Original Article

Lycopene Ameliorates Chlorpyrifos-induced Neurotoxicity via Antioxidant and Anti-inflammatory Mechanisms in Rats

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ABSTRACT

Background: Chlorpyrifos (CPF) is a common organophosphate insecticide with toxic effects on the brain, nervous system, and other organs.

Objectives: This study was designed to determine the neuroprotective efficacy of lycopene, a potential carotenoid, on CPF-induced neuronal damage in male rats.

Methods: Eighteen male Wistar rats were randomly divided into three study groups (n=6): control group received only vehicle, CPF-treated group received CPF (6 mg/kg/d), and CPF + lycopene-treated group received CPF followed by lycopene (10 mg/kg/d). After 28 days of treatment, the animals were sacrificed to assess anti-oxidative and anti-inflammatory markers.

Results: CPF intoxication significantly inhibited the acetylcholinesterase (AChE) in brain cells. As a result, excess choline accumulates in neurons, impairing neurochemical homeostasis and cognitive function. Glutathione S-transferase, catalase, peroxidase, and superoxide dismutase activities and reduced glutathione content were significantly decreased in the CPF-treated group due to excess accumulation of reactive oxygen species (ROS) in brain cells. This result is also reflected by the elevated malondialdehyde (MDA) levels and conjugated diene. Thus, cellular damage in the brain ensues. Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), the proinflammatory mediators, increased in brain tissue in the CPF-treated group. After cerebrum and cerebellum histological analysis, partial neuronal necrosis and damage in Purkinje and granular cells were observed. All the above-mentioned parameters of brain tissue were significantly recovered towards the control level in the lycopene-treated group.

Conclusion: We may conclude that lycopene protects rat brain tissue against CPF-induced neurotoxicity by acting as an antioxidant, anti-inflammatory, and anticholinergic agent.

Keywords: Antioxidant activity, Catalase, Acetylcholinesterase (AChE), Tumour necrosis factor-α, Interleukin-6

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Introduction

Pesticides are extensively used in industry, household, and agriculture. As a result, there are major health and environmental risks worldwide (Rani et al., 2021). Organophosphate pesticides (OPs) are a group of synthetic chemicals that commonly cause toxicity to humans and farm animals (Maroni et al., 2000; Sidhu et al., 2019). Chlorpyrifos (CPF), a lipophilic, broad-spectrum organophosphate insecticide (Shou et al., 2019; ur Rahman et al., 2021), causes hepatotoxicity, renal toxicity, genotoxicity, teratogenicity, neurotoxicity, and changes in neurobehavioral patterns (Mazuryk et al., 2024; Ncibi et al., 2008). Recent research has focused on the cognitive and psychiatric problems that occur in occupational workers who are exposed to OPs on a long-term basis (Kaur et al., 2019). The airways frequently absorb pesticides due to occupational and residential exposure, and one of their main targets is neurons. The OPs generally produce excessive reactive oxygen species (ROS) that are significantly associated with organ toxicity (Badr, 2020; Ogut et al., 2011), leading to various neurological impairments, including motor and cognitive deficits (Maroni et al., 2000).

Numerous research studies on human exposure to OPs, whether acute or chronic, have indicated the involvement of the cholinergic system. Acetylcholinesterase (AChE) inhibition is the primary cause of different cholinergic transmission-related issues (Marucci et al., 2021). CPF can pass through the blood-brain barrier (Ogut et al., 2011) and causes neuro-inflammation, apoptosis, and molecular changes in the nervous tissue (Lee et al., 2014). As a result, it alters the neuropsychological performance (Kuelz et al., 2004). The cognitive function of the brain and other associated processes like visuo-perceptual ability, visual attention, processing speed, problem-solving memory impairment, etc, are altered by CPF (Basaure et al., 2019).

Lycopene, a naturally occurring carotenoid in tomatoes and tomato-based products has promising antioxidant potential (Kong et al., 2010). Lycopene can scavenge singlet oxygen and free radicals (Kelkel et al., 2011). Thus, it mitigates oxidative stress by lowering the lipid peroxidation end product and elevating antioxidant enzymes. This study was designed to search out the potential anti-inflammatory and anticholinergic activities, including the antioxidant properties of lycopene against CPF-induced neurotoxicity in male rats.

