfer latency; WSF: *Withania Somnifera.*

Correspondence:
Dr. Sanvidhan G. Suke,
Department of Biotechnology, Priyadarshini Institute of Engineering and Technology,
Nagpur - 440 019,
Maharashtra, India.
E-mail: sgsuke@hotmail.com
DOI: 10.4103/pr.pr_77_18

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Dieldrin (DLD) is an organochlorine pesticide (OCP) that was once heavily used in agriculture to control pests. Its use was prohibited or restricted in many countries like North America. But, still in India, DLD (a chlorinated cyclopentadiene derivative) used as a broad-spectrum insecticide to protect food crops and control disease vectors, locusts, and termites. DLD detected in environments because it is highly persistent and resistant to biodegradation. Due to persistent in nature it get accumulate in the environment and food chain.[1] DLD coexists at significant levels in the environment, food supply and human sera, adipose tissues, and breast milk.[2] In addition to being resistant to biodegradation, DLD is one of the few OCPs that has a strong body of literature that suggests that exposure is significantly associated with an increased risk to Parkinson's disease.[3,4] Thus, this pesticide continues to be a human health concern because it is an example of a persistent pesticide that may exacerbate human diseases, especially for those diseases that have a long prodromal period such as Parkinson's disease. Widespread exposures of the human population are likely to contribute to what has been termed a "silent pandemic" of neurodevelopmental disorders.[5,6] Despite underlying differences in originating mechanisms of action of various neurotoxicants, they often produce convergent outcomes, while at the same time, there are sometimes unexpected disparities between related chemicals from the same class. One likely route by which different agents produce similar neurotoxic outcomes is through their shared ability to produce oxidative stress.[7] Compared to other tissues, the brain is especially vulnerable to oxidant damage because its membrane lipid composition is enriched in oxidizable polyunsaturated fatty acids and because of its high oxygen consumption.[8] This is exacerbated in the developing brain, which faces the increased metabolic demand required for growth, and yet has lower

Cite this article as: Ghosh T, Suke SG, Yadav C, Ahmed R, Banerjee BD. Ashwagandha reverses the dieldrin-induced cognitive impairment by modulation of oxidative stress in rat brain. Phcog Res 2019;11:92-7.

antioxidant reserves and a reduced complement of glia, the cells that protect neurons from oxidative damage.[9,10] Indeed, many developmental neurotoxicants elicit oxidative stress in the immature central nervous system.[11,12]

Memory, one of the most complex brain functions, involving multiple neuronal pathways and neurotransmitters, is considered the ability of an individual to record, retain, and recall the information when needed.[13,14] Considering the multiple hypotheses regarding the mechanisms that lead to neuronal dysfunction, for example, inflammation, oxidative stress, mitochondrial dysfunction, and axonal transport deficits, the need of an alternative therapy that may provide some symptomatic relief is highly needed.[15-17] However, neuroprotection does not seem to fully inhibit the disease progression, at least a delay can be achieved.

Ayurvedic medicine, Withania somnifera (ashwagandha), is commonly being used for its broad spectrum of pharmacological actions. The active principle of *W. somnifera* (WSF) such as sitoindosides VII-X and withaferin A (glycowithanolides) have shown an antioxidant effect in the brain which may be responsible for its diverse pharmacological properties.[18-20] The WSF is known for its memory boosting and restorative functions and is also reported to reverse loss of memory in mice model of Alzheimer's disease by promoting the neurogenesis and growth of brain cells.[21,22] Similarly, root extract of the plant and one of its active components withanolide A has been shown to improve spatial memory and cognitive deficits in temporal lobe epilepsy and experimental model of stroke.[23,24] WSF is traditionally used as a rasayana (tonic) that works in a holistic manner to promote the overall health and vitality. In the present study, the preventive effect of WSF has been investigated on cognitive impairment and oxidative stress in DLD-treated animals.

MATERIALS AND METHODS

Chemicals

Technical grade DLD (purity 99%) was purchased from Sigma-Aldrich (analytical standard, CAS Number 60-57-1). *W. somnifera* (ashwagandha) pure root powder (WSF) was procured from Himalaya Drug Company, Bengaluru, Karnataka, India. All other reagents used were of analytical grade and obtained either from Sisco Research Laboratories or Qualigens Fine Chemicals, Mumbai, Maharashtra, India.

