Ameliorative Effect of Berberine on Thiacloprid-Induced Hippocampal Oxidative Stress and Cognitive Deficits

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Abstract

Background: Thiacloprid is a commonly used neonicotinoid insecticide that is used to control insects on farms. It has been found that thiacloprid causes neurotoxicity due to oxidative stress, mitochondrial dysfunction, inflammation, and neuronal damage. Berberine and Chlorogenic acid are effective antioxidants with neuroprotective potential. Thiacloprid (65 mg/kg), berberine (50 and 100 mg/kg), and chlorogenic acid (5 and 10 mg/kg) and their combinations were administered to Wistar albino rats randomly grouped into ten sets (n = 6) for 40 days. The behavioural activity of rats was determined using the open field test and Morris water maze test. Measurement of biochemical parameters such as malondialdehyde (MDA), glutathione (GSH), and acetylcholinesterase (AChE) was conducted on brain tissue homogenates. Activities of mitochondrial complex I, as well as pro-inflammatory cytokines like IL-6 were assessed. Histopathological evaluation of brain tissues was done by HE staining. Administration of thiacloprid led to a significant increase in MDA, nitric oxide, IL-6, and AChE, as well as significant decreases in GSH, IL-6, and mitochondrial enzyme activities when compared to controls ($P<0.05$). Behavioural assessment showed a lack of locomotor activity and anxiety behaviour in the thiacloprid-exposed group, alongside memory impairment. There were findings of neuronal pyknosis and degeneration in the hippocampus following pathological assessment. The use of berberine and chlorogenic acid, individually or in combination, had a significant impact on all the biochemical, behavioral, inflammatory, mitochondrial, and histological findings in a dose dependent. Both berberine and chlorogenic acid showed strong neuroprotective properties against thiacloprid-induced neurotoxicity due to the presence of antioxidants, anti-inflammatory factors, and mitochondrial protection. In combination, they showed increased efficacy, indicating that they could be used as natural remedies to counteract oxidative neuronal damage and cognitive impairment caused by pesticides.

Keywords: Thiacloprid, Berberine, Chlorogenic acid, Neurotoxicity, Oxidative stress, Cognitive impairment, Neuroprotection, Neonicotinoids, Neuroinflammation.

Introduction

The neonicotinoid pesticides are some of the most widely used types of pesticides in the world due to their great efficiency at controlling pests and their relatively low toxicity compared to other conventional pesticides like organophosphates and carbamates. A chloronicotinyl insecticide belonging to the neonicotinoid class, has been widely used in agriculture for controlling sucking pests [1]. While thiacloprid was initially believed to be less toxic to non-target organisms, accumulating evidence from toxicological studies has shown that thiacloprid is detrimental to mammalian health due to its neurotoxicity. Current research indicates that exposure to neonicotinoids for an extended period can cause oxidative stress, dysfunction in mitochondria, neuroinflammation, and neuronal degeneration. This research has led to increased interest in finding natural compounds that could protect neurons from the toxicity caused by pesticides [2]. Berberine is an isoquinoline alkaloid extracted from plants that has been shown to have excellent antioxidant, anti-inflammatory, and neuroprotective effects [3]. Thiacloprid works mainly by acting on nicotinic acetylcholine receptors (nAChRs) in insects, which results in constant neural stimulation, paralysis, and ultimately death. Nonetheless, nAChRs in mammals may also be targeted after long-term exposure to the chemical. Experiments have found out that thiacloprid exposure may lead to an imbalance of neurotransmitters, synapse malfunction, and cell death in the neurons of animals [4]. Apart from the effects on cholinergic neurotransmission, thiacloprid has also been associated with high levels of reactive oxygen species (ROS), which cause oxidative stress, and tissue injury in different organs such as the brain. The brain is especially vulnerable to oxiznWSHAZBN dative insults because of its high oxygen utilization rate, high levels of lipids, and limited ability to act as an antioxidant. Lipid peroxidation, protein oxidation, mitochondrial dysfunctions, and DNA damage, which arise from oxidative stress, could result in neurotoxicity and subsequent impairment of cognitive function. In recent studies on toxicity, neonicotinoids such as thiacloprid were shown to induce neurobehavioral changes, including learning, memory, motor, and affective behaviors, through oxidative and inflammatory mechanisms [5]. The natural bioactive substances have recently attracted significant interest due to their safety profiles in treating oxidative stress-induced neurological disorders. The berberine substance belongs to the isoquinoline alkaloid class and is naturally obtained from medicinal plants such as Berberis vulgaris, Coptis chinensis, and Hydrastis canadensis. The berberine compound has been studied extensively for its diverse pharmacological properties like antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, and neuroprotective actions [6]. Significantly, berberine has proven its capability to pass through

