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ORIGINAL ARTICLE

Curcuma longa impact on behavioral, brain oxidative stress, and systemic inflammation in rats exposed to inhaled paraquat

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Abstract

Introduction *Curcuma longa* (Cl) shows potential effects on oxidative stress and inflammation in various organs. This research sought to explore how Cl affects learning and memory deficits caused by inhaled paraquat (PQ), along with oxidative stress in the brain, as well as systemic inflammation in rats.

Methods Rats inhaled either saline (Ctrl) or PQ aerosols during the experiment. The PQ groups were administered saline (PQ group), 0.03 mg/kg/day dexamethasone (Dexa), 150 and 600 mg/kg/day Cl (Cl-L and Cl-H), 5 mg/kg/day pioglitazone (Pio), and Cl-L + Pio for 16 days during the PQ exposure period. The evaluation of learning and memory abilities was conducted by implementing the Morris water maze (MWM) and passive avoidance tests.

Results and discussion Total and differential WBC numbers were increased in the PQ group, along with malondialdehyde (MDA) levels in the serum and brain despite the levels of thiol, catalase (CAT), and superoxide dismutase (SOD) which were lower compared to the control group ($p < 0.001$). The escape latency and traveled distance were increased in the PQ group. However, the time spent in the target quadrant in the MWM test and duration of time latency in the dark room were reduced after receiving an electrical shock ($p < 0.05$ to $p < 0.001$). In all treated groups, measured values were improved than the PQ group ($p < 0.05$ to $p < 0.001$). The combination of Cl-L + Pio showed more pronounced effects compared to either treatment alone ($p < 0.05$ to $p < 0.001$).

Conclusion Cl, Pio and their combination improved PQ-induced memory dysfunction, brain oxidative stress and systemic inflammation in rats, and PPARγ receptor contribution on the effects of Cl was suggested.

Keywords *Curcuma longa* · Pioglitazone · Paraquat · Memory · Oxidative stress · Inflammation

Introduction

In lower and middle-income territories worldwide, approximately 130 countries, paraquat (PQ), a potent herbicide and pesticide, is used [1, 2]. This herbicide exerts control over various crops, including but not limited to grains, vegetables, fruits, and various other types [3, 4]. Despite the large-scale utilization, PQ poisoning showed a mortality rate between 60 and 90% as a highly toxic herbicide [1, 5]. PQ is one of the momentous causes of fatal poisoning in many Asian and African regions [1]. Depending on the different quantities of PQ ingested, the manifestation of toxic effects follows a particular sequence, even the most minute quantity of PQ (30 mg/kg) proves fatal for an adult individual [1, 6].

Inhalation and dermal transmission are two common ways of exposure to PQ [7] and the unclear functional mechanism of PQ toxicity adds to its complexity [8], but it is supposed that the producing of superoxide anion may be the reason of inflammation and oxidative stress caused by PQ toxicity and its involvement in the redox cycle of oxygen [5, 9]. Through generation O2, PQ interacts with mitochondria and produces reactive oxygen species (ROS),

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leading to apoptosis of tissue's cells, secondary inflammation and peroxidation of lipids followed by organs failure [4, 5, 10, 11]. The main causes of death in PQ poisoning are related to lung and kidney injuries [1, 11]. In addition to renal and pulmonary dysfunctions, various other organs and systems are also influenced, including the hepatobiliary system, cardiovascular system, and nervous system [1]. It is reported that there have been an association between neurodegenerative diseases with mitochondrial dysfunction that appears in some diseases such as Alzheimer and Huntington disease due to the dysfunction of brain cortex mitochondria affecting by superoxide productions and destroying dopaminergic neurons associated with Parkinson disease (PD). Accordingly, inhibiting and reducing the effects of PQ on human health is so important. However, no special antidote or therapeutic approach has been defined for PQ poisoning complaints, but the present treatments include activated charcoal, immunosuppressive and anti-inflammatory drugs as well as antioxidants such as acetylcysteine and salicylate which act as radical scavengers, although, these drugs are not so effective [1, 3]

Curcuma longa (Cl) or Turmeric is a common spice, especially used in Iran, Malaysia, India, China, Polynesia, and Thailand [12]. The Cl main active component is curcumin, which is a proved antioxidant, anti-inflammatory combination, anti-cancer and neuroprotective [9, 13, 14]. It seems curcumin as an activator of apoptosis by inhibiting ROS regarding antioxidant features [15].

The scientific community is greatly intrigued by the neuroprotective and inflammation-reducing properties of Peroxisome proliferator-activated receptor γ (PPAR-γ) agonists, as they effectively hinder the progression of neurodegenerative processes [16]. Neurons were found to be more susceptible to ischemic brain damage when lacking in PPARγ. In the treatment of type 2 diabetes, pioglitazone (Pio), as an agonist of PPAR-γ, is effective [16]. Pio exhibits anti-inflammatory effects by reducing the production of cytokines and oxidative stress in models of neurodegenerative disease [17]. PPARγ is known to be expressed constitutively in the brain, and its gene expression in neurons is up regulated in response to cerebral ischemia. In addition, Pio further enhances the expression of PPARγ in peri-focal cortical areas [18, 19].

The interaction of Pio in the brain with various pathophysiological events occurring in ischemic brain tissue has been highlighted in previous studies. After experiencing an ischemic stroke, Pio decreased inflammatory responses in the initial phases [20, 21]. Despite the scarcity of studies focusing on the impact of brain PPARγ activation on inflammatory responses in ischemic brain tissue, there remains an unanswered question regarding the exact contribution of cerebral PPARγ to the neuroprotective effects of Pio. Thus, this research sought to analyze memory impairment in rats post-PQ exposure, along with the beneficial effects of Cl and Pio on cognitive ability and neuroinflammation. New therapeutic methods for treating and preventing cerebral disorders from PQ exposure could be influenced by these findings.

Methods

Study groups

Male Wistar rats, weighing 200 ± 20 g, were utilized under controlled conditions (22 ± 2 °C temperature, 12-h light/dark cycle, unrestricted access to food and tap water, humidity maintained at 54 ± 2%) as per the guidelines of the Animal House at the School of Medicine, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. Confirmation from MUMS Ethics Committee, using authorization Code: 992,197, the experiment was authorized and that animal rights were adhered to following NIH directives in the US. Only male rats in this experiment to avoid hormonal influence among animals.