Animals

Male Wistar rats, weighing between 150 and 200 g, were used. The animals were procured from the Central Animal House, University College of Medical Sciences (UCMS), Delhi, India. The animals were housed in standard laboratory conditions (natural hours of light and dark cycle; 23°C ± 1°C temperature and 50% ± 2% humidity) with pellet diet and water available *ad libitum*. The experimental works on rats were performed with the approval of institutional animal ethical committee reference no. 2008/RC/03.

Experimental design

The rats were randomly divided into four groups (eight animals each group) and received treatment orally using syringe and 20-gauge Ryle's tube. Group I (control) received castor oil. DLD was dissolved in castor oil and administered to Group II animals at a dose of 5 mg/kg body weight/day (i.e., one-tenth of LD_{50}).[25] Group III received daily a dose of both DLD (5 mg/kg) and WSF powder (100 mg/kg) aqueous suspension.[26] Group IV was administered with WSF (100 mg/kg) alone. The rats were treated with vehicle or test chemicals for 28 days.

Assessment of cognition

All animal groups were evaluated for a cognitive function 1 day before the start of the treatment and on the day of completion of the treatment schedule. Animals were trained on each day before assessment of cognition. All tests were conducted in the Neuropharmacology Laboratory, Department of Pharmacology, UCMS (University of Delhi), Delhi, India, between 0900 and 1600 h. Cognition functions were assessed on the basis of two separate experiments, as described below.

Step-down latency

This apparatus consisted of a wooden block placed in the center of a grid floor of a continuous avoidance apparatus. The block served as a shock-free zone (SFZ). The rat was placed on the SFZ and, on stepping down was given electric shock (20 V) through the grid floor. The experiment was repeated after ½ h and the time taken by the rat to step-down latency was measured (SDL; acquisition of memory). The procedure was repeated after 24 h without shock (SDL; retention of memory). A cutoff time of 180 s was chosen and for the animal which did not step down in this period, the time to step down was taken as 180 s.[27]

Transfer latency

The elevated plus maze consists of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 40 cm) with an open roof. The maze is elevated to a height of 50 cm from the floor. The arms were connected with a central square (10 cm × 10 cm). The animals were placed individually at either ends of the open arms and allowed to enter either of the closed arms, facing away from the central square. The animals were trained 24 h before testing. On retesting, the time taken to enter the closed arm was taken as transfer latency (TL). A time of 180 s was taken as cutoff and animals not entering the closed arm in this period were assigned the TL of 180 s.[28]

Assessment of oxidative stress

At the end of the experiment, each animal was euthanized by cervical dislocation and their whole brains quickly dissected out, washed with ice-cold sodium phosphate buffer, weighed, and stored over ice. Brains were further processed within ½ h of dissection, and estimations of oxidative stress were performed on the same working day. Brain tissue was homogenized with 10% sodium phosphate buffer (pH 7.4, ice-cold, mixture of KH_2PO_4 and Na_2HPO_4). The homogenates were centrifuged at 1500 × g for 10 min at 4°C.

Determination of proteins

Proteins were determined in the homogenized tissue using bovine serum albumin as standard.[29]

Determination of malondialdehyde

The intensity of lipid peroxidation (LPO) in the brain tissue was spectrophotometrically measured, based on the thiobarbituric acid response products.[30] Homogenate absorption was measured at 532 nm. Malondialdehyde (MDA), an LPO end product, concentration was expressed in nmol/g of brain tissue.

Determination of protein carbonyl

Carbonyl group concentration, as the level of oxidative modified proteins, was determined spectrophotometrically using 2,4-dinitrophenylhydrazine (DNPH), a traditional carbonyl reagent.[31] Reactive carbonyl derivatives were calculated using the DNPH molar extinction coefficient at 370 nm (22 l/mol/cm × 103 l/mol/cm) and expressed in μmol/g of protein.

Determination of glutathione

The nonenzymatic antioxidant glutathione (GSH) was estimated.[32] This assay estimates the activity of GSH reductase that reduces GSH disulfide to the sulfhydryl form, GSH, which is an important cellular antioxidant.

Statistics

All values are expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA (SPSS version 12, Statistical Product and Service Solutions, Chicago, IL, USA). *Post hoc* comparisons between groups were made using Tukey's test and $P < 0.05$ was considered statistically significant.