Materials and Methods

Chemical constituents

Lycopene was purchased from Tokyo Chemicals Industry Co. Ltd. (TCI) (Zwijndecht, Belgium), while CPF was acquired from Sigma-Aldrich Co. (St. Louis, USA). Dimethyl sulfoxide (DMSO) was also purchased from Sigma-Aldrich Co. (St. Louis, USA). Tween-20 was supplied by Beijing Solarbio Science & Technology Co. (Beijing, China). Interleukin-6 (IL-6) was purchased from Thermo Fisher Scientific (Waltham, MA USA, catalog number - ERIL1B), and tumor necrosis factor-alpha (TNF-α) was bought from R&D Systems (Minneapolis, MN, catalog number - RTA00). Alanine aminotransferase (ALT) (catalog number - 1102210075) and aspartate aminotransferase (AST) (catalog number - 1102200075) were purchased from Coral Chemicals Pvt Ltd. (New Delhi, Delhi, India).

Animal treatment schedule

The Institutional Animal Ethical Committee approved the selection of eighteen male albino rats of the Wistar breed for this study. All methods adhered to the established norms for animal care according to the National Institutes of Health (1985). Before the experiment, the animals were kept in polypropylene cages for 15 days at 25±2 ˚C, with a 12-hour light and 12-hour dark cycle. Eighteen rats were randomly assigned into three groups, with six in each group.

Animals in the control group were administered 1.0 mL of redistilled water per kilogram of body weight daily.

Rats in the CPF-treated group were given CPF at a 6 mg/kg/d dose.

Animals under the CPF + Lycopene group were treated with CPF (6 mg/kg/d), followed by lycopene 10 mg/kg/d.

Rats were sacrificed by euthanasia at the end of the 28-day treatment period using an overdose of carbon dioxide. Blood was collected, and the separated serum was stored at -20 ˚C for subsequent analysis. Brain tissue, like the cerebrum and cerebellum, was weighed and documented. The tissue was stored at -20 ˚C for enzymatic analysis, while a portion of the left brain of each animal was preserved in Bouin's fluid for histological examination.

Determination of the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in brain tissue

The SOD activity was determined by the standard method (Kakkar et al., 1984). The brain tissue was homogenized and centrifuged. After adding 50 mmol of Tris-HCl (pH=8.4) and 10 mmol of pyrogallol to the supernatant, the absorbance at 420 nm was measured in a spectrophotometer with Tris-HCl as a blank.

The CAT activity in brain tissue was assessed using a standard biochemical method (Sinha, 1972). The tissue was centrifuged after homogenizing the tissue in 0.5 M Tris-HCl buffer solution (pH=7.0). The catalase activity of the obtained supernatant was measured at 240 nm.

A standard approach was used to measure the POD activity (Chance & Maehly, 1955). To obtain the supernatant, the brain tissue was homogenized and centrifuged. The enzyme activity was measured at 420 nm using the supernatant.

Measurement of AChE activity:

The AChE activity was determined following the method of Ellman et al. (1961). Thiocholine reacted with 5,5-dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent) to form a yellow product (5-mercapto-2-nitrobenzoic acid and its dissociated forms), which was subsequently measured at 412 nm (Ellman et al., 1961).

Activity of reduced glutathione (GSH)

The reduced GSH content was measured using Ellman's (1959) method. Brain tissues were combined with 25% TCA in this technique and centrifuged for 15 minutes at 2000× g. The resultant supernatant was diluted using sodium phosphate buffer (pH=8.0, 0.2 M). The yellow-colored complex resulting from the reaction between GSH and DTNB was assessed for optical density at 412 nm after freshly prepared 10 mM DTNB in 0.2 M phosphate buffer (pH 8.0) was added to the diluted solution (Ellman, 1959).

Activity of glutathione s-transferase (GST)

The approach was used to measure the activity of GST. 2,4-dinitrochlorobenzene (CDNB) and sodium phosphate buffer were used to make up the reaction mixture combined with the sample. GSH was added to the reaction mixture to start the reaction, and for 3 minutes, OD measurements were taken at 340 nm. The enzyme activity was measured in micromoles of GSH-CDNB conjugate formed per minute per milligram of protein (Habig et al., 1974).

Native gel electrophoresis study

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done at 40 mA after loading 50 µg of proteins from the cerebrum and cerebellum onto two independent 8% gels.

After electrophoresis, the first gel was treated with a 0.003% H_2O_2 solution to measure the CAT gel activity. The samples were washed with distilled water (d/w) and stained using 2% ferric chloride and 2% potassium ferricyanide solutions. The gel was once more cleaned with water after the stain was emptied until achromatic bands started to show (Weydert & Cullen, 2010).

Another gel was treated with 1% (v/v) Triton X-100 two times for 15 min each with gentle shaking. The reactive solution was discarded, and the gel was stained with 20 mL of POD staining solution containing 10 µL of 25% (v/v) guaiacol dissolved in 1.5 mL ethanol, 50 mM phosphate buffer at pH 7.0, and 1 mL of 0.3% (v/v) H_2O_2. A photograph of POD bands was captured before it faded out (Castro et al., 2017).