In each of the seven groups, ten rats were selected at random (Table 1, Fig. 1). Previous studies provided a detailed explanation of how rats were exposed to saline and PQ [9]. The process was repeated 8 times over a period of 16 days, on days 1, 3, 5, 7, 9, 11, 13, and 15. Each repetition lasted for 30 min. These animals were then examined to assess

Table 1 Different groups, their exposing to saline or paraquat and treatment

No	Group	Abbreviation	Exposure	Treatment
1	Control	Ctrl	Salin aerosol	–
2	Paraquat	PQ	Paraquat aerosol 54 mg/m^3	–
3	*C. longa*	Cl-L	Gavage	150 mg/kg/day
4		CL-H	Gavage	600 mg/kg/day
5	Pioglitazone	Pio-L	i.p	5 mg/kg/day
6	Combined treatment	Pio + CL-L	i.p. and Gavage	5 + 150 mg/kg/day
7	Dexamethasone	Dexa	Gavage	0.03 mg/kg/day

In control group the rats were exposed to saline and in other groups to PQ aerosols. Treated groups were treated with mentioned for 16 days during the exposure period [22, 56]

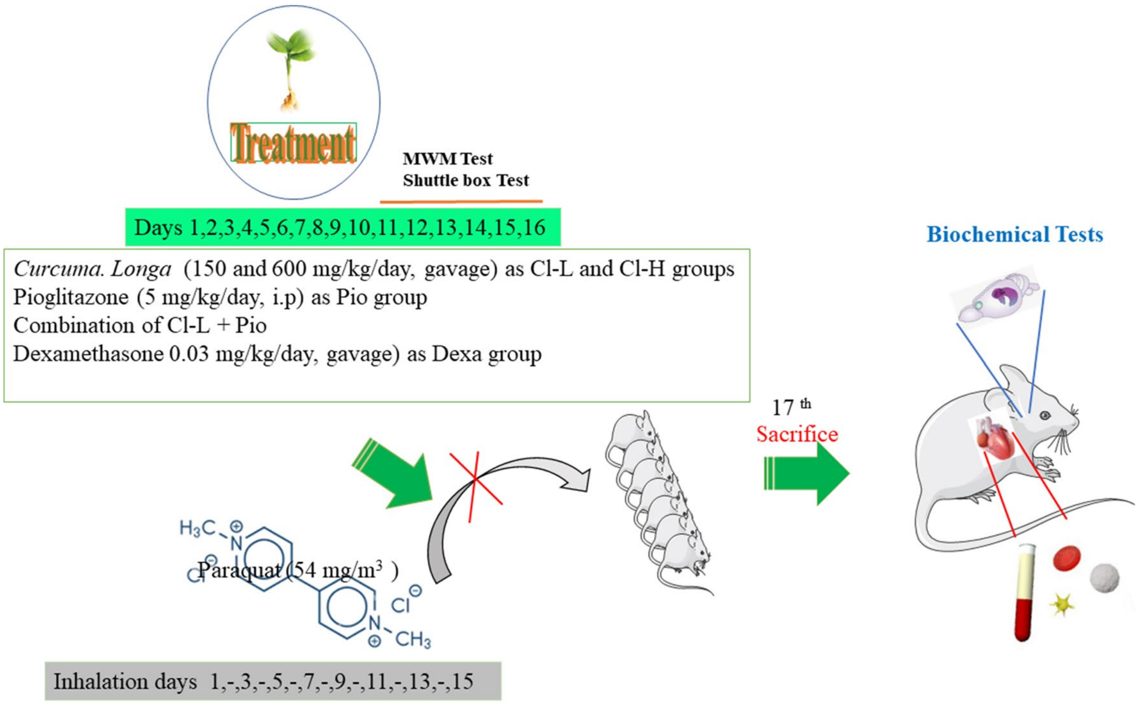


Fig. 1 Protocol of animal's exposure to PQ aerosol and treatments

systemic inflammation, oxidative stress and behavioral evaluations.

Extract preparation and exposure of animals to PQ

The extract preparation was done according to the previous study [22]

Exposure to PQ

The method of exposure of rats to saline and PQ was fully described in previous studies [23].

WBC (total and differential) determination

Following the completion of the treatment period on day 16, the animals received anesthesia by an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Total number of white blood cells was determined using a Neubauer chamber, counting in duplicate, while the differential count was done by preparing BALF smear stained with Wright-Giemsa as previously outlined [24].

Oxidant stress markers determination

A previously described the levels of malondialdehyde (MDA), thiol, catalase (CAT), and superoxide dismutase (SOD) were measured in serum or cortex tissue to determine oxidant stress markers [25]. WBC count and oxidative stress markers were measure in 6 animals of each group randomly.

Behavioral evaluations

The evaluation of detrimental reinforcement via the shuttle box examination was done on the ultimate day of therapy employing the inactive avoidance examination. Additionally, the examination of spatial memory was carried out through the utilization of the Morris water maze (MWM) assessment for a period of 6 days, in accordance with the published reports [26, 27].

Statistical analysis

The normal distribution of data was analyzed using the Kolmogorov–Smirnov test. Comparisons underwent one-way ANOVA analysis, followed by Tukey's multiple comparison test. Means ± SEM were used to convey the results [25, 28]. The behavioral data of MWM were compared by repeated measure ANOVA test. Significance was regarded at $p < 0.05$ level. GraphPad Prism 8 statistical software program was used to illustrate and present the data (GraphPad Software, Inc, San Diego, CA, USA).

Results

Oxidative and inflammatory markers

Total and differential WBC counts in the blood

Figure 2 illustrates that the PQ group had higher numbers of total and differential WBCs versus the Ctrl group (for all, $p < 0.001$). In contrast to the PQ group, there was a decline observed in total and differential WBCs counts in all treated groups ($p < 0.01$ to $p < 0.001$). The counts of differential WBCs, excluding the counts of total WBCs, showed a considerably higher improvement in the Cl-H versus the Cl-L group ($p < 0.05$ to $p < 0.001$). Total WBC count, monocyte, and lymphocyte levels in the Pio and Cl-L groups, eosinophil counts only in the Pio group, and neutrophil counts only in