the blood-brain barrier, thus making it very important in the treatment of neurological disorders. Recently, studies have shown that berberine has neuroprotective activity through the regulation of various signaling pathways related to oxidative stress, apoptosis, mitochondrial dysfunction, and neuroinflammation [7]. The antioxidant properties of berberine are mainly due to its ability to scavenge free radicals and support antioxidant mechanisms. Studies have proven that berberine enhances the antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), along with minimizing lipid peroxidation and ROS production. Additionally, berberine activates the nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway, whose function is to regulate the detoxifying and antioxidant genes in order to defend cells from oxidation. Activation of this pathway helps in preserving neurons and protecting them from toxic-induced oxidative stress. Besides its role as an antioxidant, berberine acts as a neuroprotective agent by suppressing inflammation processes in neurons due to its ability to inhibit the NF-κB pathway and limit the secretion of pro-inflammatory cytokines like IL-6 [8]. Experimental studies carried out recently have shown that berberine improves cognitive impairments in animal models of toxicity and neurodegeneration. According to [9], berberine improved cognitive functions via regulation of oxidative stress and inflammation mechanisms. In the same way, [10] emphasized the role of berberine as a potent neuroprotectant by its capability to enhance mitochondrial activity, apoptosis, and increase neuronal survival. It is also important to note that berberine can regulate various signalling pathways including PI3K/AKT, AMPK, and MAPK signalling pathways that play an important role in neuronal growth and survival. Therefore, it can be said that berberine acts as a potent neuroprotective agent for chemical-induced neurotoxicity. While there are more reports on the toxicity of neonicotinoids, little research has been conducted on the protection offered by berberine against neurobehavioral and oxidative changes caused by thiacloprid. With the extensive use of pesticides like thiacloprid in agriculture and the rising number of cases of pesticide-related neurodegeneration, it is necessary to identify treatment modalities that would mitigate these problems. Hence, this experiment seeks to investigate the protective impact of berberine against oxidative stress and neurobehavioral damage triggered by thiacloprid pesticide. In this research, neurobehavioral assessment, oxidative stress markers, and brain tissue histopathology will be used to unravel the underlying neuroprotective actions of berberine. This experiment is expected to reveal insights into potential treatment strategies for pesticide-induced oxidative neurotoxicity.

Material & Methods

Lists of drugs & chemicals used in the experimental protocol

Thiacloprid, berberine, 2-nitrobenzoic acid (DTNB), Folin -Ciocalteu reagent, N-Naphthyl ethylene diamine, Bovin serum albumin, Ethylene glycol tetra acetic acid (EGTA), Nitrazo-blue tetrazolium (NBT). Wistar albino rats (150-160g) were selected as a study model of neurotoxicity. Wistar albino rat maintain low body weight compared to another model. They have smaller body size which offer easy handling and maintenance. Significant histological data is available selected species for the evaluation of neurotoxicity. Experiment animals and their study period as per protocol was designed.

Number of experimental animals and their study period as per protocol.

Groups	No. of animals	Durations (Days)
Control	06	40
Berberine (100 mg/kg) Perse	06	40
Chlorogenic acid (10mg/kg) Perse	06	40
Thiacloprid (65mg/kg)	06	40
Berberine (50mg/kg) + Thiacloprid (65mg/kg)	06	40
Berberine (100 mg/kg) + Thiacloprid (65mg/kg)	06	40
Chlorogenic acid (5mg/kg) + Thiacloprid (65mg/kg)	06	40
Chlorogenic acid (10 mg/kg) + Thiacloprid (65mg/kg)	06	40
Berberine (50mg/kg) + Chlorogenic acid (5mg/kg) + Thiacloprid (65mg/kg)	06	40
Berberine (100 mg/kg) + Chlorogenic acid (10 mg/kg) + Thiacloprid (65mg/kg)	06	40

Experimental Induction of neurotoxicity & animal care: Wistar albino rats at the age of 14 days will be procured and were used as experimental animal model. All animals will be randomly housed 6 per cage with a 12 h-12 h light-dark cycle and were given access to standard food pellets and water ad libitum. Room temperature was maintained at 25±2° C with relative humidity of 55-65%. The weights of the albino rat on the first day of experiment will be measured.

Determination of Behaviour Assessment:

Open field test: The Actophotometer (INCO Pvt Ltd, Ambala) was employed to measure locomotion activity of rats individually. Actophotometer was designed using three infrared beams placed on the x and y axis, along with equal numbers of photoreceptors placed on the opposing walls. When conducting the experiment, it was important to place the instrument in an illuminated, soundproof, and well-ventilated chamber. The locomotor activity was measured using photobeam breakdowns. The frequency of the beam breaks recorded for 20 minutes of testing time in two intervals of 10 minutes was used to quantify the locomotor activity [11].

Morris Water Maze: Given that previous literature stated that neurotoxicity often results in memory deficits (Morris, 1984), an assessment of memory function was performed. A rectangular pool containing water (25°C) and being 40 cm in

depth was divided into four parts (Northeast; NE, Southeast; SE, Southwest; SW. and Northwest; NW). Water was used from the tap and some non-noxious milk was placed inside the pool. In one quadrant of the water surface, there was a removable platform that rats had to remember based on cues located at the periphery of the water tank. The time taken to find the submerged platform [12].

Dissection and homogenization

Sacrifice was performed by cervical dislocation following the removal of vertebrae on PND 41. Brains were extracted and weighed separately. Next, 10% (w/v) homogenate from the tissue was prepared using Teflon homogenizer in 0.1M phosphate buffer solution pH 7.4. Homogenate was then centrifuged for 15 minutes at 4°C and 3000 rpm/10,000 g. Supernatant of the homogenate was collected and used for determination of oxidative stress parameters.

Determination of Antioxidant parameters

Estimation of brain Thio-barbituric acid reactive species (TBARS)

The TBA reactive substances were estimated from the interaction between malondialdehyde (MDA) which is produced during the process of lipid peroxidation and TBA to generate a coloured complex. Newly made tissue homogenates were mixed with each other in test tubes containing equal amounts of 60 mM Tris-HCl buffer (pH 7.4), 0.1 mM DTPA, 12% TCA, and 0.73% TBA, followed by vigorous shaking after each addition. These solutions were heated at 100°C for an hour and cooled down to room temperature and centrifuged at 10,000 x g for five minutes. The absorbance of the supernatant was spectrophotometrically measured at 535 nm. The extent of lipid peroxidation was evaluated using the molar extinction coefficient of MDA at 535 nm (153 M^-1cm^-1). Duplicate analyses were done for all samples and reported as micromoles of MDA per gram of tissue [13].