RESULTS

Cognitive assessment parameters

Step-down latency

At beginning day 0 of experiment, no significant differences were found among the SDLs of all the groups (data not shown). A significant reduction in both acquisition and retention in SDL was found for the DLD-treated group after 4 weeks of treatment as compared to the control ($P < 0.001$). Treatment with DLD + WSF, a significant reversal in SDL, was noted, when administered 1 h before pesticide treatment and caused a significant decrease in acquisition and retention ($P < 0.001$), as compared to DLD alone [Figure 1a].

Transfer latency

At day 0, no significant differences were found among the TLs of all studied groups (data not shown). A significant prolongation in both acquisition and retention in TL was found for DLD-treated group for 28 days, as compared to the TL values of control group ($P < 0.001$). A significant increase in TL values was also noted for the WSF + DLD at week 4 ($P < 0.001$) as compared to pesticide-treated group. Treatment of WSF exhibited antagonization to the effect of DLD on TL [Figure 1b].

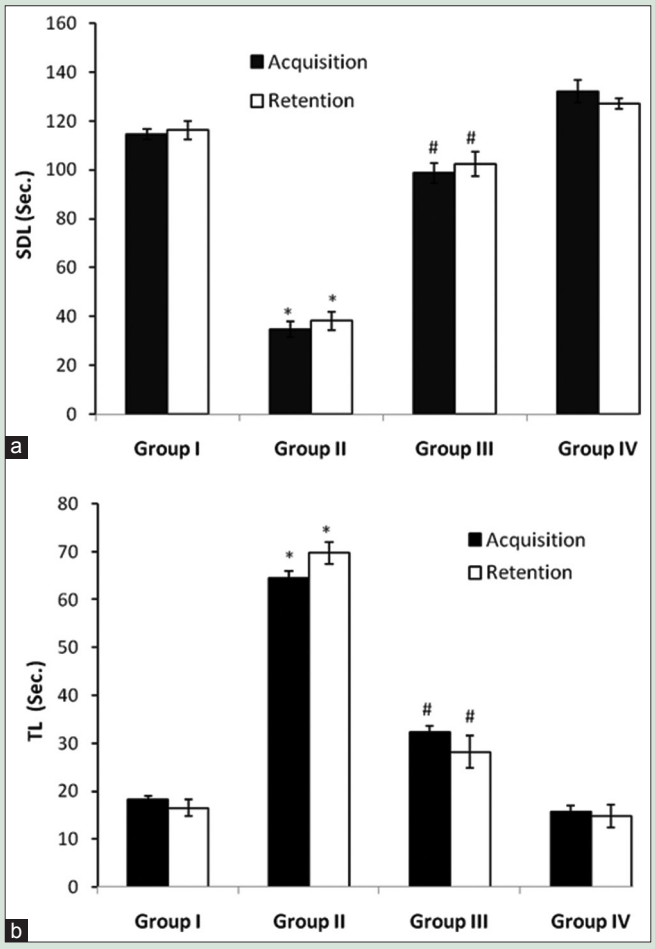


Figure 1: Effect of dieldrin and *Withania somnifera* on (a) acquisition and retention on step-down latency; (b) acquisition and retention on transfer latency in male rats. Values are expressed as mean ± standard deviation. $n = 8$ animals in each group.*Significantly different from control Group I and #significantly different from dieldrin Group II ($P < 0.001$)

Oxidative stress parameters

Malondialdehyde

There was a marked and statistically significant increase in brain MDA levels of the group treated with DLD only ($P < 0.001$). Treatment with WSF attenuated the effect of DLD on the MDA level; the difference between DLD alone and DLD + WSF was found to be statistically significant ($P < 0.001$). No difference was observed in the brain MDA levels of the groups treated with WSF only, as compared to the control group of animals [Figure 2].

Protein carbonyl

The DLD treatment induced a significant increase in the protein carbonyl (PC) content in brain tissue compared to the control group ($P < 0.001$). A significant decrease in the PC contained was noted for the WSF + DLD ($P < 0.001$) as compared to DLD-alone-treated group. The PC content of the DLD group was attenuated by treatment with DLD + WSF. The WSF-only treatment did not cause a significant effect on the PC content as compared to the control group [Figure 3].

Reduced glutathione

A significant decrease in the brain reduced GSH activity was observed for DLD-treated group, as compared to the control ($P < 0.001$). A significant increase was noted for the DLD + WSF ($P < 0.001$) treated group as compared to DLD-only-treated group. The WSF reversed the effect of DLD on GSH. The difference between the DLD-alone-treated group and the DLD + WSF groups was found to be significant ($P < 0.001$) [Figure 4].