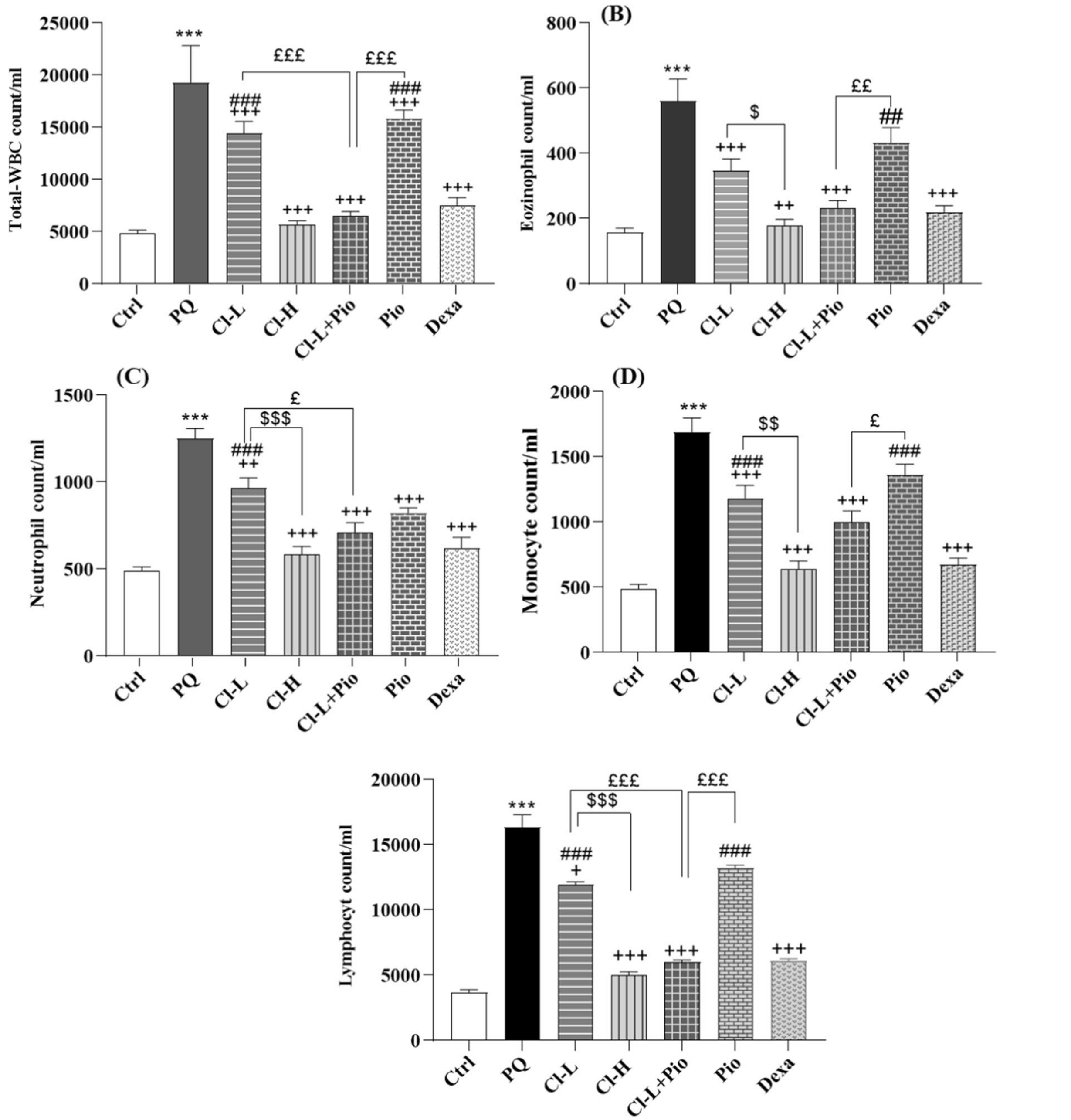


Fig. 2 The effects of exposure of rats to 54 mg/m^3 paraquat (PQ) aerosol on total WBC, (**A**) neutrophil (**B**) eosinophil (**C**) lymphocyte (**D**), and monocyte (**E**) counts in the blood (per ml) compared to the control group (Ctrl), group (***$p < 0.001$) and the influence of treatment with 150 and 600 mg/kg/day *Curcuma Longa* (Cl-L and Cl-H), 5 mg/kg pioglitazone (Pio), combination of Cl-L + Pio or 0.03 mg/kg dexamethasone (Dexa), (++$p < 0.01$ and +++$p < 0.001$ versus PQ group). ≠≠$p < 0.01$, ≠≠≠and $p < 0.001$ versus Dexa group. \$ $p < 0.05$, \$\$ $p < 0.01$ and \$\$\$ $p < 0.001$, Cl-H versus Cl-L. £ $p < 0.05$, ££ $p < 0.01$, and £££ $p < 0.001$, Cl-L + Pio versus Cl-L and Pio. The results are expressed as mean ± SEM ($n = 6$ in each group). One-way ANOVA followed by Tukey's multiple comparison test was applied for comparisons among different groups

the Cl-L group were considerably less improved versus Dexa group ($p<0.01$ to $p<0.001$). When comparing Cl-L versus Cl-L + Pio group, there was a smaller improvement in total WBC, lymphocyte, and neutrophil counts for those treated with just Cl-L. Conversely, considerably more improvements were seen in the numbers of differential and total WBCs except neutrophil count for the Cl-L + Pio treatment group than the Pio group ($p<0.05$ to $p<0.001$).

Oxidant and anti-oxidants in the serum

As it is shown in Fig. 2, the PQ group displayed an increase in MDA level while reductions in CAT and SOD activities, as well as thiol level, versus the control group (for all, $p<0.001$). Except for the Pio groups, all treated groups demonstrated considerable improvements in oxidative stress markers versus the PQ group ($p<0.01$ to $p<0.001$). The group that received Dexa treatment experienced considerably more improvement in thiol, SOD, and CAT levels versus the group treated with Pio and Cl-L ($p<0.05$ to $p<0.001$). In comparison with both the Pio and Cl-L groups, all oxidative stress markers were notably improved in the Cl-L + Pio treated group, excluding MDA levels in the Cl-L group ($p<0.05$ to $p<0.001$), (Fig. 3).