Determination of glutathione (GSH) levels:

The amount of reduced glutathione in the brain was determined through the spectrophotometric analysis at 412 nm (Singh et al., 2015). Trichloroacetic acid solution (10%, w/v) was mixed in a ratio of 1:1 with the supernatant of the homogenate. The mixture was then subjected to 10 minutes of centrifugation at 1000g and temperature of 40°C. The resultant supernatant (0.5 ml) was then added to 2 ml disodium hydrogen phosphate solution (0.3M). After adding 0.25 ml freshly prepared 5,5'-dithiobis (2-nitrobenzoic acid), DTNB (DTNB was dissolved in 1% w/v sodium citrate), the absorbance was read at 412 nm. The results were expressed in micro molar reduced glutathione per mg protein, and standard curve was obtained from the range of 10-100 µM reduced glutathione [14].

Estimation of Acetylcholinesterase in brain

Homogenized brain tissue (500 µL) was then incubated with 1% Triton X-100 (w/v) diluted in 0.03 M sodium phosphate buffer (pH 7.0). This mixture was then centrifuged at 30 minutes to obtain the supernatant that was further kept in the refrigerator at 4°C for future assessment of the enzyme acetylcholinesterase (AChE). The activity of the enzyme was assessed through spectrophotometric analysis at 412 nm using a time interval of 15 seconds. One unit of AChE activity was determined as the quantity of enzyme hydrolyzing one micromole (µmol) of acetylthiocholine iodide per minute per milligram (mg) of protein [15].

Determination of mitochondrial complex I (NADH dehydrogenase) activity:

The spectrophotometric test was performed for evaluating Complex I (NADH dehydrogenase activity). This procedure involves the reduction of cytochrome-C by catalyzing the oxidation of NADH into 10 NAD*. The reaction mixture consists of 10.5mM cytochrome-C, 6mM NADH in 2mM glycyl glycine buffer, and 0.2M glycyl glycine buffer at a pH level of 8.5. With the addition of the required quantity of the solubilized mitochondrial extract, the reaction starts. The absorbance change was measured for two minutes at 550nm wavelength [16].

Determination of inflammatory parameters:

Estimates of CREB, antibody plates, IL-6, BDNF, TNF- a, and IL-6 levels will be calculated using Alba Science Rat ELISA kits. They were evaluated using the sandwich technique of the enzyme-linked immunosorbent assay of the brain. Following the exact steps outlined in the product info booklet, samples were processed in triplicate for optical density measurements, and the mean optical density was considered for the concentration's final computation. On 96-well percolated to BDNF, and CREB quantification was performed sing supernatant at 450 nm. Results were expressed in pg/ml and ng/ml [17]. The instructions in each kit outlined the following basic steps: All reagents were brought to room temperature (18 - 25°C) before use. To each well of the ELISA plate 100µl of each standard and sample were added. Wells were covered and incubated for 2.5 hours at room temperature with gentle shaking. The solution in each well was discarded and each well was washed 4 times with IX Wash Solution. The Wash Buffer (300µl) was used to wash each well using a multi-channel Pipette. The remaining wash buffer in the wells was removed after each wash by aspirating or decanting. The plate was inverted and blotted against some clean paper towels. To the above cleaned and empty well plate 100µl of 1X prepared biotinylated antibody was added. The plate was than incubated for 1 hour at room temperature with gentle shaking. Solution was discarded and the washing step was repeated as described above. To the above cleaned and empty well plate 100µl of prepared Streptavidin solution was added to each well. The resulting plate was than incubated for 45 min at room temperature with gentle shaking. Solution was discarded and the washing step was repeated as described above. To the above clean well plate 100µl of TMB One-Step Substrate Reagent was added to each well. This was followed by incubation at 30 minutes at room temperature in the dark with gentle shaking. Finally, 50µl of Stop Solution was added to each well and the plate was read at 450 nm.

Histopathological studies

Histopathological investigations were conducted to examine brain tissue. Brain tissue slices, each measuring 5 micrometres in thickness, were prepared in the sagittal plane. These brain slices were stained using Haematoxylin and Eosin (H&E) staining techniques. After staining, the brain slices were photographed using a microscope equipped with a light source to assess any alterations in the Purkinje cell layer. This histopathological analysis using H&E staining allowed for the visualization and examination of the brain tissue, specifically focusing on the Purkinje cell layer, to identify any structural changes or abnormalities.

Statistical analyses

The data were statistically analyzed using Graph pad prism 5 and represented using the Mean ± Standard Error of the Mean (SEM) terminology. Statistical analysis was performed using Analysis of Variance (ANOVA) to determine statistical significance. Following ANOVA, a post hoc test, specifically the Tukey test, was applied to further analyze and compare the data for significant differences among different groups. The P value (0.05, 0.01, 0.001) is elaborated for each obtained result.

Result

Estimation of Behavioural Parameters

Effect of thiacloprid, chlorogenic acid and berberine on locomotor activity

Thiacloprid treated animals had significantly increased (F (9,59) =0.097, p< 0.050, ^{a}p<0.050 vs control, ^{b}p<0.050 vs thiacloprid. **Fig. 1** While there is no significant changes in all groups.

