OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.XX.XX

Research Article
Ciproxifan Attenuates Lipopolysaccharide-Induced Neuroinflammation and Mitochondrial Dysfunctions in Mouse Brain

[1]Vasudevan Mani, [2]Minhajul Arfeen, [3,4]Abdel-Moneim Hafez Abdel-Moneim and [1,5]Hussein M. Ali

[1]Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraydah, Kingdom of Saudi Arabia
[2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Buraydah, Kingdom of Saudi Arabia
[3]Department of Physiology, Faculty of Medicine, Qassim University, Kingdom of Saudi Arabia
[4]Department of Physiology, Faculty of Medicine, Mansoura University, Egypt
[5]Department of Biochemistry, Faculty of Medicine, Al-Azhar University, Assiut 71524, Egypt

Abstract

Background and Objective: Presently, Histamine H_3 Receptor (HH_3R) antagonists have been assured as a potential therapeutic candidate for several CNS-related disorders. In animal experiments, ciproxifan, HH_3R antagonist/inverse agonist causes arousal and attentiveness. This study examined the neuroprotective potential of ciproxifan against Lipopolysaccharides (LPS)-induced neuroinflammation and mitochondrial dysfunctions in mice. **Materials and Methods:** Ciproxifan(1 and 3 mg kg^{-1}) was treated orally for 30 days and 4 doses of LPS (250 μg kg^{-1}, i.p.) was injected for the last 4 days of treatment (27-30 days) to induce neuroinflammation. Brain homogenates were collected at end of the dosing (30 days) for estimation of cytokines [Interleukin-6 (IL-6) and Tumour Necrosis Factor-alpha (TNF-α) as pro-inflammatory cytokines, IL-10 and Transforming Growth Factor-beta 1 (TGF-$β_1$) as anti-inflammatory cytokines], Cyclooxygenase-2 (COX-2) and Mitochondrial Respiratory Chain Complex (MRCC) enzymes (complexes I, II and IV). **Results:** Treatment of ciproxifan exhibited its anti-inflammatory effects by significantly reducing the release of both pro-inflammatory cytokines (IL-6 and TNF-α) and COX-2 levels, further, it improved the anti-inflammatory cytokines (IL-10 and TGF-$β_1$) levels in LPS-challenged mouse brain. Additionally, ciproxifan reversed the brain MRCC (I, II and IV) levels in LPS-induced mice. **Conclusion:** According to the findings, ciproxifan assured its neuroprotection against LPS-induced neurotoxicity in mice models by reducing neuroinflammation and restoring MRCC enzymes.

Key words: Ciproxifan, neuroinflammation, lipopolysaccharides, cyclooxygenase-2, pro-inflammatory cytokines, anti-inflammatory cytokines, mitochondrial dysfunctions

Citation: Mani, V., M. Arfeen, A.M.H. Abdel-Moneim and H.M. Ali, 2022. Ciproxifan attenuates lipopolysaccharide-induced neuroinflammation and mitochondrial dysfunctions in mouse brain. Int. J. Pharmacol., 18: XX-XX.

Corresponding Author: Vasudevan Mani, Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraydah, Kingdom of Saudi Arabia Tel: + 966-508695644

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inflammation of the brain is thought to play a role in the etiology of major chronic neurodegenerative diseases such as Alzheimer's Disease (AD) and Parkinson's Disease (PD). It causes neurodegeneration by activating the microglia and brain's resident immune cells, which are trigger excessive production of pro-inflammatory substances[1]. Considering neurodegenerative diseases, the prevalence of AD-related dementia is currently predicted to be approximately 40-50 M cases, with the number expected to rise to 74.7 M cases by 2030[2]. A prominent feature in AD is the formation of Amyloid-beta (Aβ) plaques and the development of intracellular neurofibrillary tangles. Neuroinflammation and mitochondrial dysfunction also play a role in AD and other neurodegenerative illnesses[3,4]. In AD, astrocytes and microglia are arguably the most important generators of cytokines. The productions of cytokines are involved in practically every facet of neuroinflammation, including pro- and anti-inflammatory processes, induce neuronal deficits, chemoattraction and Aβ related microglia responses[5]. Other than cytokines, several factors like prostaglandin E_2, oxidative stress and reactive nitrogen species also act as the key factors in the brain inflammatory process[6]. An endotoxin, Lipopolysaccharide (LPS) derived from the cell wall component of gram-negative bacteria is identified as a pathogen-associated molecular pattern by higher vertebrates' immune systems. In mononuclear myeloid cell surface, LPS binds to the Toll-Like Receptor 4 (TLR4)/CD14 complex and activates the pro-inflammatory cytokines IL-6, IL-1β and TNF-α[1].