Oxidant and anti-oxidants in the brain tissue

Cortex tissue. The MDA level was considerably increased in the cortex of the non-treated PQ group, while CAT and SOD activities, as well as thiol level, showed a considerable decrease versus the control group (for all $p<0.001$). When comparing to the PQ group, there was a considerable improvement in oxidative stress markers in the cortex for all experimental groups, except Cl-L group for all-oxidative stress markers, Pio group for thiol, CAT, and SOD levels ($p<0.01$ to $p<0.001$). Exception MDA levels, all oxidative stress markers in the cortex tissues showed considerably greater improvement in the Cl-H group versus the Cl-L group ($p<0.01$ to $p<0.001$). In the Dexa-treated group, there was a considerable improvement in all oxidative stress markers in the cortex versus the Cl-L and Pio group ($p<0.05$ to $p<0.001$). Oxidative stress markers showed

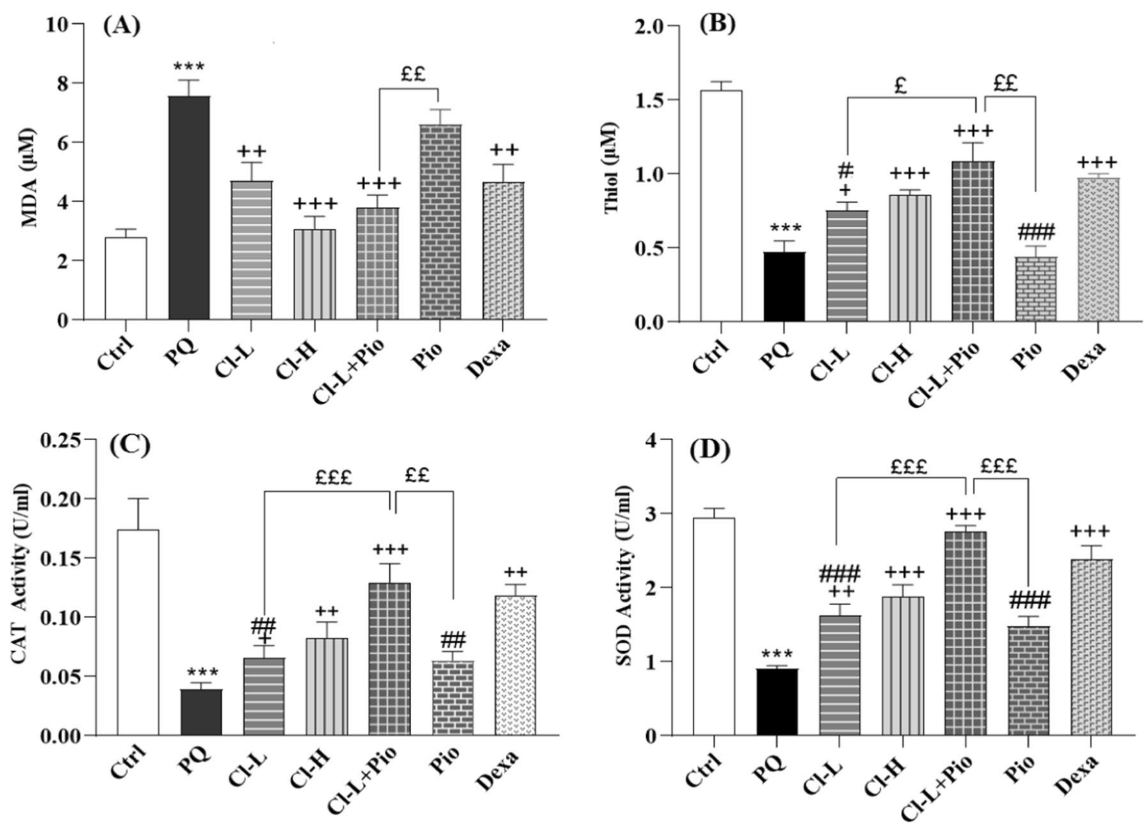


Fig. 3 The effects of exposure of rats to 54 mg/m^3 paraquat (PQ) aerosol on oxidant and anti-oxidant biomarkers in the serum (per ml) compared to the control group (Ctrl), group (***$p<0.001$) and the influence of treatment with 150 and 600 mg/kg/day *Curcuma Longa* (Cl-L and Cl-H), 5 mg/kg pioglitazone (Pio), combination of Cl-L + Pio or 0.03 mg/kg dexamethasone (Dexa). +$p<0.05$, ++$p<0.01$ and +++$p<0.001$ versus PQ group. ≠≠$p<0.01$ and ≠≠≠$p<0.001$ versus Dexa group. £ $p<0.05$, ££ $p<0.01$, and £££ $p<0.001$, Cl-L + Pio versus Cl-L and Pio. The results are expressed as mean ± SEM ($n=6$ in each group). One-way ANOVA followed by Tukey's multiple comparison test was applied for comparisons among different groups

more improvement in the Cl-L + Pio treated group versus the Pio and Cl-L groups, except MDA in the Pio group ($p < 0.05$ to $p < 0.001$), (Fig. 4).

Hippocampus tissue. The hippocampus tissue of the non-treated PQ group exhibited a marked increase in MDA level but, CAT and SOD activities, alongside thiol level, demonstrated a notable decrease vs the control group (for all $p < 0.001$). All experimental groups demonstrated a considerable improvement in oxidative stress markers in the hippocampus tissue versus the PQ group, excluding the Pio group for CAT, and SOD activity in the Cl-L group ($p < 0.05$ to $p < 0.001$). Except for the MDA level, all oxidative stress markers showed more enhancement in the hippocampus tissue of the Cl-H versus Cl-L group ($p < 0.05$). In the Dexa-treated group, there was more improvement in all oxidative stress markers except MDA in the hippocampus versus the Pio group and only more improvement in the SOD activities than the Cl-L-treated group ($p < 0.05$ to $p < 0.01$). All oxidative stress markers in the Cl-L + Pio treated group were more improved than Pio, and the levels of thiol and SOD showed more improvement versus the Cl-L group ($p < 0.05$ to $p < 0.001$), (Fig. 5).

Behavior effects

MWM results

Time latency to reach the stand. The Morris water maze (MWM) test indicated a considerable increase in the time it took for rats in the PQ group to find the platform versus the control group across all 5 days ($p < 0.05$ for the fourth day and $p < 0.001$ for other days). Every treatment group present a substantial decrease in the time it took to reach the platform over the course of 5 days, with the exception of the Pio group on days three and five and the Cl-L group versus the PQ group ($p < 0.05$ to $p < 0.001$). In the first, second, and 4 days, reaching the platform took substantially more time for rats in the Pio and Cl-L treatment groups days Dexa group ($p < 0.05$ to $p < 0.001$). In comparison with Cl-L + Pio, both the Pio and Cl-L treated groups exhibited