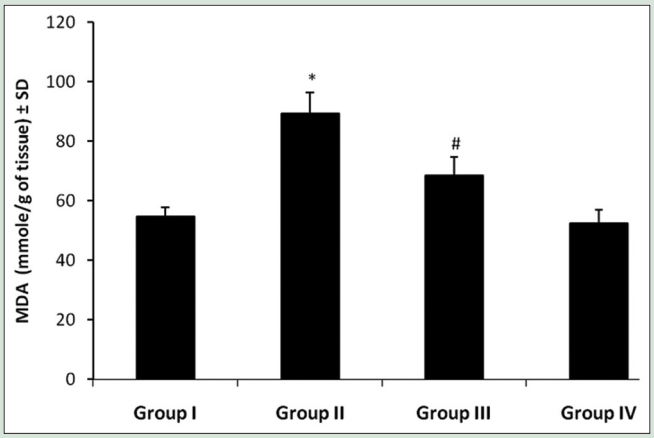


Figure 2: Effect of dieldrin and *Withania somnifera* on the malondialdehyde level in brain tissue of rats. Values are expressed as mean ± standard deviation. $n = 8$ animals in each group. *$P < 0.001$ as compared to the control Group I for dieldrin (one-way ANOVA followed by Tukey's test). #$P < 0.001$ as compared to the dieldrin-alone-treated Group II (one-way ANOVA followed by Tukey's test)

DISCUSSION

Among various pesticides, OCPs belong to a family of highly persistent environmental contaminants due to their resistance to biodegradation.[33] Environmental exposure to DLD may be particularly relevant to human health because of the coexistence of this OCP in the environment and food supply.[34] In addition, several studies have detected elevated levels of DLD in postmortem PD brains relative to age-matched controls.[35-37] In recent years, several reports have suggested that OCPs may cause oxidative stress, characterized by exposure to excessive reactive oxygen species (ROS). Oxidative stress, which is known to be involved in the pathogenesis of several diseases, has been described in acute, chronic, and developmental exposure to OCPs, in both animals and humans, as well as in some *in vitro* studies.[38] Overall, these findings suggest that OCPs can induce oxidative stress.[39-41] Mechanisms related to DLD-induced inhibition of mitochondrial respiration may include the production of ROS, the activation of stress kinases, compromised mitochondrial permeability transition, and activation of caspases and other proteases. The molecular mechanisms that underlie pesticide-induced neurotoxicity are numerous and can involve multiple pathways, substrates, and proteins.[42]

A previous study in our laboratory shows that administration of WSF attenuated the cognitive dysfunction induced by propoxure.[43] In the present study, we investigated the effect of WSF on neurological function (SDL and TL) and oxidative damage (MDA, PC, and GSH) in the experimental animals induced by DLD. The rats exposed to DLD (5 mg/kg p.o.) for 28 days produced no overt toxicity signs and symptoms. No significant differences were noted in mortality rate, body weight, and food intake between control and treated rats (data not mentioned). The selection of DLD exposure was based on the LD_{50} of DLD, which is 50.8 mg/kg body weight for oral treatment in rats. Figure 1a and b indicates that both acquisition and retention functions of SDL parameter were reduced in long-term administration of DLD. This pesticide also caused a significant prolongation on both acquisition and retention on the TL parameter. WSF produced a significant reversal of DLD-induced cognitive dysfunction, when administered 1 h before DLD treatment and caused a significant decrease in SDL and an increase in TL shown in Table 1.

Earlier studies reported that toxicity of several pesticides results from increased LPO in the brain regions, accompanied by decreased levels of GSH, and an accompanied decrease in the activity of GSH peroxidase, GSH reductase, CAT and SOD.[44,45] The present study