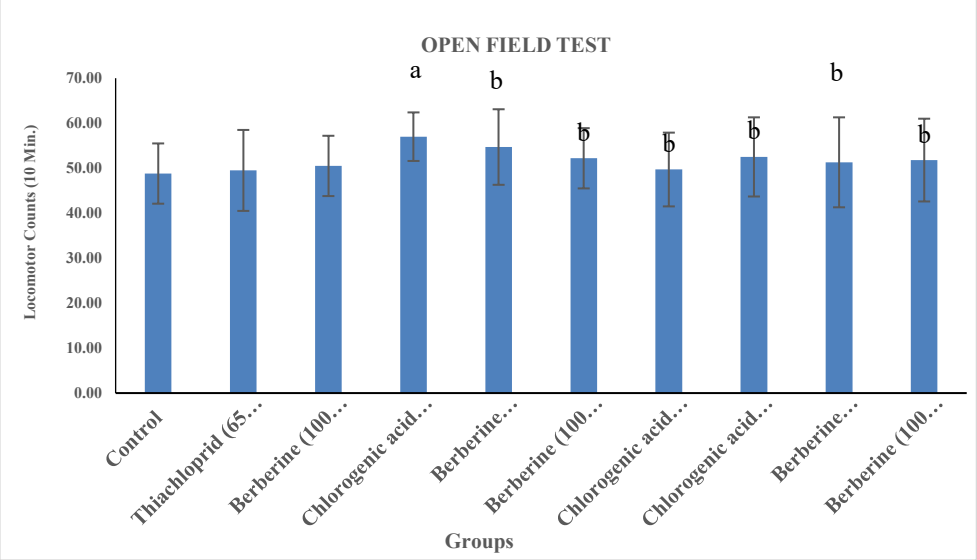


Fig. 1

Results are represented as mean ± SEM (n=6), one-way ANNOVA followed by Tukey's multiple comparison test. OFT ((9,59) =0.097, p< 0.050, ^{a}p<0.050 vs control, ^{b}p<0.050 vs thiacloprid]. There are no significant changes observed in any of the groups. TLO (Thiacloprid), CGC (Chlorogenic), BER (Berberine), OFT (Open field test).

Effect of thiacloprid, berberine and chlorogenic acid (time consumed in target quadrant) on Morris water maze

Result is represented as mean ± SEM (n=6), one-way ANNOVA followed by Tukey's multiple comparison test. **Fig. 2.** ELT = (F (9,59) =3.498, P<0.05): ^{a}p<0.05 vs control: ^{b}p<0.05 vs ELT in thiacloprid. TLO (Thiacloprid), CGC (Chlorogenic), BER (Berberine), TSTQ (Time spent in target quadrant).

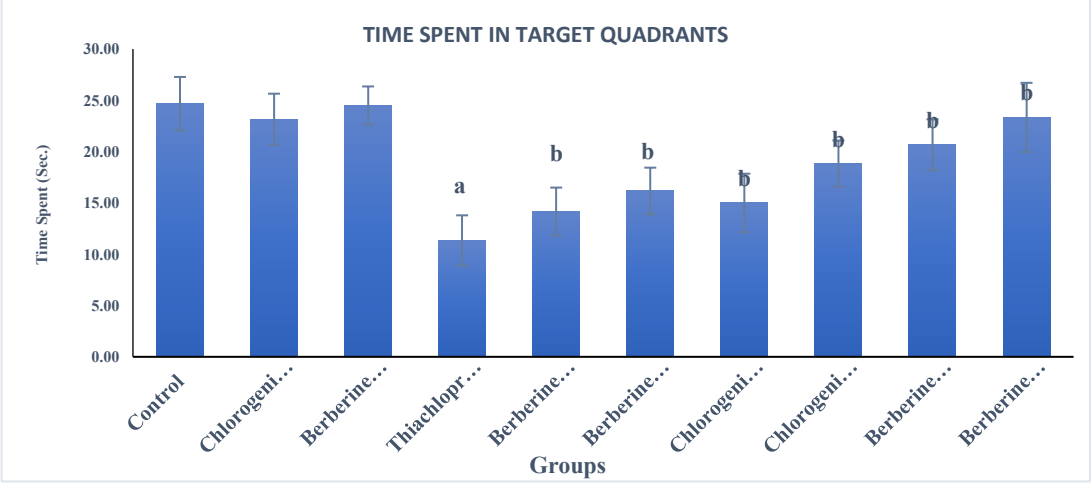


Fig. 2

Estimation of biochemical parameters

Effect of thiacloprid, berberine and chlorogenic acid on Acetylcholinesterase activity

Thiacloprid treated animals had significantly increased (F (9,59) =6.525, p< 0.050, [a]p<0.050 vs control, [b]p<0.050 vs Thiacloprid]. **Fig. 3.** However, various doses of berberine and chlorogenic acid reversed these effects.

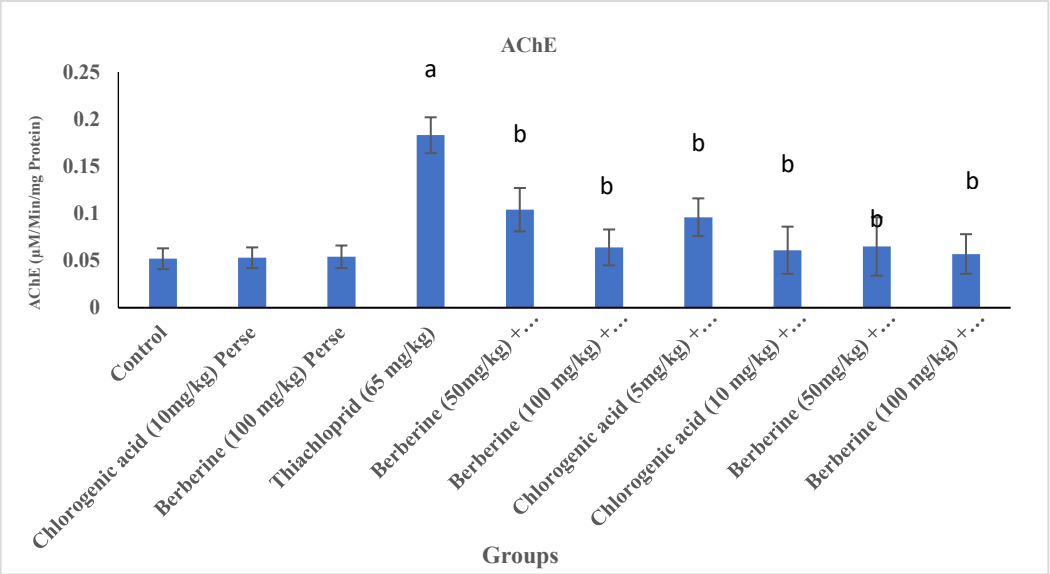


Fig. 3

Results are represented as mean ± SEM (n=6), one-way ANNOVA followed by Tukey's multiple comparison test. AChE (F (9,59) =6.525, p< 0.050, [a]p<0.050 vs control, [b]p<0.050 vs thiacloprid. TPO (Thiacloprid), CGC (Chlorogenic), BER (Berberine).