For the electrozymographic investigation of SOD, brain tissue proteins weighing 50 µg were loaded onto a 12% native gel and electrophoresed at 40 mA. After electrophoresis, the gel was left in the light for 15 minutes before being stained with SOD native gel stain. The presence of achromatic bands is suggestive of SOD (Weydert & Cullen, 2010).

Conjugated diene and malondialdehyde (MDA) level in brain tissue:

Tris-HCl buffer solution (0.1 M, pH 7.4) was used to homogenize the neural tissue. Chloroform and methanol (2:1) were combined to remove the lipid layer, which was then centrifuged at 1000 ×g for 5 minutes and allowed to evaporate. A UV spectrophotometer set at 233 nm was used to measure absorbance after the lipid residue had been dissolved in cyclohexane (Lawal et al., 2022).

The neuronal tissue was homogenized using a cold Tris-HCl buffer solution (0.5 M, pH=7.0). The homogenates were treated with a thiobarbituric acid–trichloroacetic acid (TBA–TCA) mixture, boiled for 10 minutes at 100 ˚C, and then allowed to cool to room temperature. After centrifugation at 4000× g for 10 minutes, the supernatant was collected, and the optical density was measured at 535 nm (Saad et al., 2017).

Determination of proinflammatory markers, IL-6, and TNF-α

Proinflammatory cytokines (IL-6) and TNF-α were measured using enzyme-linked immune sorbent assay (ELISA) kits. Their levels were determined according to the manufacturer's instructions (Drafta et al., 2021).

Estimation of serum ALT and AST

ALT and AST activities, which are toxicity biomarkers, were measured according to the instructions provided with specific kits (Ikemoto et al., 2001).

Histology of brain tissue

Paraffin blocks of the cerebrum and cerebellum were sectioned at 5 µm thickness and stained with hematoxylin and eosin. The stained slices were examined under 100x magnification using a trinocular microscope, and a particular field was captured on camera (Miller et al., 2002).

Statistical analysis

The data were expressed as mean with a 95% confidence interval (CI). The means of the various groups were compared using a one-way analysis of variance (ANOVA) post-hoc Tuckey test (Brown, 2005). The mean value of each column was compared with the mean of every other column at significance levels of $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, and $P \leq 0.0001$, which was carried out with GraphPad Prism software, version 10.4.0 on a Microsoft Windows.

Results

Body weight and brain tissue weight

The weight of the cerebrum and cerebellum and the body weight of each rat were measured. Still, no significant alteration was observed among the control, CPF-treated, and lycopene-treated groups (Table 1).

Activities of SOD, CAT, POD, and expression pattern of proteins in brain tissue

The activities of CAT, SOD, POD, and the protein expression patterns were assessed through electrozymographic analysis in the CPF-treated group. All these parameters were significantly reduced. After treating lycopene at a dose of 10 mg/kg/d for 28 days, these parameters were recovered considerably in the control group (Figure 1).

The activity of AChE level in brain tissue

AChE activity in brain tissue significantly decreased in the CPF-treated group compared to the control group. In contrast, a significant increase in the activity of this parameter was observed after treatment with lycopene at a dose of 10 mg/kg/d for 28 days (Figure 2).

The activity of GSH level and GST in brain tissue

GSH levels and GST activities in brain tissue were significantly reduced in the CPF-treated group. Following lycopene treatment, these two parameters showed a significant recovery in the control group compared to the CPF-treated group (Figure 3).

Conjugated dienes (CD) and MDA levels in brain tissue

Levels of CD and MDA in brain tissue were significantly increased in the CPF-treated group. However, these parameters showed significant recovery towards control levels after treatment with lycopene at a dose of 10 mg/kg body weight for 28 days (Figure 4).

ALT and AST activities in the serum

Serum ALT and AST enzyme activities were significantly elevated in the CPF-treated group. These parameters were recovered considerably towards the control level after treating lycopene at 10 mg/kg/d for 28 days (Figure 5).

Determination of proinflammatory markers

IL-6 and TNF-α levels in serum were significantly increased in the CPF-treated group. However, these parameters were recovered considerably to the control levels following 28 days of lycopene treatment (Figure 6).

Histological observation

CPF-treated rats displayed partial neuronal necrosis in the cerebral tissue and degeneration of cells in the cerebellar tissue compared to the control group. Conversely, the lycopene-treated group significantly improved cerebral and cerebellar tissues (Figure 7).