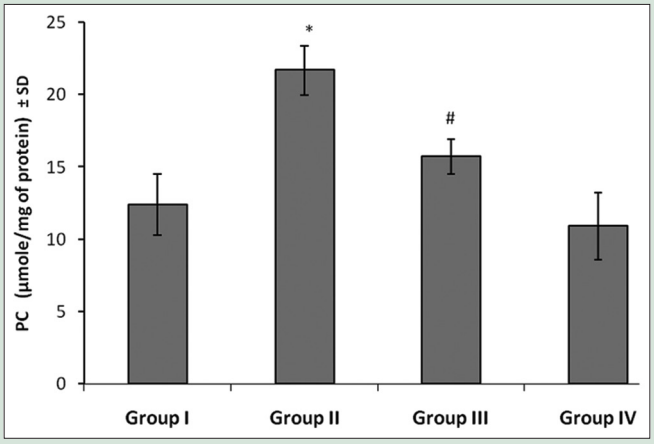


Figure 3: Effect of dieldrin and *Withania somnifera* on the protein carbonyl level in brain tissue of rats. Values are expressed as mean ± standard deviation. n = 8 animals in each group. *P < 0.001 as compared to the control Group I for dieldrin (one-way ANOVA followed by Tukey's test). #P < 0.001 as compared to the dieldrin-alone-treated Group II (one-way ANOVA followed by Tukey's test)

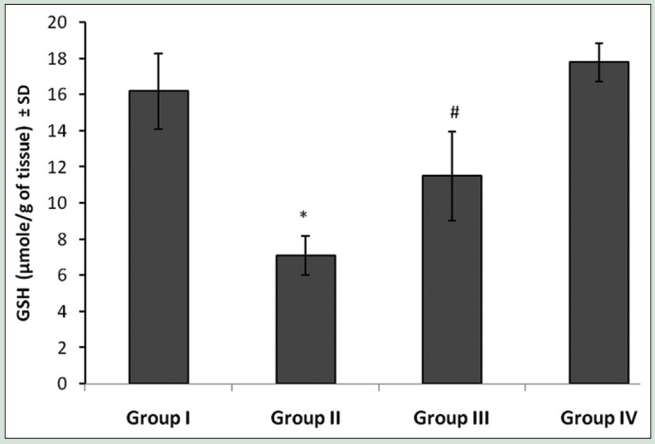


Figure 4: Effect of dieldrin and *Withania somnifera* on reduced glutathione level in brain tissue of rats. Values are expressed as mean ± standard deviation. n = 8 animals in each group. *P < 0.001 as compared to the control Group I for dieldrin (one-way ANOVA followed by Tukey's test). #P < 0.001 as compared to the dieldrin-alone-treated Group II (one-way ANOVA followed by Tukey's test)

Table 1: Effect of dieldrin and *Withania somnifera* on (A) acquisition and retention on step-down latency and (B) acquisition and retention on transfer latency in male Wistar rats

Animal group	Treatment (28 days)	Acquisition		Retention	
		0 day	29th day	0 day	29th day
		(A) SDL (s)			
I	Control	112.9±3.21	114.6±2.17	113.7±3.42	116.2±3.62
II	DLD (5 mg/kg)	111.6±3.11	34.8±3.2*	114.1±4.02	38.2±3.68*
III	DLD + WSF	109.4±4.17	98.7±4.17#	106.9±5.32	102.4±5.12#
IV	WSF (100 mg/kg)	114.2±4.21	132.1±4.62	115.3±4.37	127.2±2.18
		(B) TL (s)			
I	Control	16.4±1.52	18.2±0.76	15.1±2.36	16.5±1.78
II	DLD (5 mg/kg)	15.7±1.48	64.4±1.51*	14.1±2.44	69.7±2.31*
III	DLD + WSF	15.3±1.37	32.4±1.28#	15.7±1.32	28.2±3.42#
IV	WSF (100 mg/kg)	15.9±1.63	15.7±1.32	15.9±2.31	14.8±2.36

*P<0.001 as compared to control group for DLD and #P<0.001 as compared to DLD alone group at 29th day of the animal experiment. WSF: *W. somnifera*; SDL: Step-down latency; TL: Transfer latency; DLD: Dieldrin; *W. somnifera*: *Withania somnifera*

confirmed that DLD alters parameters used assessing oxidative stress. DLD increased MDA levels and PC levels in the animal brain tissue. MDA, a product of LPO, is increased during xenobiotic-induced oxidative stress. The assay of MDA is often considered as an index of ROS generation. High levels of MDA in DLD-exposed rats indicate that the compound enhances LPO and produces oxidative stress. These oxidative stress-related alterations, which are in agreement with previous reports, were attenuated by WSF administration as shown in Figure 2. The level of PC enhanced significantly after administration of a subchronic dose of DLD ad shown in Figure 3. This might indicate an increased oxidatively modified protein in the brain with the increase of neurotoxin. Thus, good agreement exists between the present study involving PC induction and previous studies involving induction of LPO in various regions of the brain.[46] Hence, our study further confirmed that DLD may alter the parameters involved in oxidative stress *in vivo*.