Effect of Thiacloprid, berberine and chlorogenic acid on GSH

Thiacloprid treated animals had significantly decreased [F (9,59) =2.760, p< 0.050, [a]p<0.050 vs control, [b]p<0.050 vs Thiacloprid]. **Fig 4.** While these effects were reversed with different dose of berberine and chlorogenic acid.

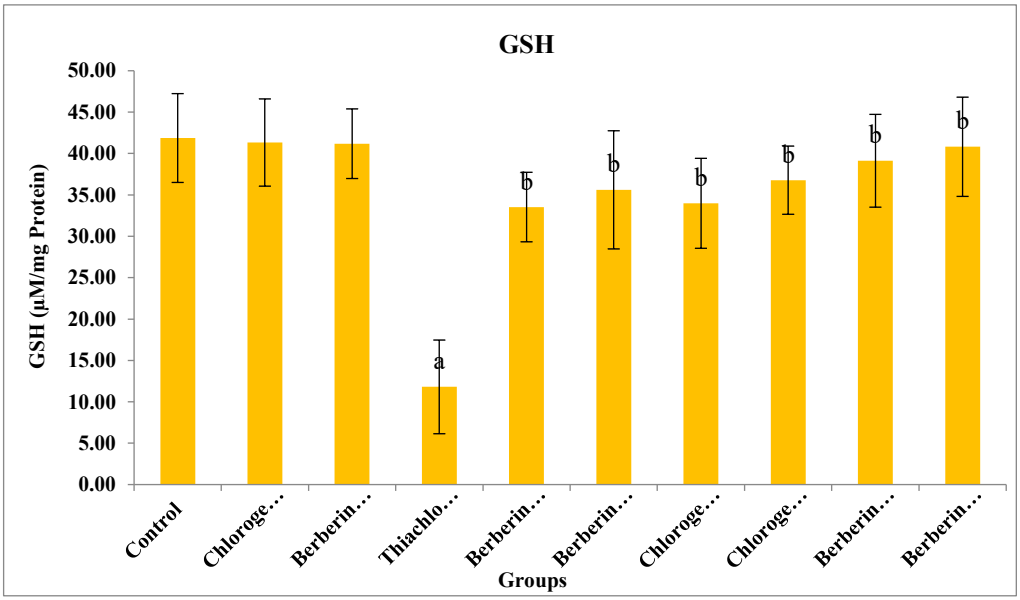


Fig 4

Results are represented as mean ± SEM (n=6), one-way ANNOVA followed by Tukey's multiple comparison test. GSH (F (9,59) =2.760, p< 0.050, [a]p<0.050 vs control, [b]p<0.050 vs thiacloprid. TPO (Thiacloprid), CGC (Chlorogenic), BER (Berberine) GSH (Glutathione)

Effect of Thiacloprid, berberine and chlorogenic acid on MDA

Thiacloprid treated animals had significantly increased (F (9,59) =61.15, p< 0.050, [a]p<0.050 vs control, [b]p<0.050 vs Thiacloprid]. **Fig. 5.** While these effects were reversed with different dose of berberine and chlorogenic acid.

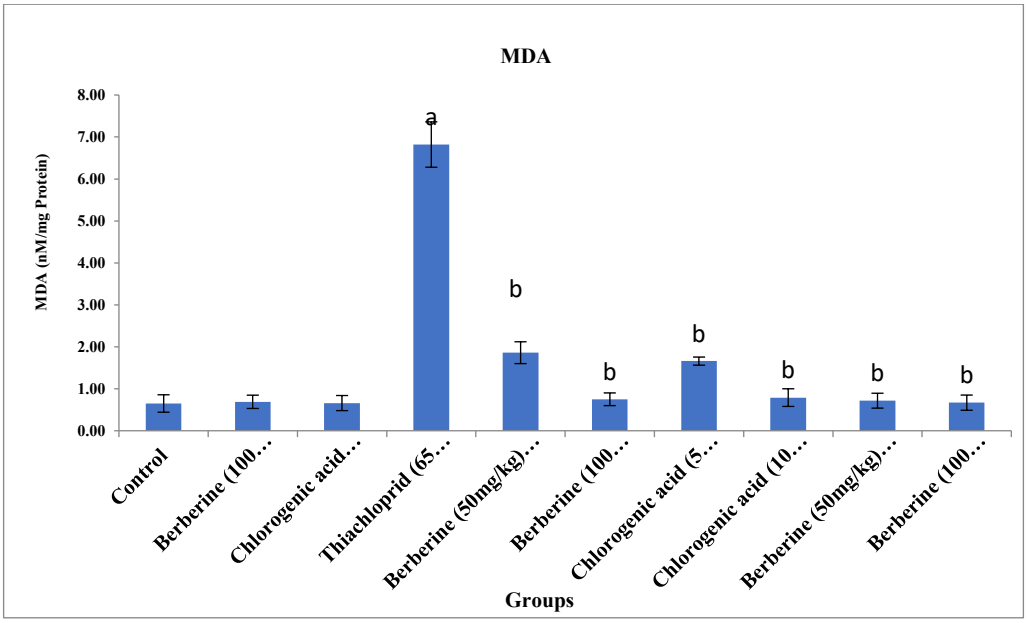


Fig. 5

Results are represented as mean ± SEM (n=6), one-way ANNOVA followed by Tukey's multiple comparison test. MDA, F (9,59) =61.5, p< 0.050, [a]p<0.050 vs control, [b]p<0.050 vs TPO (Thiacloprid), CGC (Chlorogenic), BER (Berberine).