Table 1. Body weight and brain tissue weight in the control, CPF-treated, and lycopene-treated groups

Group	Mean±SE			
	Body Weight (g)		Brain Tissue Weight (g)	
	Initial	Final	Cerebrum	Cerebellum
Control	123.5±1.38	157.83±1.88	1.1566±0.47	0.3466±0.008
CPF-treated	123.66±1.97	154.5±1.97^{ns}	1.1533±0.47^{ns}	0.3483±0.01^{ns}
Lycopene-treated	122.66±1.49	156±1.82^{ns}	1.1583±0.47^{ns}	0.3566±0.02^{ns}

Note: The data were expressed as Mean±SE, with a 95% CI. The means of the various groups were compared using ANOVA post-hoc Tuckey test. The significance levels of data are $^{*}P\leq0.05$, $^{**}P\leq0.01$, $^{***}P\leq0.001$, $^{****}P\leq0.0001$ compared with the control, CPF-treated group, and lycopene-treated group to each other, respectively. There is no significant change between the three groups.

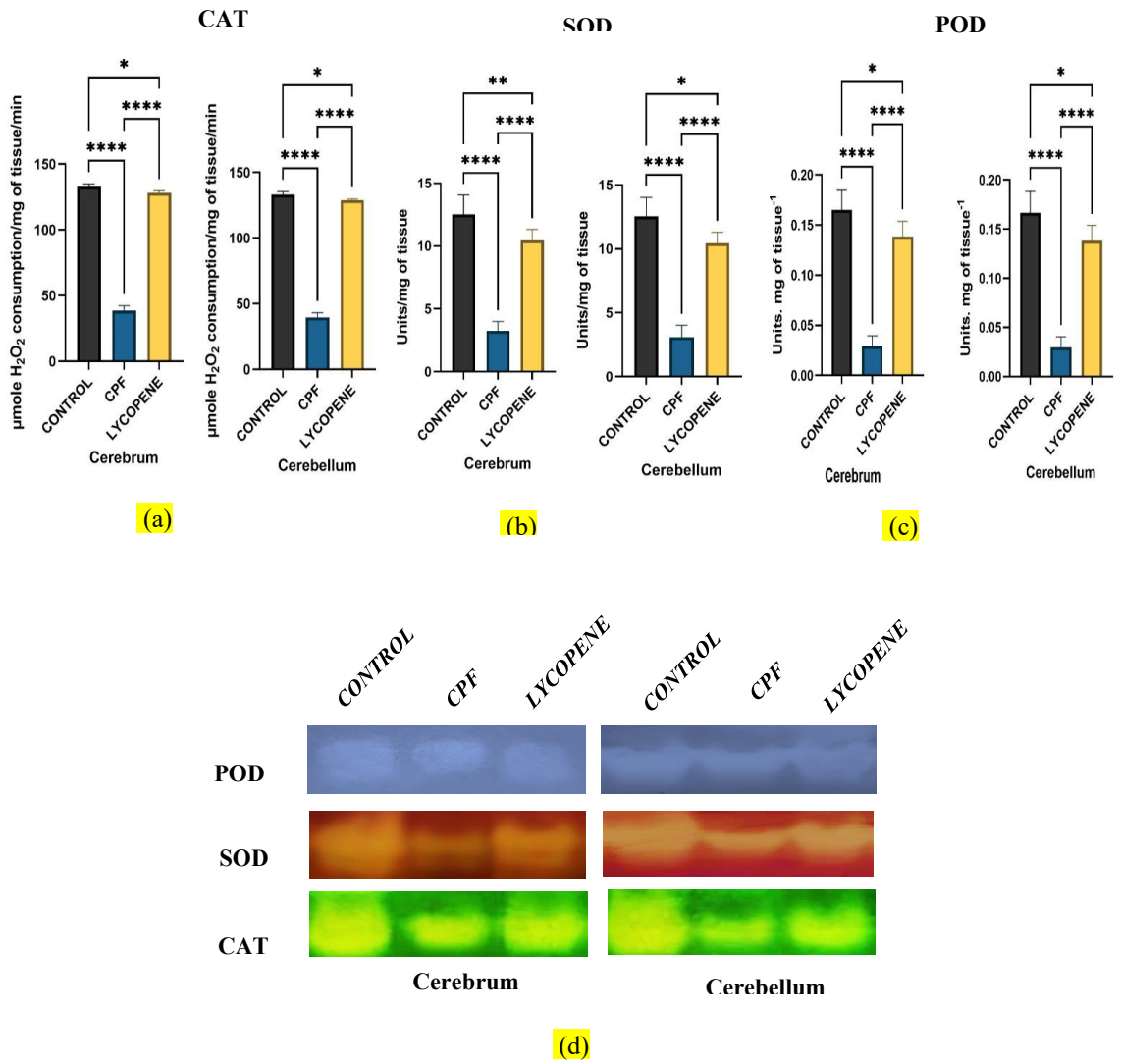

Figure 1. Effect of lycopene on CAT, SOD, and POD in the cerebrum and cerebellum in CPF-treated rats