Mitochondrial dysfunction is associated with several factors including activated immune-inflammatory pathways, oxidative and nitrosative stress and metabolic problems in the brain. Several neurodegenerative diseases, including AD and PD, have been linked to changes in the mitochondrial respiratory chain and related enzymes and coenzymes[7]. Inflammatory mediators, particularly cytokines, can alter cellular mitochondrial metabolism by decreasing mitochondrial oxidative phosphorylation and ATP generation while also causing mitochondrial Reactive Oxygen Species (ROS) generation. Mitochondrial-derived ROS prolongs neuroinflammation by activating NF-κB in the brain, which causes the generation of proinflammatory cytokines[6]. Experimentally, LPS could induce mitochondrial dysfunctions through alteration of Mitochondrial Respiratory Chain Complex (MRCC) activities. Intracerebroventricular (ICV) injection of LPS established mitochondrial electron transfer chain dysfunction by decreasing the MRCC (I, II, IV and V) activities in the rat model[6,8].

The HH_3R antagonists regulate the release of histamine and other neurotransmitters pre-synoptically, making it a promising target for CNS disorders such as narcolepsy, cognitive impairments, Attention-deficit and hyperactivity disorder and pain[9]. Ciproxifan, a strong HH_3R antagonist, has been demonstrated to elevate histamine, acetylcholine, dopamine and norepinephrine production in the brain's cortex and hippocampus[4]. Recently, a study from our laboratory demonstrated the anti-Alzheimer potential of ciproxifan by improving cognitive functions, enhancing cholinergic transmission, improving anti-oxidant properties and attenuating neuroinflammation in B6.129-Tg (APPSw) 40Btla/J transgenic AD mice[4]. Early, ciproxifan reversed the cognitive deficit in the Tg2576 mouse model of AD also supported the results of the previous study[10]. However, data are scarce on ciproxifan's impact on neuroinflammation and mitochondrial dysfunctions. The objective of the existing investigation was to evaluate the effect of ciproxifan pre-treatment for 30 days on LPS-induced neuroinflammation and mitochondrial dysfunctions in a mouse model.

MATERIALS AND METHODS

Study area: The study was carried out at Pharmacology Research Laboratory, Department of Pharmacology and Toxicology, Qassim University, Saudi Arabia from March-September, 2021.

Drugs and chemicals: Fine chemicals such as ciproxifan maleate and lipopolysaccharides extracted from *Escherichia coli* were acquired from Sigma-Aldrich Co (St. Louis, MO, USA). ELISA kits for mouse IL-6, TNF-α, IL-10, TGF-$β_1$ and COX-2 were procured from Cloud-Clone Corp., Texas, USA. Mouse MRCC-I, MRCC-II and MRCC-IV were obtained from MyBioSource, Inc., San Diego, USA. All other chemicals and solvents were obtained from local suppliers and were used of analytical grade.

Animals: In the current experiment protocols included a total number of 24 male ICR mice with adult age (between 8-12 weeks, body weight between 25-35 g) were obtained from animal house maintained by the Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, KSA. The animals were randomly divided into 4 groups with each group containing 6 mice and maintained 3 animals in a cage for entire experiment periods. During 7 days of adaptation and 30 days of drug treatment, animals were allowed free access to water and food. The present experimental protocols and use of animals were reviewed

and approved by the Institutional Animal Ethical Committee of the College of Pharmacy (Approval ID 2020-CP-7) and the Deanship of Scientific Research of Qassim University (research grant number: 5601-pharmacy-2019-2-2-I), Qassim University, KSA.

Experimental design: A total of 4 groups of animals were used in this present protocol. Among, the control group received only vehicle (normal saline: 10 mL kg^{-1}) by oral for continuously 30 days and 4 doses of normal saline (10 mL kg^{-1}) by Intraperitoneal Injection (IP) for the last 4 days (27th-30th days) of treatment schedule. The second group was the LPS-induced group administrated with a vehicle similar to the control group and neuronal inflammation induced with 4 injections of LPS (250 µg kg^{-1}, i.p.) for the last 4 days of the treatment schedule. The dose of the LPS was selected from our previous researches[11,12]. The remaining 2 groups (LPS+CIP1 and LPS+CIP3) were treated with 2 doses (1 or 3 mg kg^{-1}, respectively)of ciproxifan orally for 30 days and induced neurotoxicity with 4 doses of LPS (250 µg kg^{-1}, i.p.) as followed LPS-induced group.

Collection of brain homogenate: After successive treatment with 30 days, each of the animals was sacrificed and brain tissue was isolated from the skull. Using a homogenizer, the isolated tissues were homogenized with phosphate buffer (4°C, pH 7.4) and the homogenates were collected after centrifuging at 4000 rpm for10 min. The biuret colorimetric technique was used to determine the total protein content of the homogenates (Crescent Diagnostics, Saudi Arabia).