In biological systems, GSH importantly serves the functions of quenching electrophilic chemical species, circumvention of cellular oxidative stress, and maintenance of intracellular thiol redox status.[47] GSH can also spontaneously react with and scavenge a number of ROS. It acts as an intracellular reservoir of cysteine and plays a central role in coordinating synergism of various crucial antioxidants.[48] Reduction or increase in cerebral GSH may be regarded as an index of oxidative stress. Depletion of GSH reserves in the brain can cause neurological deficits.[49] A number of studies showed a decline in GSH concentrations in substantia nigra, which may be a presymptomatic phase of PD.[50] GSH is the most prevalent and important intracellular antioxidant. The result of the present study demonstrated that DLD induced a marked loss in neuronal GSH content. This decline in GSH content indicates that it is consumed to challenge the prevailing oxidative stress as shown in Figure 4. Hence, the increase in oxidative stress could be a mechanism for the cognitive dysfunction observed after administration of DLD. The administration of WSF reversed the effect of DLD on the parameters used for measuring oxidative stress. The administration of WSF resulted in a decrease in MDA levels, signifying its role in decreasing LPO and reduced PC level. WSF also caused an increase in GSH levels, indicating an increase in free radical scavenger activity. These observed effects of WSF point to its possible role as an antioxidant and suggest that this feature may contribute to its cognition-enhancing properties. WSF is used in the Indian traditional medicine Ayurveda and is believed to have a variety of health-promoting effects.[51] The plant exhibits varying degrees of therapeutic values and useful in the treatment of cognitive dysfunction, epilepsy, insomnia, rheumatism, gout, and dyspepsia.[52]

CONCLUSION

Our study revealed that WSD exerts a significant protective effect on DLD-induced oxidative stress parameters (MDA, PC, and GSH), as well as acquisition and retention functioning parameters of cognition in SDL and TL test paradigms. Thus, this study demonstrates a possible correlation between memory impairment and oxidative damage in the brain following pesticide exposure, which can be attenuated by herbal medicine treatment. It may be suggested that WSF has a neuroprotective effect. The detection of behavioral changes induced by a low dose of DLD suggested the need to analyze several other behavioral parameters that might be altered by such pesticides.