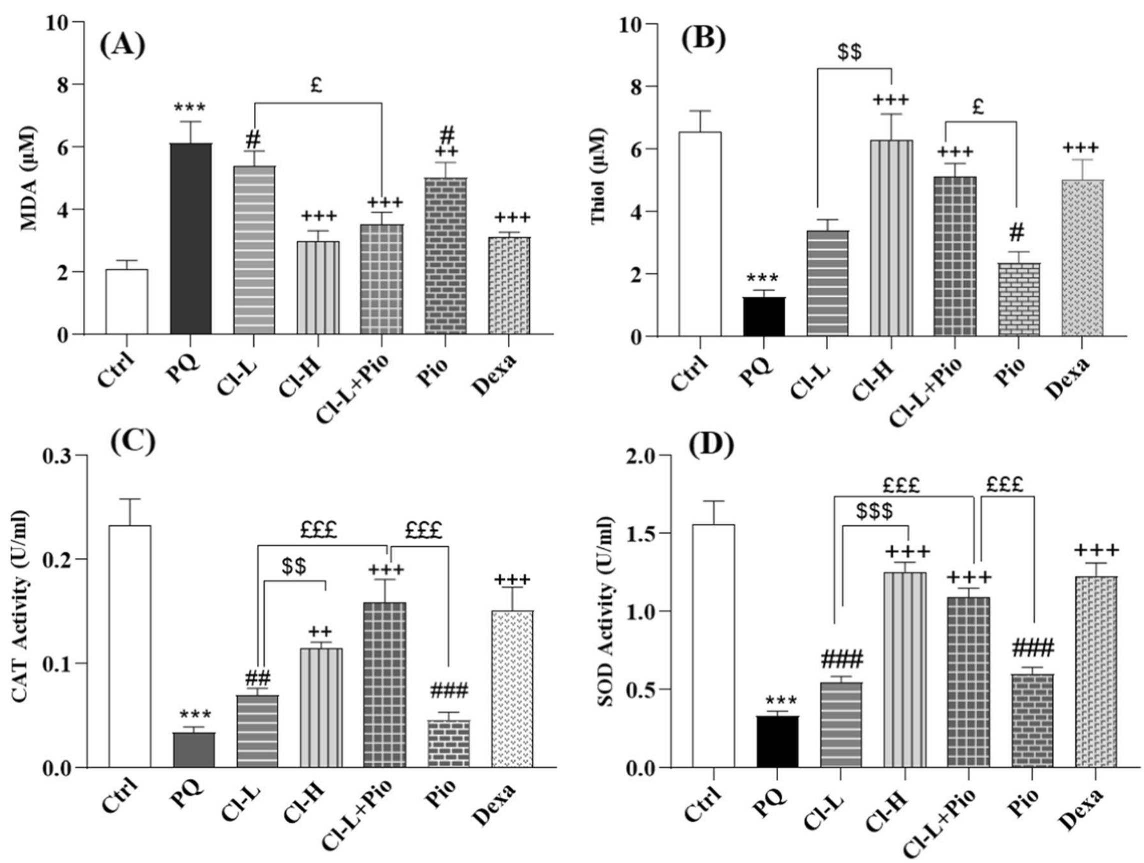


Fig. 4 The effects of exposure of rats to 54 mg/m^3 paraquat (PQ) aerosol on oxidant and anti-oxidant biomarkers in the cortex compared to the control group (Ctrl), group (***$p < 0.001$) and the influence of treatment with 150 and 600 mg/kg/day *Curcuma Longa* (Cl-L and Cl-H), 5 mg/kg pioglitazone (Pio), combination of Cl-L + Pio or 0.03 mg/kg dexamethasone (Dexa). ++$p < 0.01$ and +++$p < 0.001$ versus PQ group. ≠$p < 0.05$, ≠≠$p < 0.01$, ≠≠≠$p < 0.001$ versus Dexa group. $$ $p < 0.01$ and $$$ $p < 0.001$, Cl-H versus Cl-L. £ $p < 0.05$ and £££ $p < 0.001$, Cl-L + Pio versus Cl-L and Pio. The results are expressed as mean ± SEM ($n = 6$ in each group). One-way ANOVA followed by Tukey's multiple comparison test was applied for comparisons among different groups