Note: Data are represented as mean with a 95% CI, n=6. Significance levels of data were $^{*}P\leq0.05$, $^{**}P\leq0.01$, $^{***}P\leq0.001$, $^{****}P\leq0.0001$ compared with the control and treated groups to each other—changes of CAT, SOD and POD expression on the native gel in control, CPF, and lycopene groups.

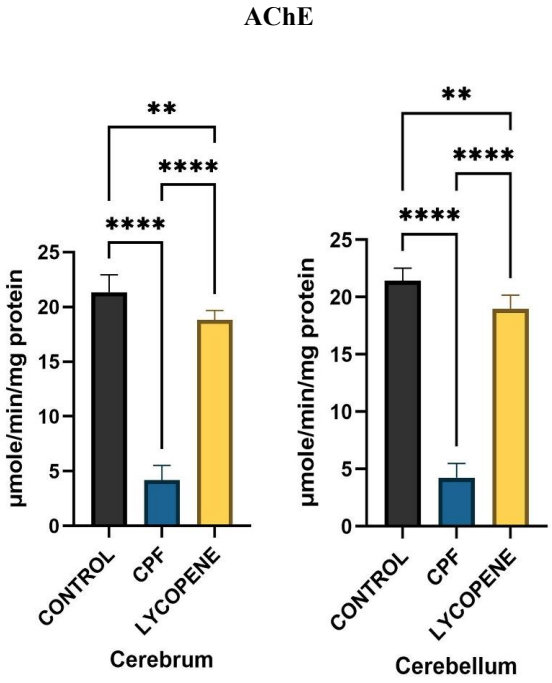

Figure 2. Effect of lycopene on AChE in cortical tissues in the cerebrum and cerebellum in CPF-treated rats

Note: Data are represented as mean with a 95% CI, n=6. Significance levels of data were *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001 compared with the control and treated groups to each other.

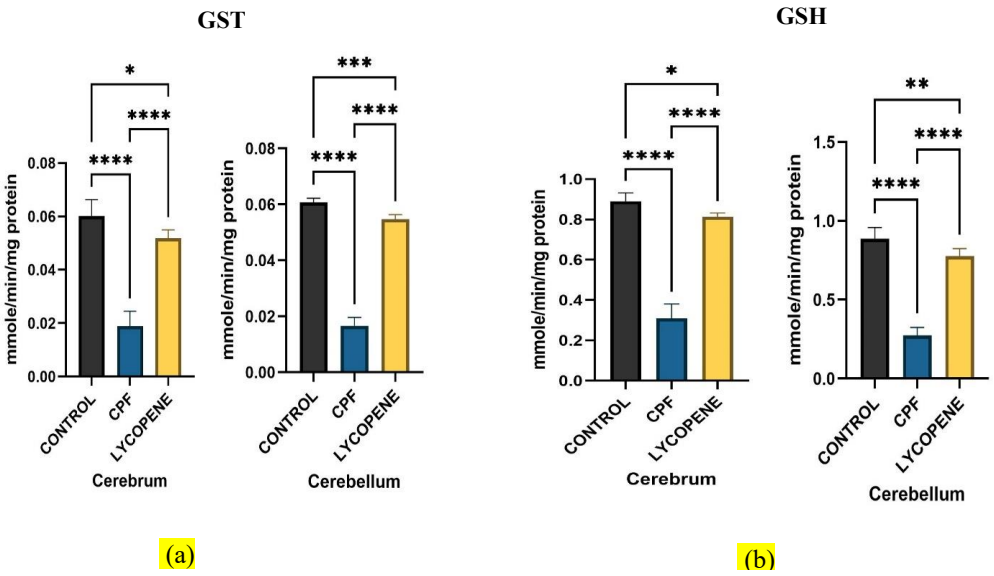

Figure 3. Effect of lycopene on GST and reduced GSH in the cerebrum and cerebellum in CPF- treated rats

Note: Data are represented as mean with a 95% CI, n=6. Significance levels of data are *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001 compared with the control and treated groups to each other.