Acknowledgements

The authors are grateful to the University College of Medical Science (University of Delhi), Delhi, India, for providing the research facility for completing this work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. El Nemr A, Moneer AA, Khaled A, El-Sikaily A. Levels, distribution, and risk assessment of organochlorines in surficial sediments of the red sea coast, Egypt. Environ Monit Assess 2013;185:4835-53.
2. Lee DH, Porta M, Jacobs DR Jr., Vandenberg LN. Chlorinated persistent organic pollutants, obesity, and type 2 diabetes. Endocr Rev 2014;35:557-601.
3. Chhillar N, Singh NK, Banerjee BD, Bala K, Mustafa M, Sharma D, *et al.* Organochlorine pesticide levels and risk of Parkinson's disease in North Indian population. ISRN Neurol 2013;2013:371034.
4. Steenland K, Mora AM, Barr DB, Juncos J, Roman N, Wesseling C, *et al.* Organochlorine chemicals and neurodegeneration among elderly subjects in Costa Rica. Environ Res 2014;134:205-9.
5. Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. Lancet Neurol 2014;13:330-8.
6. Barrett ES, Patisaul HB. Endocrine disrupting chemicals and behavior: Re-evaluating the science at a critical turning point. Horm Behav 2017;96:A1-6.
7. De Felice A, Ricceri L, Venerosi A, Chiarotti F, Calamandrei G. Multifactorial origin of neurodevelopmental disorders: Approaches to understanding complex etiologies. Toxics 2015;3:89-129.
8. Slotkin TA, Seidler FJ. Oxidative stress from diverse developmental neurotoxicants: Antioxidants protect against lipid peroxidation without preventing cell loss. Neurotoxicol Teratol 2010;32:124-31.
9. Mulholland PJ, Stepanyan TD, Self RL, Hensley AK, Harris BR, Kowalski A, *et al.* Corticosterone and dexamethasone potentiate cytotoxicity associated with oxygen-glucose deprivation in organotypic cerebellar slice cultures. Neuroscience 2005;136:259-67.
10. Ojo JO, Rezaie P, Gabbott PL, Stewart MG. Impact of age-related neuroglial cell responses on hippocampal deterioration. Front Aging Neurosci 2015;7:57.
11. Kitazawa M, Anantharam V, Kanthasamy AG. Dieldrin-induced oxidative stress and neurochemical changes contribute to apoptopic cell death in dopaminergic cells. Free Radic Biol Med 2001;31:1473-85.
12. Amri F, Ghouili I, Amri M, Carrier A, Masmoudi-Kouki O. Neuroglobin protects astroglial cells from hydrogen peroxide-induced oxidative stress and apoptotic cell death. J Neurochem 2017;140:151-69.
13. Başar E, Düzgün A. The brain as a working syncytium and memory as a continuum in a hyper timespace: Oscillations lead to a new model. Int J Psychophysiol 2016;103:199-214.
14. Sevastre-Berghian AC, Făgărăsan V, Toma VA, Bâldea I, Olteanu D, Moldovan R, *et al.* Curcumin reverses the diazepam-induced cognitive impairment by modulation of oxidative stress and ERK 1/2/NF-κB pathway in brain. Oxid Med Cell Longev 2017;2017:3037876.
15. Golde TE. The therapeutic importance of understanding mechanisms of neuronal cell death in neurodegenerative disease. Mol Neurodegener 2009;4:8.
16. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 2009;7:65-74.
17. Stepien KM, Heaton R, Rankin S, Murphy A, Bentley J, Sexton D, *et al.* Evidence of oxidative stress and secondary mitochondrial dysfunction in metabolic and non-metabolic disorders. J Clin Med 2017;6. pii: E71.
18. Bhattacharya A, Ramanathan M, Ghosal S, Bhattacharya SK. Effect of withania somnifera glycowithanolides on iron-induced hepatotoxicity in rats. Phytother Res 2000;14:568-70.
19. Sarbishegi M, Heidari Z, Mahmoudzadeh-Sagheb H, Valizadeh M, Doostkami M. Neuroprotective effects of *Withania coagulans* root extract on CA1 hippocampus following cerebral ischemia in rats. Avicenna J Phytomed 2016;6:399-409.
20. Dar NJ, Hamid A, Ahmad M. Pharmacologic overview of *Withania somnifera*, the Indian Ginseng. Cell Mol Life Sci 2015;72:4445-60.
21. Pandey V, Ansari WA, Misra P, Atri N. *Withania somnifera*: Advances and implementation of molecular and tissue culture techniques to enhance its application. Front Plant Sci 2017;8:1390.
22. Kuboyama T, Tohda C, Komatsu K. Effects of ashwagandha (roots of *Withania somnifera*) on neurodegenerative diseases. Biol Pharm Bull 2014;37:892-7.
23. Choudhary D, Bhattacharyya S, Bose S. Efficacy and safety of *Ashwagandha* (*Withania somnifera* (L.) Dunal) root extract in improving memory and cognitive functions. J Diet Suppl 2017;14:599-612.
24. Manchanda S, Kaur G. *Withania somnifera* leaf alleviates cognitive dysfunction