Effect of thiacloprid, berberine and chlorogenic acid on IL-6

Thiacloprid treated animals had significantly increased (F (9,59) =23.76, p< 0.050, [a]p<0.050 vs control, [b]p<0.050 vs thiacloprid).

Fig. 6. Both doses of chlorogenic acid and berberine treatment successfully reversed the observed effects.

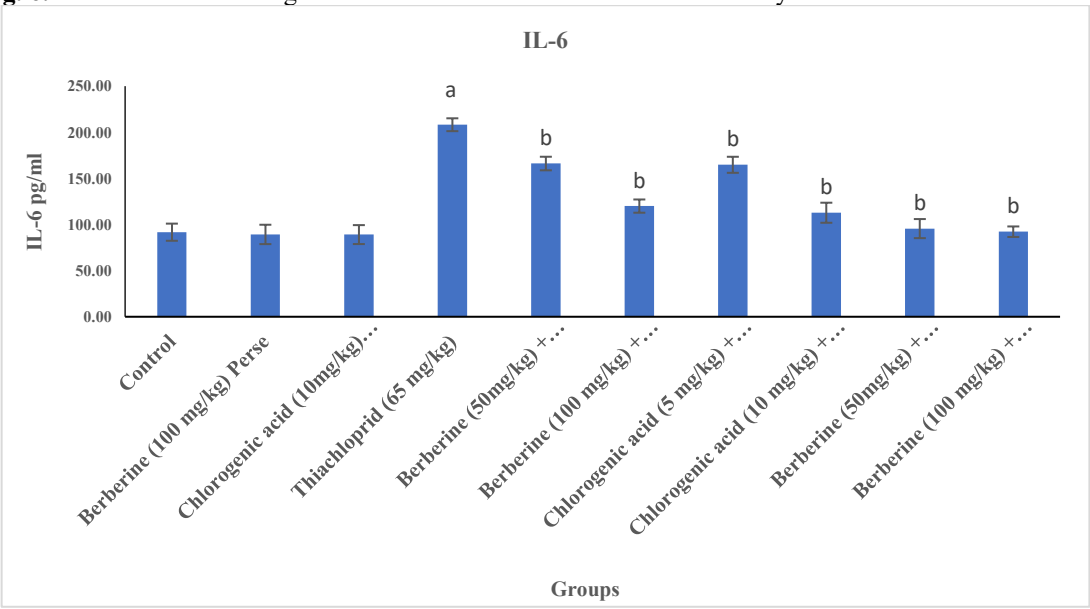


Fig 6

Results are represented as mean ± SEM (n=6), one-way ANNOVA followed by Tukey's multiple comparison test. II-6, (F (9,59) =23.76 p< 0.050, [a]p<0.050 vs control, [b]p<0.050 vs thiacloprid]. TLO (Thiacloprid), CGC (Chlorogenic), BER (Berberine), IL-6 (Interleukin).

Effect of thiacloprid, berberine and chlorogenic acid on Mitochondrial complex I

Mitochondrial complex I-treated animals had significantly decreased (F (9,59) =3.47 p< 0.050, [a]p<0.050 vs control, [b]p<0.050 vs thiacloprid).

Fig. 7. Both doses of chlorogenic acid and berberine administration markedly alleviated the observed effects.