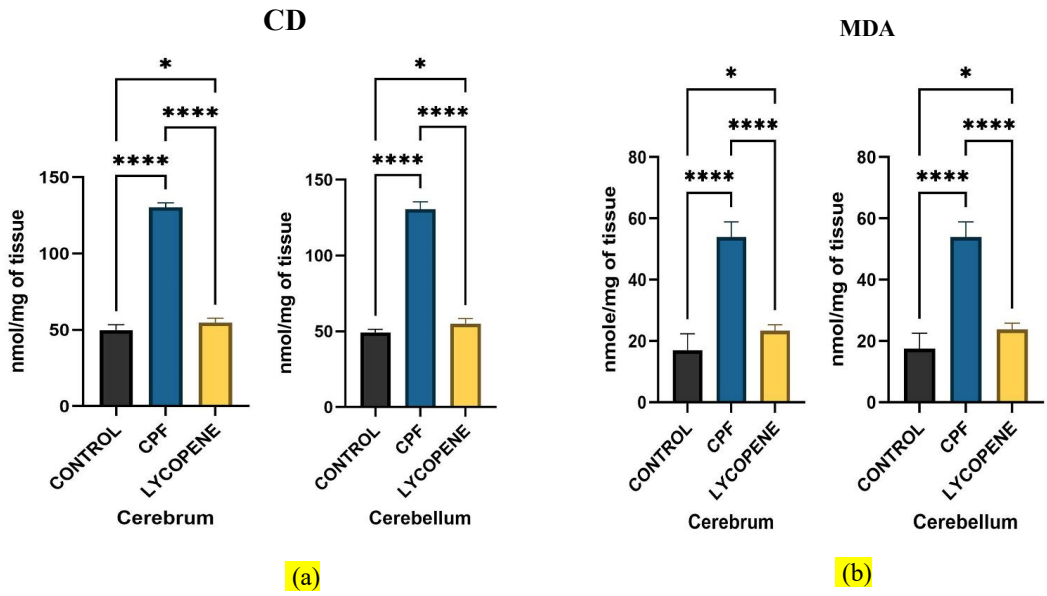

Figure 4. Effect of lycopene on CD and MDA in the cerebrum and cerebellum in the CPF-treated rats

Note: Data are represented as mean with a 95% CI, n=6. Significance levels of data are *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001 compared with the control and treated groups to each other.

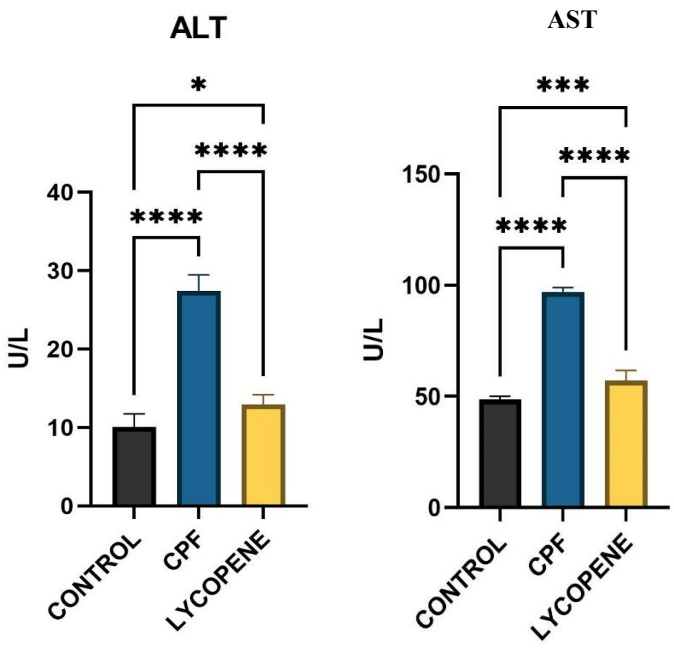

Figure 5. Effect of Lycopene on ALT and AST in serum in CPF-treated rats

Note: Data are represented as mean with a 95% CI, n=6. Significance levels of data are *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001 compared with the control and treated groups to each other.