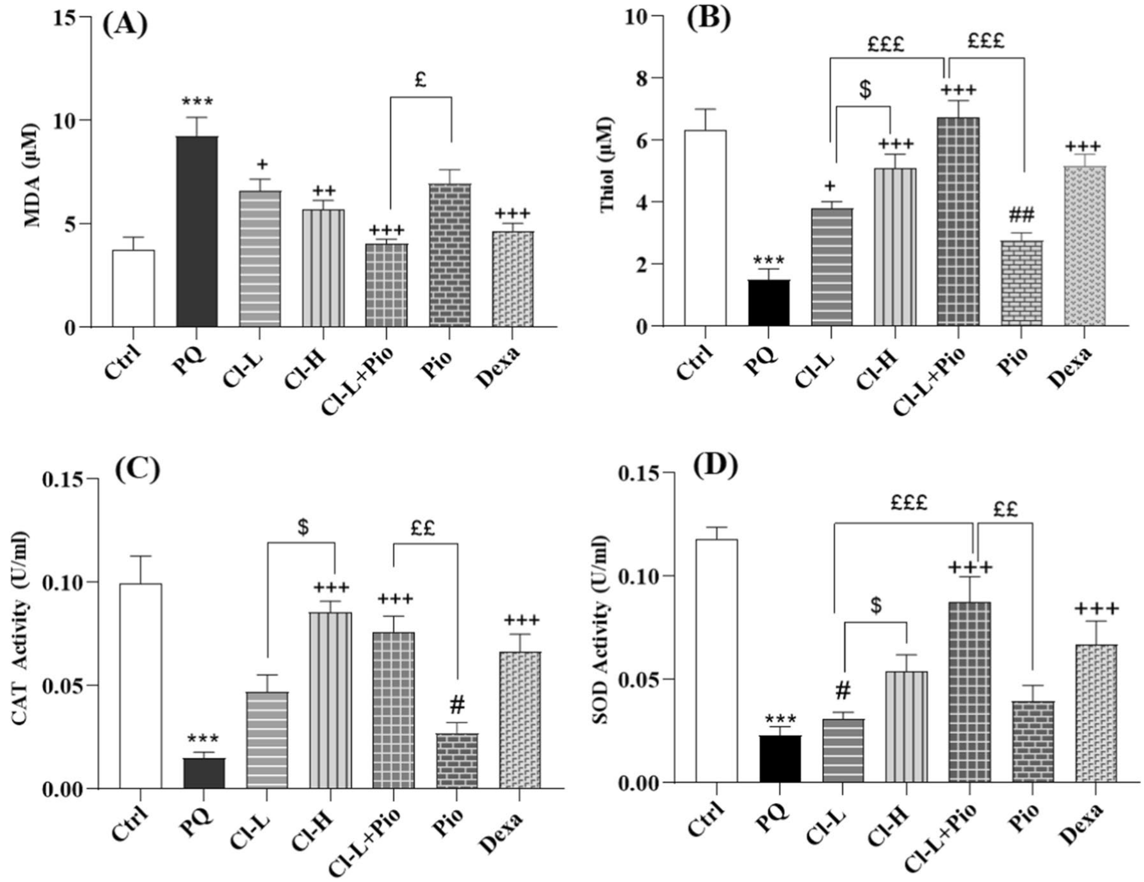


Fig. 5 The effects of exposure of rats to 54 mg/m^3 paraquat (PQ) aerosol on oxidant and anti-oxidant biomarkers in the hippocampus compared to the control group (Ctrl), group (***$p<0.001$) and the influence of treatment with 150 and 600 mg/kg/day *Curcuma Longa* (Cl-L and Cl-H), 5 mg/kg pioglitazone (Pio), combination of Cl-L + Pio or 0.03 mg/kg dexamethasone (Dexa). + $p<0.05$, ++ $p<0.01$ and +++ $p<0.001$ versus PQ group. ≠ $p<0.05$ and ≠≠ $p<0.01$ versus Dexa group. $ $p<0.05$, Cl-H versus Cl-L. £ $p<0.05$, ££ $p<0.01$, and £££ $p<0.001$, Cl-L + Pio versus Cl-L and Pio. The results are expressed as mean ± SEM ($n=6$ in each group). One-way ANOVA followed by Tukey's multiple comparison test was applied for comparisons among different groups

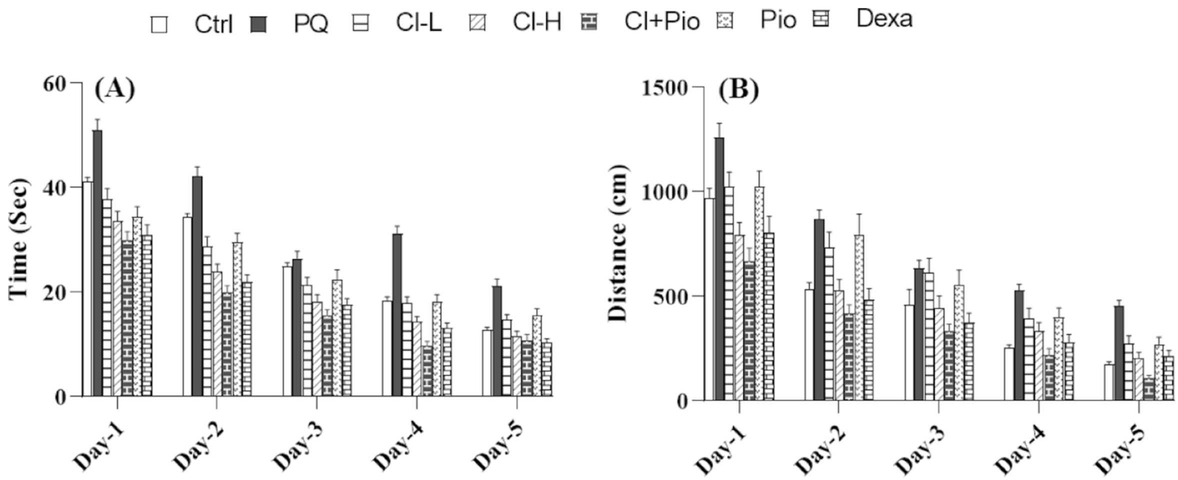
a less decrease in the time latency to reach the platform on days second to fifth (excluding the Pio-L group on days first and fifth and the Cl-L group on days fifth), ($p<0.05$ to $p<0.001$), (Fig. 6A).

Traveling distance to reach the stand. With the exception of the third day, throughout all days of the MWM assessment, the PQ group exhibited a longer distance covered to reach the platform versus the control group ($p<0.05$ to $p<0.001$). In all treated groups, there was a reduction in the distance traveled to achieve the platform (except for the Pio group on days second to fifth, Cl-L group on days third to fifth, and the Cl-H group on days third to fourth) days the PQ group ($p<0.05$ to $p<0.001$). On the first and third days, the Dexa group exhibited a greater decrease in distance versus the Cl-L-treated group, while greater decrease than Pio group on only the first and second days ($p<0.05$ to $p<0.001$). Compared to the Cl-L + Pio combined group, the Pio-treated group presented a lower decrease in distance on the first to fourth days, and a lower reduction on the first to third days versus the Cl-L treated group ($p<0.05$ to $p<0.001$), (Fig. 6B).

Probe Day. Compared to the control group, administering PQ led to a considerable decrease in the amount of time rats spent in Q4 ($p<0.001$). Except for the Pio group, there was a considerable rise in the duration spent by rats in Q4 in all treated groups, versus to the PQ group ($p<0.01$ to $p<0.001$). The time spent in Q4 considerably increased in the Cl-H group versus the Cl-L group ($p<0.001$). In the Dexa group, there was a considerably more increase in the time spent in Q4 versus the Pio group ($p<0.01$). When compared to the Pio and Cl-L group, the rats in the Cl-L + Pio group displayed considerably more increase in the time spent in Q4 ($p<0.01$ for Cl-L and $p<0.001$ for Pio), (Fig. 6C).

Shuttle box (passive avoidance) assay

Following administration of PQ, the delay time was reduced within 24, 48, and 72 h after the electric shock (for all,



Ctrl&PQ	***	***	*	***	***		**	***	-	**	**
PQ&Cl-L	+++	+++	-	+++	+		+	-	-	-	-
PQ&Cl-H	+++	+++	+++	+++	+++		+++	+++	-	-	+
PQ&Cl+Pio	+++	+++	+++	+++	+++		+++	+++	+++	+++	+++
PQ&Pio	+++	+++	-	+++	-		+	-	-		-
PQ&Dexa	+++	+++	+ +	+++	+++		+++	+++	++	+	+
Daxa&Cl-L	##	##	-	##	-		#	##	#	-	-
Dexa&Cl-H	-	-	-	-	-		-	-	-	-	-
Daxa&Cl+Pio	-	-	-	-	-		-	-	-	-	-
Dexa&Pio	#	###	-	-	-		#	###	-	-	-
Pio& Cl-L+Pio	-	£££	££	£££	-		£££	£	£	£	-
Cl-L&Cl-L+Pio	£££	£££	£	£££	-		£££	£££	££	-	-
Cl-L&Cl-H	-	-	-	-	-		-	-	-	-	-