Determination of cytokines and cyclooxygenase levels: Using mouse Enzyme Linked Immunosorbent Assay (ELISA) (Cloud-Clone Corp., Texas, USA) procedure 2 pro-inflammatory (TNF-α and IL-6), 2 anti-inflammatories ($TGF\beta_1$ and IL-10) cytokines and COX-2 were analyzed. Sandwich enzyme immunoassay for *in vitro* quantitative detection of particularly targeted proteins was used after the assay kits.

Determination of mitochondrial respiratory chain complexes (MRCC): The mouse ELISA kits of MRCC-I, MRCC-II and MRCC-IV were employed from MyBioSource Inc., (San Diego, USA). In the end, using a Microplate Reader, absorbance was taken at 450 nm (ELx800, BioTek Instruments, Inc.)

Statistical analysis: Results were presented as Mean±Standard Error (SEM). Followed one-way ANOVA (GraphPad version 9 from GraphPad Software Inc., USA. to display the variations among all groups and a Tukey-Kramer *post hoc* test was utilized for finding significance levels between the targeted 2 groups. The $p<0.05$ were refereed statistically significant.

RESULTS

Ciproxifan reduced pro-inflammatory cytokine levels in LPS-induced mice brains: Effect of the ciproxifan on pro-inflammatory cytokine markers such as TNF-α and IL-6 validated in Fig. 1. It is known that pro-inflammatory cytokines up-regulate the inflammatory reactions. Refers to IL-6 levels, there was a substantial difference ($F(3,20) = 13.38$, $p<0.001$) among the treated groups when analyzed with one-way ANOVA in Fig. 1a. The LPS-induced group showed a significant elevation (87.03±3.98 pg mg^{-1} protein, $p<0.01$) in IL-6 levels

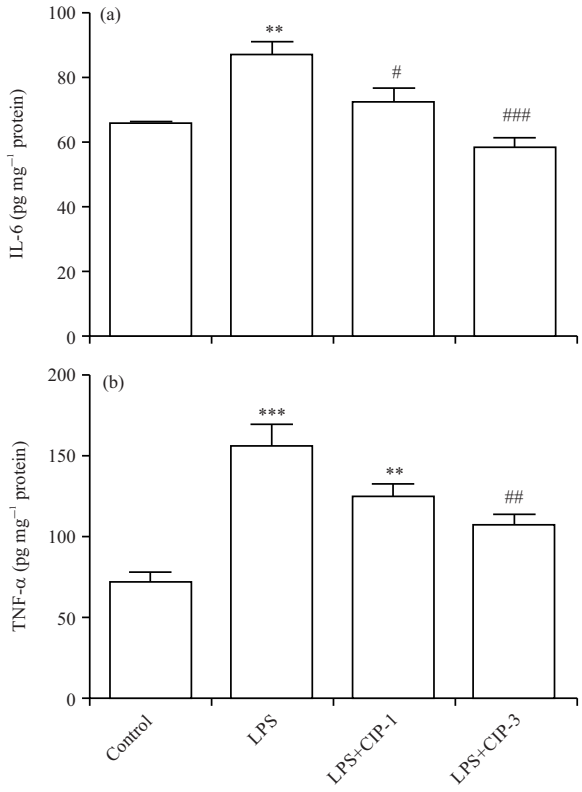

Fig. 1(a-b): Effect of ciproxifan (CIP) on pro-inflammatory cytokines levels in lipopolysaccharide (LPS)-induced mice brains, (a) IL-6 and (b) TNF-α levels
Results are expressed by Mean±SEM (n = 6), one-way ANOVA ($F(3,20) = 13.38$, $p<0.001$for IL-6 and $F(3,20) = 14.74$, $p<0.001$ for TNF-α) followed by Tukey-Kramer multiple comparisons test, **$p<0.01$ and***$p<0.001$ as compared to the control group, #$p<0.05$, ##$p<0.01$ and ###$p<0.001$ as compared to the LPS-induced group