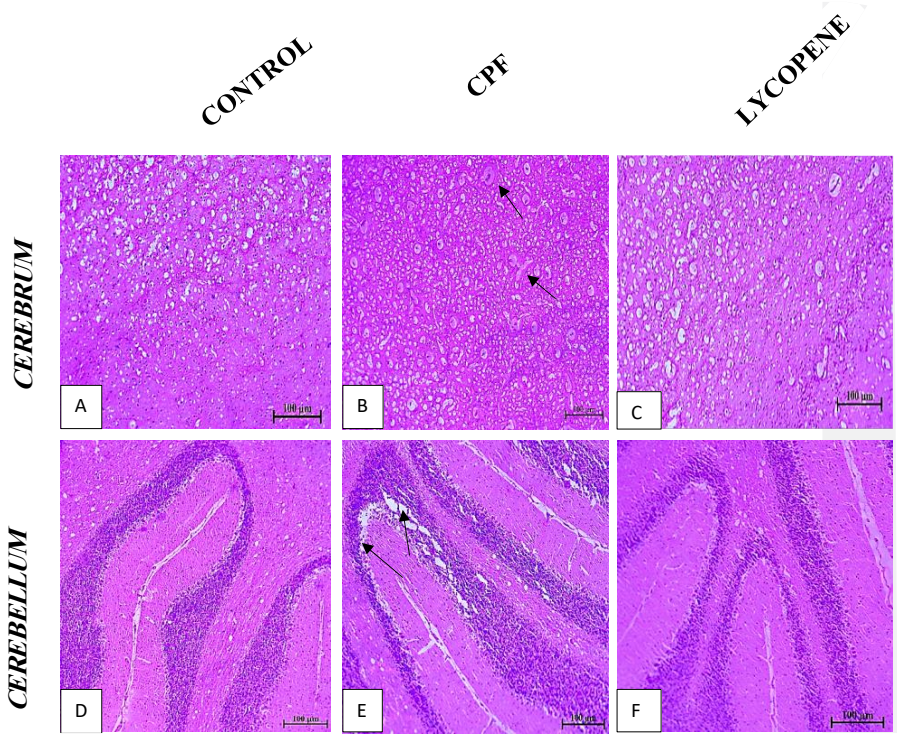

Figure 6. Effect of lycopene on proinflammatory marker IL-6 and TNF-α) cortical tissues in CPF-treated rats

Note: Data are represented as mean with a 95% CI, n=6. Significance levels of data are *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001 compared with the control and treated groups to each other.

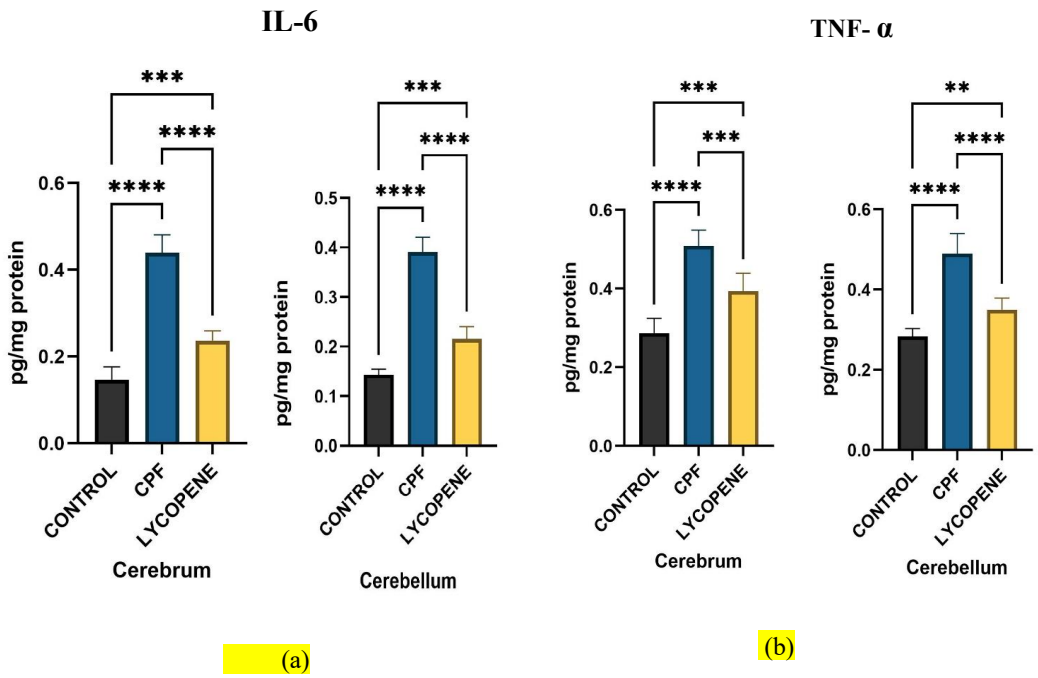

Figure 7. Histological findings (100 X) of cerebrum and cerebellum tissues by Haematoxylin-Eosin staining: (bar=100 µm)

Note: Control (A, D): The control group shows the normal structure in the cerebrum and cerebellum. CPF-treated (B, E): arrows indicate partial neuronal necrosis and cell degeneration in the cerebrum and cerebellum. Lycopene (C, F): The lycopene-treated group showed recovery of both the cerebrum and cerebellum.