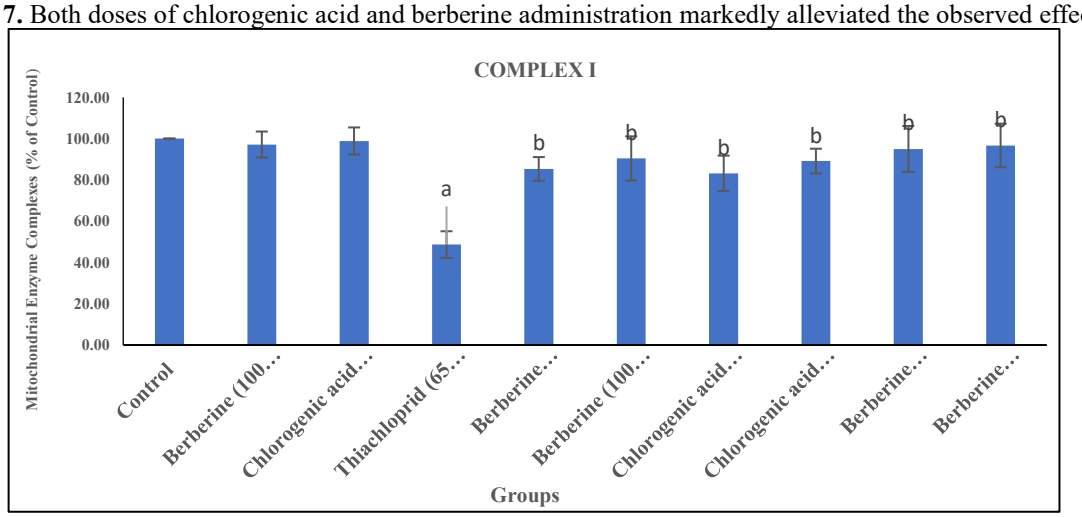


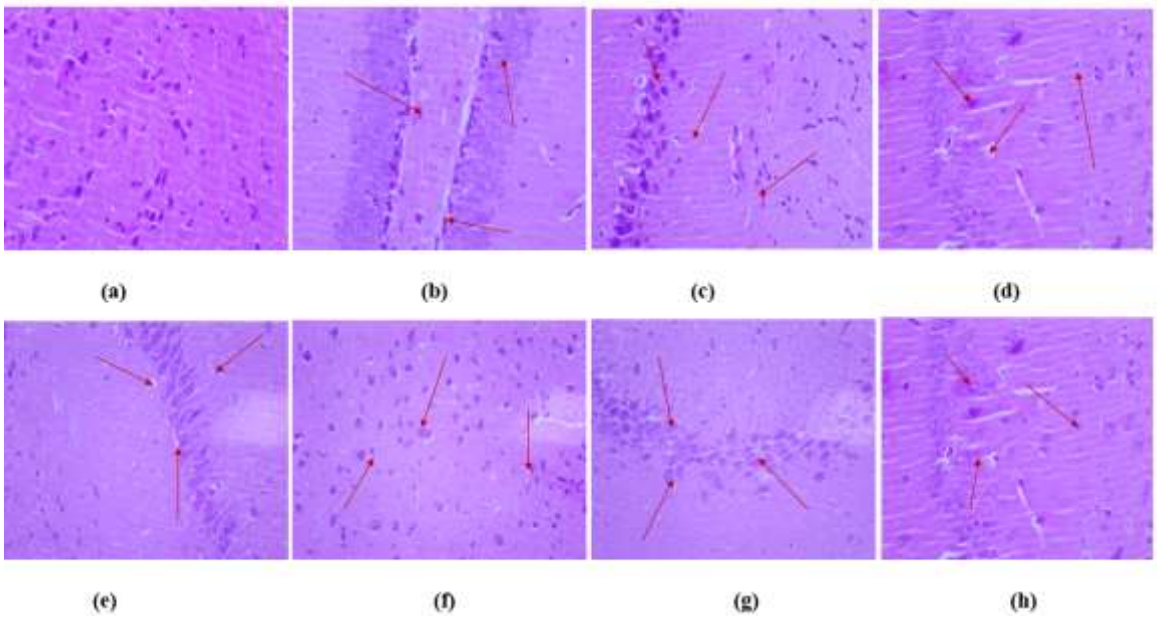
Fig 7

Results are represented as mean ± SEM (n=6), one-way ANNOVA followed by Tukey's multiple comparison test. Mitochondrial complex I, ($F(9,59) = 3.47$ $p < 0.050$, $^{a}p<0.050$ vs control, $^{b}p<0.050$ vs thiacloprid, TLO (Thiacloprid) CGC (Chlorogenic), BER (Berberine).

Histopathology

Effect of Thiacloprid, Berberine, and Chlorogenic Acid on Histopathology

Illustrative pictures of H&E staining (magnification x400) in the cortex area. Ordinary neurons displayed regulatory, round, and bright blue nuclei. However, dead cells exhibited pyknotic nuclei and shrunken cell bodies. Arrows indicate shrunken cells, or pyknosis, as well as the unevenly shaped and tangled cells.



a. Control: There were normal histopathological structures observed. Neurons had complete cell bodies, and nuclei seemed to be well organized; no vacuolization, pyknosis or neurodegeneration observed in the hippocampus region.

b. Thiacloprid group: Nuclear pyknosis and reduced cell bodies were observed in dead cells.

c. Berberine (50 mg/kg) p.o.+ Thiacloprid (65 mg/kg) p.o.: Nuclear pyknosis, moderate shrunken cell bodies lower the number of dead cells in comparison to clothianidin

d. Berberine (100 mg/kg) p.o.+ Thiacloprid (65 mg/kg) p.o.: Nuclear pyknosis mildly shrunken cell bodies lower the number of dead cells in comparison to clothianidin.

e. Chlorogenic acid (5 mg/kg) p.o. + Thiacloprid (65 mg/kg) p.o.: Nuclear pyknosis and slight shrinkage of cell bodies were observed, with a comparatively lower number of dead cells than in the groups treated with clothianidin.

f. Chlorogenic acid (10 mg/kg) p.o. + Thiacloprid (65 mg/kg) p.o.: Mild nuclear pyknosis and slight cellular shrinkage were evident, along with a reduced number of dead cells compared to those observed in the clothianidin groups.

g. Berberine (50 mg/kg) + Chlorogenic acid (5 mg/kg) + Thiacloprid (65 mg/kg) p.o.: In comparison to clothianidin and thiacloprid, the cells showed mild reduction in cell body size, decreased cell death, and less nuclear pyknosis.

h. Berberine (100 mg/kg) + Chlorogenic acid (10mg/kg) + Thiacloprid (65 mg/kg) p.o.: Slightly neurons pyknosis and contracted cell bodies in contrast to treated groups.

Conclusion

It was observed in the present study that there were neurobehavioral, biochemical, inflammatory, mitochondrial, and histopathological changes in experimentally induced rats due to chronic exposure to thiacloprid. The neurotoxic effect was shown by oxidative stress in the form of increased lipoperoxidation, nitric oxide formation, and acetylcholinesterase levels, along with lowered glutathione and catalase activity in the rats subjected to chronic exposure. Thiacloprid exposure. Furthermore, the rats' mitochondria exhibited decreased complex activities, while an increase in IL-6 levels and a decrease in IL-10 levels indicated inflammation. Behavioral changes included memory impairment, anxiety-like behavior, and locomotion deficiency, while histopathology showed neuronal loss, pyknosis, and cellular shrinkage.