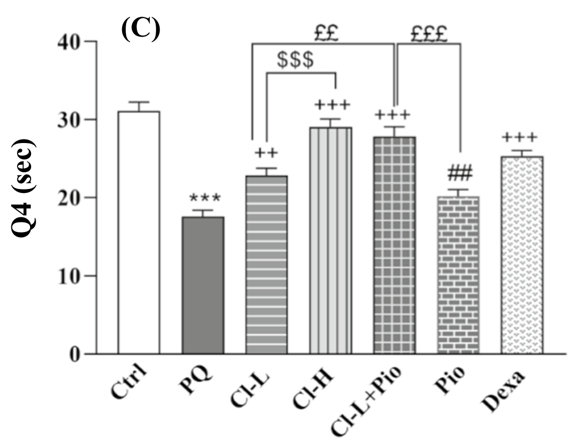


Fig. 6 Time latency measured by Morris water maze (MWM) test (A), traveled distance by MWM test (B), and time spent in the target quadrant on probe day (C). Group exposed to 54 mg/m^3 (PQ) paraquat aerosol, exposing groups to PQ and treated with 150 and 600 mg/kg/day *Curcuma Longa* (Cl-L and Cl-H), 5 mg/kg pioglitazone (Pio-L), combination of Cl-L + Pio or 0.03 mg/kg dexamethasone (Dexa). *$p<0.05$, **$p<0.01$ and ***$p<0.001$ versus control group. +$p<0.05$, ++$p<0.01$, and +++$p<0.001$ versus PQ group, **#** $p<0.05$, **##** $p<0.01$, and **###** $p<0.001$ versus Dexa group. $$$ $p<0.001$, Cl-H versus Cl-L. £ $p<0.05$, ££ $p<0.01$, and £££ $p<0.001$, Cl-L + Pio versus Cl-L and Pio. The results are expressed as mean ± SEM ($n=10$ in each group). One-way ANOVA followed by Tukey's multiple comparison test was applied for comparisons among different groups

$p<0.001$). Compared to the PQ group, the duration of time delay was increased in all treated groups at 24, 48, and 72 h, except for the Pio group at 24, 48, and 72 h as well as for the Cl-L group at 48 and 72 h after electrical shock ($p<0.01$ to $p<0.001$). In the CL-H group, the time delay was increased considerably more than in the Cl-L group at 48 h after the shock ($p<0.01$). In the groups treated with Pio and Cl-L, 72 h after the shock, there were a notable less increase in the time delay to enter the dark compartment versus the Dexa group ($p<0.001$). In the group that received combination treatment, the duration of time delay in the dark compartment displayed a higher increase when vs the Pio group at 24, 48 and 72 hours, and Cl-L group at 72 hours after the shock (for all $p < 0.001$), (Fig. 7).

Discussion

The results indicate that inhaling PQ caused inflammation throughout the body, oxidative stress, brain tissue damage, and memory impairment in rats. Conversely, administration of Cl alone (two doses), Pio, and their combination resulted in a reversal of the mentioned effects triggered by PQ. New evidence indicates that PQ may infiltrate the central nervous system by way of transporters found in the blood–brain barrier (BBB) and impact the nervous system, possibly leading to the development of neurodegenerative disorders like PD [29, 30]. In the current study, biochemical tests revealed that PQ led to reduced levels of SOD, CAT, and total thiol, while also increasing MDA levels in the hippocampus and cortex, suggesting oxidative damage in rat brain tissue. Analysis of the data collected from the behavioral tests demonstrated that PQ exposure led to delays in reaching the stand and longer travel distances. In addition, in the probe challenge of the MWM test, PQ administration reduced the distance traveled and time spent in the target quarter. In addition, PQ administration decreased time delay in shuttle box assay. In line with our findings, studies using rodent models and cell cultures have demonstrated that PQ can induce neurotoxic effects through several distinct mechanisms such as oxidative stress. In human neural progenitor cells (hNPCs), treated with PQ, increased production of reactive oxygen species (ROS) alteration of Nrf2/ARE signaling pathway was observed [31, 32]. In another in vitro study,