Discussion

Around 3000000 people suffer from pesticide poisoning each year, and approximately 250000 people die as a result of the harmful consequences of pesticides (Yang & Deng, 2007). Chlorpyriphos, a broad-spectrum insecticide (Fu et al., 2022), causes neuronal damage (Elmorsy et al., 2022). In this experiment, the potential role of lycopene was evaluated to mitigate CPF-induced neurotoxicity.

The antioxidant enzyme CAT, SOD, POD, GST activities, and GSH content in brain tissue were significantly reduced in the CPF-treated group. Earlier researchers supported this result (Saulsbury et al., 2009; Albasher et al., 2020). Superoxide, the most widely recognized free radical generated by the mitochondrial electron transport chain, is produced when oxygen levels increase to 2.5% and 5%. During oxidative phosphorylation, it is frequently generated (Kerksick & Willoughby, 2005). This level is highly elevated due to pesticide exposure and xenobiotics (Hernandez et al., 2013). The SOD is the first preventive antioxidant enzyme that neutralizes the singlet oxygen and dismutate superoxide to hydrogen peroxide (Islam et al., 2022). CAT decomposes H_2O_2, thus preventing lipid peroxidation. POD and GSH together also catalyze hydrogen peroxide and lipid peroxidation. So, the depletion of antioxidant enzymes in nerve cells causes the accumulation of hydrogen peroxide and the depletion of the antioxidant defense system. This result is also reflected by the elevation of CD and MDA levels in the cerebrum and cerebellum in the CPF-treated group concerning control (Almeer & Abdel, 2018). The free radical scavenging properties of lycopene (Heymann et al., 2015) may help to restore the levels of SOD, CAT, POD, and GST (Vicidomini et al., 2024).

Acetylcholine esterase significantly decreased in the CPF-treated group (Xing et al., 2010). Brain AChE is inhibited at the synapses, leading to an accumulation of excess acetylcholine (Greenfield, 1991). The overactivation of acetylcholine receptors in the autonomic and central nervous systems causes neuronal damage, especially in the cerebrum and cerebellum, due to excitotoxicity (Cardona et al., 2013). Lycopene treatment reduces oxidative stress and potentially protects the AChE inhibitory activity and neuronal damage (Kaur et al., 2011).

In the current investigation, the cerebrum and cerebellum of the CPF-treated group showed higher levels of proinflammatory cytokines, IL-6, and TNF-α. Chlorpyriphos activates acute-phase inflammatory responses, producing cytokines like TNF-α and IL-6, which generate ROS through the mitochondrial respiratory chain (Duramad et al., 2007). ROS generation causes damage to proteins, lipids, and DNA, and these mediators are also implicated in several cellular and biological reactions. Tumor progression, the synthesis of transcription factors, growth factors, and pro-apoptotic protein activation (Abdel Moneim, 2016). Furthermore, it has been shown that astrocyte impairment is linked to elevated secretions of TNF-α and IL-6, which are associated with the development of brain inflammation (Wang et al., 2015).

Histoarchitectural analysis revealed that the CPF-treated group has prominent degeneration in brain cells, both cerebrum, and cerebellum, especially Purkinje cells, granular cells, and other neurons, but after lycopene treatment, the neuronal damage was recovered towards control (Wittekind, 2003; Mesallam et al., 2016).

Body weight and brain tissue weight were not altered. Still, elevated serum ALT and AST activities in the CPF-treated group may be due to CPF-induced metabolic and neurotoxicity (Akpa et al., 2021). These parameters were recovered significantly after treating lycopene through its antioxidant, anti-inflammatory, and anticholinergic activity.

From this study, we may conclude that lycopene potentially mitigates CPF-induced neurotoxicity through its antioxidant and anticholinergic properties without any side effects.

Ethical Considerations

Compliance with ethical guidelines

The Institutional Animal Ethical Committee approved this study (Reg No.: 5/8/VU/IAEC, Dt 11.4.2023). The NIH (1985) animal care guidelines were adhered to during the entire experiment. The rats were supplied by a CCSEA (Committee for Control and Supervision of Experiments on Animals)-authorized dealer.

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Authors' contributions

Conceptualization, study design, supervision, and writing: Chhanda Mallick Mukherjee; Animal treatment, data collection, and performing histology: Parag Ranjita Bera; Data analysis: Sudipta Jana and Prabir Mondal.

Conflict of interest

The authors declared no conflict of interest.

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