Berberine and chlorogenic acid were found to greatly reduce thiacloprid-induced toxicity in terms of increased antioxidant enzyme activity, decreased oxidative stress, and improved mitochondrial integrity. The combined use of berberine and chlorogenic acid appeared to offer greater benefits in neuroprotection as compared to their individual use.

In summary, the current study demonstrates that berberine and chlorogenic acid have strong neuroprotective effects on oxidative damage to the brain caused by thiacloprid. The antioxidant, anti-inflammatory, and mitochondrial protection effects of these compounds may play an important role in the prevention of neurological alterations resulting from pesticide exposure. The implications of these results highlight the importance of bioactive plant compounds in the treatment of neurotoxicity induced by chemicals.

Consent for publication: This manuscript/data has not been published and is not currently under review for publication elsewhere.

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Author contribution: In the present research, AS and RKP performed the research work "Ameliorative Effect of Berberine on Thiacloprid-Induced Hippocampal Oxidative Stress and Cognitive Deficits," which was the most important contribution in making the manuscript.

References

1. Xu X, Wang X, Yang Y, Ares I, Martínez M, Lopez-Torres B, Martínez-Larrañaga MR, Wang X, Anadón A, Martinez MA. Neonicotinoids: mechanisms of systemic toxicity based on oxidative stress-mitochondrial damage. Arch Toxicol. 2022 Jun;96(6):1493-1520. doi: 10.1007/s00204-022-03267-5. Epub 2022 Mar 28. PMID: 35344072.
2. Hao Y, Li J, Yue S, Wang S, Hu S, Li B. Neuroprotective effect and possible mechanisms of berberine in diabetes-related cognitive impairment: a systematic review and meta-analysis of animal studies. Front Pharmacol. 2022;13:917375. doi:10.3389/fphar.2022.917375.
3. Cheng Z, Kang C, Che S, Su J, Sun Q, Ge T, Guo Y, Lv J, Sun Z, Yang W, Li B, Li X and Cui R (2022) Berberine: A Promising Treatment for Neurodegenerative Diseases. Front. Pharmacol. 13:845591. doi: 10.3389/fphar.2022.845591.
4. Houchat JN, Cartereau A, Le Mauff A, Taillebois E, Thany SH. An Overview on the Effect of Neonicotinoid Insecticides on Mammalian Cholinergic Functions through the Activation of Neuronal Nicotinic Acetylcholine Receptors. Int J Environ Res Public Health. 2020 May 6;17(9):3222. doi: 10.3390/ijerph17093222. PMID: 32384754; PMCID: PMC7246883.
5. Cartereau A, Taillebois E, Le Questel JY, Thany SH. Mode of Action of Neonicotinoid Insecticides Imidacloprid and Thiacloprid to the Cockroach Pameα7 Nicotinic Acetylcholine Receptor. Int J Mol Sci. 2021 Sep 13;22(18):9880. doi: 10.3390/ijms22189880. PMID: 34576043; PMCID: PMC8471617.
6. Sahu K, Singh S, Devi B, et al. A review on the neuroprotective effect of berberine against chemotherapy-induced cognitive impairment. Curr Drug Targets. 2022;23(15):1450-1462. doi:10.2174/1389450123666220518121515.
7. Shou JW, Shaw PC. Therapeutic efficacies of berberine against neurological disorders: an update of pharmacological effects and mechanisms. Cells. 2022;11(5):796. doi:10.3390/cells11050796.
8. Tian E, Sharma G, Dai C. Neuroprotective properties of berberine: molecular mechanisms and clinical implications. Antioxidants (Basel). 2023;12(10):1883. doi:10.3390/antiox12101883.
9. Hao Y, Li J, Yue S, Wang S, Hu S, Li B. Neuroprotective effect and possible mechanisms of berberine in diabetes-related cognitive impairment: a systematic review and meta-analysis of animal studies. Front Pharmacol. 2022;13:917375. doi:10.3389/fphar.2022.917375.
10. Cheng Z, Kang C, Che S, Su J, Sun Q, Ge T, Guo Y, Lv J, Sun Z, Yang W, Li B, Li X and Cui R (2022) Berberine: A Promising Treatment for Neurodegenerative Diseases. Front. Pharmacol. 13:845591. doi: 10.3389/fphar.2022.845591.
11. Bernatova, I., et al. "Determination of motor activity and anxiety-related behaviour in rodents: methodology and significance." Interdisciplinary Toxicology, 2012.
12. Schneider. T., & Przewłocki, R. (2005). Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 30(1), 80-89. https://doi.org/10.1038/SJ.NPP.1300518.
13. H Ohkawa et al., (1979)- Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction 1979.
14. Singh, P., Gupta, S & Sharma, B (2015) . Melatonin receptor and KATP channel modulation in experimental vascular dementia. Physiology & behaviour, 142, 66-78 https://doi.org/10.1016/j.PHYSEEH.2015.02.009.
15. Ellman GL, Courtney DK, Andres V, Feathstone RM., (1961)- A new and rapid colorimetric determination of acetylcholinestrase activity. Biochem Pharmacol1961;.

16. Singh, P., Gupta, S & Sharma, B (2015) . Melatonin receptor and KATP channel modulation in experimental vascular dementia. Physiology & behaviour, 142, 66-78 https://doi.org/10.1016/j.PHYSEEH.2015.02.009.
17. Luhach, K., Kulkarni, G. T., Singh, V. P., & Sharma, B. (2021). *Cilostazol attenuated prenatal valproic acid-induced behavioural and biochemical deficits in a rat model of autism spectrum disorder*. Journal of Pharmacy and Pharmacology, 73(11), 1460–1469. DOI: 10.1093/jpp/rgab115.