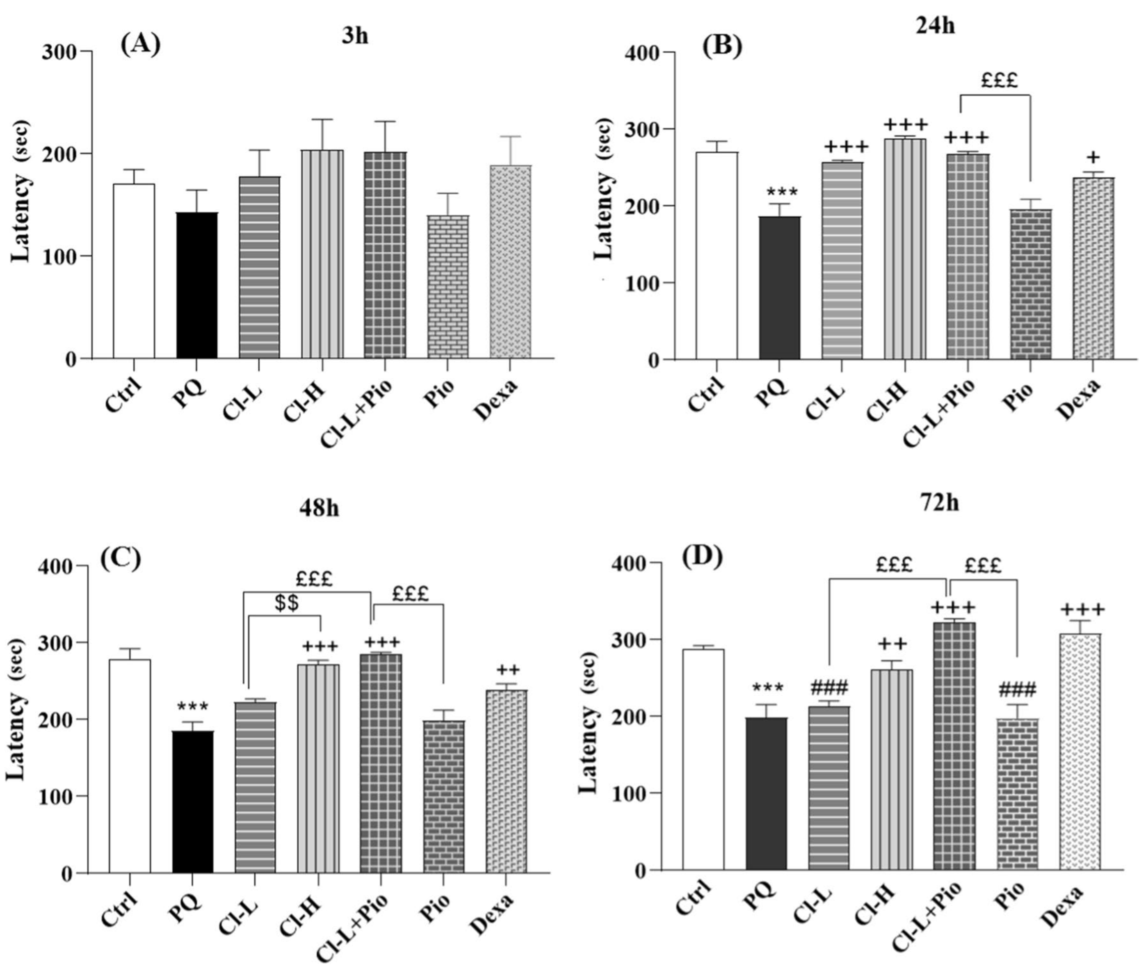


Fig. 7 The results of delay for entering the dark 3 (A), 24 (B) 48, and (C) 72 h (D) after electric shock in passive avoidance (Shuttle) test in different studied groups. ***$p < 0.001$ versus control group. $+p < 0.05$, $++p < 0.01$ and $+++p < 0.001$ versus PQ group. ### $p < 0.001$ versus Dexa group. $$ $P < 0.01$, Cl-H versus Cl-L. £££ $p < 0.01$, Cl-L + Pio versus Cl-L and Pio. The results are expressed as mean ± SEM ($n = 10$ in each group). The results are expressed as mean ± SEM ($n = 10$ in each group). One-way ANOVA followed by Tukey's multiple comparison test was applied for comparisons among different groups

PQ treatment increased oxidative stress indicators such as MDA and 4-hydroxynonenal (4-HNE) in cortical neurons and astrocytes [33]. Chen et al. [34] reported that PQ had a toxic effect on the hippocampus neurons of mice through ROS generation.

Moreover, destructive effect of PQ on memory reported in previous experimental studies, which is in line with our findings. For example, administration of PQ negatively impacts memory abilities in adult mice in the Y-maze test, while also elevating interleukin-1β levels in the hippocampal dentate gyrus to hinder neurogenesis [35]. Increased lipid peroxidation in the hippocampus as a result of PQ administration was shown to lead cognitive deficits, such as spatial memory and passive avoidance, in rats [36].

Free radicals are known to be involved in the pathogenesis of neurocognitive disorders in particular; a strict connection between oxidative stress and memory dysfunction has been widely demonstrated in previous studies [37, 38]. In support of this view, our study reveals the relationship between systemic and brain oxidative damage and the occurrence of memory impairment.

Treating animals with Cl, Pio, or a combination treatment effectively mitigates oxidative stress in systemic and brain tissues and cognitive decline. The protective effect of Cl on memory impairment and brain oxidative damage was reported in several published studies. In scopolamine-induced amnesia model, Cl administration alleviates memory dysfunction by suppressing oxidative stress and inflammation [39]. In D-galactose-induced cognitive impairment model rats, treatment with Cl, improved spatial memory by upregulating CREB signaling and BDNF in the hippocampal dentate gyrus [40]. In rats exposed to trimethyltin, the administration of Cl improved spatial memory deficits and prevented the decrease in the number of pyramidal neurons in the CA2-CA3 regions [41]. Aged rats showed improved learning and spatial memory, along with decreased glutamate-induced excitotoxicity and neurodegeneration processes in the hippocampus following long-term administration of Cl [42]. It was shown that Pio administration also improved memory ability and brain oxidative stress in fructose-drinking insulin resistance rats [43], lipopolysaccharide-treated rats [44], streptozotocin-induced diabetic mice [45], scopolamine-challenged rat [46] and L-methionine treated rats [47].

In the current investigation, treatment of animals with PQ caused a systemic inflammatory response, which was characterized by increased level of WBC in the blood. In addition, PQ inhalation changed biochemical markers of serum in favor of oxidative damage. Our earlier research findings were consistent with the current study, supporting the impact of PQ on systemic inflammation and oxidative damage [28]. In addition, this study may support previous evidence regarding the effect of systemic inflammation [48, 49] or oxidative stress [50, 51] on cognitive decline and behavioral disturbances in clinical studies. Furthermore, the findings of this research could potentially offer empirical proof for the link between systemic inflammation, oxidative stress, and cognitive decline in animals exposed to PQ. The combination of Cl and Pio treatment significantly reduced systemic inflammation and oxidative stress. In line with our finding, Cl extract administration improved the marker of systemic inflammatory [52] and oxidative stress [53, 54] in previous studies. In addition, Pio administration suppressed systemic inflammation and oxidative stress in our previous studies [25, 55]. The comparison of the effects between two doses of Cl suggested the dose-dependency effect for Cl. Therefore, we recommend that future studies evaluate different doses of Cl on PQ-induced. Moreover, when evaluating the treated groups, it was found that the impact of Cl-H and Cl-l + Pio groups on several measures closely resembled that of Dexa.

Previous research has shown that combining Cl-L and Pio had a greater impact on PQ-induced insults compared to either substance alone, indicating a potential role of the PPARγ receptor activation in Cl effects on PQ-induced changes [23, 25]. Additional research is necessary to understand the impact of a PPARγ receptor antagonist on Cl in PQ-induced changes and to gain clarity on the potential memory-boosting, antioxidant, and anti-inflammatory properties of Cl.

Conclusion

The Administration of Cl, Pio and their combination improved memory dysfunction and brain oxidative stress as well as systemic inflammation in PQ exposed rats. The results suggest that Cl may induce these effects through activation of PPARγ receptor.

Author contributions The experimental work was conducted by Parisa Sarbaz, Sima Beigoli, Bahareh Payami, Mohammad Hossein Eshagh Ghalibaf, Sabiheh Amirahmadi, Reyhaneh Khosravi, Mahmoud Hosseini, and Mohammad Hossein Boskabady. They also analyzed the statistics, created figures, and wrote the initial draft of the manuscript. Mohammad Hossein Boskabady and Sima Beigoli collaborated on the study's design, oversaw its progress, contributed to statistical analysis, and finalized the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

Declarations

Conflict of interest Parisa Sarbaz, Sima Beigoli, Bahareh Payami, Mohammad Hossein Eshaghi Ghalibaf, Sabiheh Amirahmadi, Mahmoud Hosseini, Mohammad Hossein Boskabady declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human subjects or animals performed by any of the authors.

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