by enhancing hippocampal plasticity in high fat diet induced obesity model. BMC Complement Altern Med 2017;17:136.
25. Barnes JM, Heath DF. Some toxic effects of dieldrin in rats. Br J Ind Med 1964;21:280-2.
26. Singh A, Naidu PS, Gupta S, Kulkarni SK. Effect of natural and synthetic antioxidants in a mouse model of chronic fatigue syndrome. J Med Food 2002;5:211-20.
27. Joshi H, Parle M. Brahmi rasayana improves learning and memory in mice. Evid Based Complement Alternat Med 2006;3:79-85.
28. Naidu PS, Singh A, Kulkarni SK. Reversal of reserpine-induced orofacial dyskinesia and cognitive dysfunction by quercetin. Pharmacology 2004;70:59-67.
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
30. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
31. Levine RL, Wehr N, Williams JA, Stadtman ER, Shacter E. Determination of carbonyl groups in oxidized proteins. Methods Mol Biol 2000;99:15-24.
32. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.
33. Jayaraj R, Megha P, Sreedev P. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdiscip Toxicol 2016;9:90-100.
34. Mazlan N, Ahmed M, Muharam FM, Alam A. Status of persistent organic pesticide residues in water and food and their effects on environment and farmers: A comprehensive review in Nigeria. Semina Ciênc Agrárias Londrina 2017;38:2221-36.
35. Sharma H, Zhang P, Barber DS, Liu B. Organochlorine pesticides dieldrin and lindane induce cooperative toxicity in dopaminergic neurons: Role of oxidative stress. Neurotoxicology 2010;31:215-22.
36. Baltazar MT, Dinis-Oliveira RJ, de Lourdes Bastos M, Tsatsakis AM, Duarte JA, Carvalho F, *et al.* Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative diseases – A mechanistic approach. Toxicol Lett 2014;230:85-103.
37. Costa LG. The neurotoxicity of organochlorine and pyrethroid pesticides. Handb Clin Neurol 2015;131:135-48.
38. Marouani N, Hallegue D, Sakly M, Benkhalifa M, Ben Rhouma K, Tebourbi O, *et al.* Involvement of oxidative stress in the mechanism of p, p'-DDT-induced nephrotoxicity in adult rats. Gen Physiol Biophys 2017;36:309-20.
39. Ghosh R, Siddharth M, Singh N, Kare PK, Banerjee BD, Wadhwa N, *et al.* Organochlorine pesticide-mediated induction of NADPH oxidase and nitric-oxide synthase in endothelial cell. J Clin Diagn Res 2017;11:BC09-12.
40. Mangum LC, Borazjani A, Stokes JV, Matthews AT, Lee JH, Chambers JE, *et al.* Organochlorine insecticides induce NADPH oxidase-dependent reactive oxygen species in human monocytic cells via phospholipase A2/arachidonic acid. Chem Res Toxicol 2015;28:570-84.
41. Pathak R, Suke SG, Ahmed T, Ahmed RS, Tripathi AK, Guleria K, *et al.* Organochlorine pesticide residue levels and oxidative stress in preterm delivery cases. Hum Exp Toxicol 2010;29:351-8.
42. Schmidt JT, Rushin A, Boyda J, Souders CL 2nd, Martyniuk CJ. Dieldrin-induced neurotoxicity involves impaired mitochondrial bioenergetics and an endoplasmic reticulum stress response in rat dopaminergic cells. Neurotoxicology 2017;63:1-12.
43. Yadav CS, Kumar V, Suke SG, Ahmed RS, Mediratta PK, Banerjee BD, *et al.* Propoxur-induced acetylcholine esterase inhibition and impairment of cognitive function: Attenuation by *Withania somnifera*. Indian J Biochem Biophys 2010;47:117-20.
44. Suke SG, Shukla A, Mundhada D, Banerjee BD, Mediratta PK. Effect of phosphamidon on cognition and oxidative stress and its modulation by ascorbic acid and 4'-chlorodiazepam in rats. Pharmacol Biochem Behav 2013;103:637-42.
45. Suke SG, Ahmed RS, Pathak R, Tripathi AK, Banerjee BD. Attenuation of phosphamidon-induced oxidative stress and immune dysfunction in rats treated with N-acetylcysteine. Braz J Med Biol Res 2008;41:765-8.
46. Sharma A, Bist R, Bubber P. Thiamine deficiency induces oxidative stress in brain mitochondria of *Mus musculus*. J Physiol Biochem 2013;69:539-46.
47. Harris C, Hansen JM. Oxidative stress, thiols, and redox profiles. Methods Mol Biol 2012;889:325-46.
48. Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, *et al.* Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol 2015;6:183-97.
49. Sharma AK, Mehta AK, Rathor N, Chalawadi Hanumantappa MK, Khanna N, Bhattacharya SK, *et al.* Melatonin attenuates cognitive dysfunction and reduces neural oxidative stress induced by phosphamidon. Fundam Clin Pharmacol 2013;27:146-51.
50. Smeyne M, Smeyne RJ. Glutathione metabolism and Parkinson's disease. Free Radic Biol Med 2013;62:13-25.
51. Chauhan NS, Sharma V, Dixit VK, Thakur M. A review on plants used for improvement of sexual performance and virility. Biomed Res Int 2014;2014:868062.
52. Russo A, Izzo AA, Cardile V, Borrelli F, Vanella A. Indian medicinal plants as antiradicals and DNA cleavage protectors. Phytomedicine 2001;8:125-32.