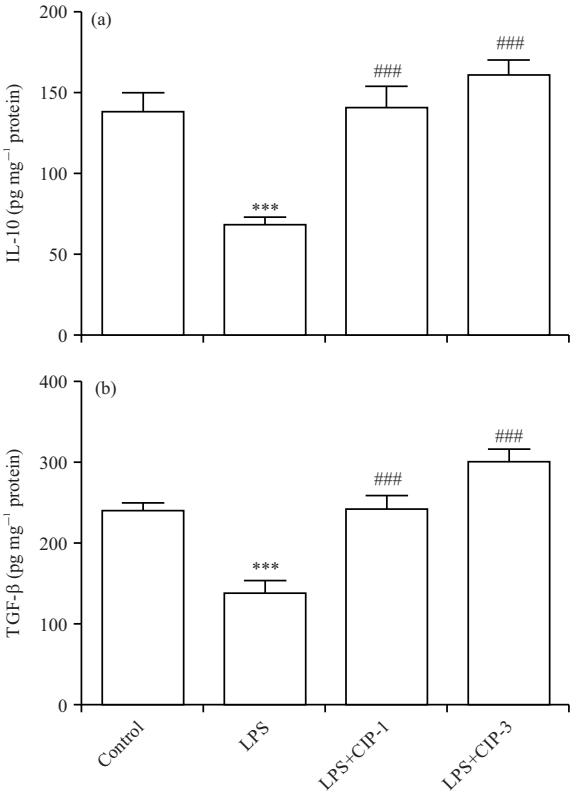

Fig. 2(a-b): Effect of ciproxifan (CIP) on anti-inflammatory cytokines levels in lipopolysaccharide (LPS)-induced mice brains, (a) IL-10 and (b) TGF-β levels
Results are expressed by Mean±SEM (n = 6), one-way ANOVA (F(3,20) = 15.83, p<0.001 for IL-10 and F(3,20) = 21.28, p<0.001 for TGF-β) followed by Tukey-Kramer multiple comparisons test, ***p<0.001 as compared to the control group and ###p<0.001 as compared to the LPS-induced group

in the brain as paralleled to control (65.75±1.46 pg mg^{-1} protein) indicated the induction of neuronal inflammation by 4 doses of LPS (250 μg kg^{-1} i.p.). However, oral treatment of ciproxifan with 1 mg kg^{-1} (72.45±4.11 pg mg^{-1} protein, p<0.05) and 3 mg kg^{-1} (58.08±3.22 pg mg^{-1} protein, p<0.001) extensively reduced the level of IL-6 in mice brains as compared to LPS-induced animals.

Figure 1b highlights the effect of ciproxifan on the brain cytokine TNF-α production in LPS-treated mice. It was found that, when comparing among groups, differences in TNF-α levels (F(3,20) = 14.74, p<0.001) were exhibited in brain homogenates. In continuation of multiple *post hoc* analysis represented that LPS administration was noted with extensive elevation (156.30±13.68 pg mg^{-1} protein, p<0.001) of TNF-α levels in brain tissues as matched to the control mice (72.08±5.90 pg mg^{-1} protein). However, treatment with ciproxifan (3 mg kg^{-1}, p.o.) considerably (106.90±7.04 pg mg^{-1} protein, p<0.01) reversed with declining the brain TNF-α levels associated with the LPS-induced group.

Ciproxifan improved anti-inflammatory cytokine levels in LPS-induced mice brains: Figure 2 showed the results of ciproxifan on 2 selected anti-inflammatory cytokine markers such as IL-10 and TNF-β_1 in the brain homogenates of LPS-induced mice. Anti-inflammatory cytokines prevent the inflammatory pathways by regulating the production of proinflammatory cytokines. Comparing the variations among the groups, there were significant changes (F(3,20) = 15.83, p<0.001) in IL-10 levels by One-way ANOVA analysis in Fig. 2a. The levels of the cytokine IL-10 in LPS-induced animals were noted extensively decreased (68.08±4.29 pg mg^{-1} protein, p<0.001) as associated with the control mice (138.0±11.22 pg mg^{-1} protein). It is explained that LPS-induction reduced anti-inflammatory activity in the mouse brain. Pretreatment with ciproxifan (1 and 3 mg kg^{-1}, p.o.), however, extensively (139.9±13.43 pg mg^{-1} protein and 160.0±9.14 pg mg^{-1} protein, correspondingly, p<0.001) increased IL-10 levels in mice brains as compared to the LPS-induced group.

Reference to TGF-β_1 levels, there were notable variations showed (F(3,20) = 21.28, p<0.001) as compared among all the treatment groups in Fig. 2b. It was also found that LPS-induction resulted in significantly declined (137.8±14.47 pg mg^{-1} protein, p<0.001) brain TGF-β_1 levels in mice as compared to the control group (238.8±9.76 pg mg^{-1} protein). The reduction of TGF-β_1 levels in LPS-induced mice showed further validation of neuroinflammation by LPS injection. Interestingly, treatment with both doses of ciproxifan (1 and 3 mg kg^{-1}, p.o.) extensively elucidated (241.3±16.63 pg mg^{-1} protein and 299.0±16.20 pg mg^{-1} protein, respectively, p<0.001) the TGF-β_1 levels in the LPS-induced brain. The restore of both IL-10 and TGF-β_1 levels by ciproxifan with both doses was equivalent to the control group of animals.

Ciproxifan reduced cyclooxygenase 2 (COX-2) activities in LPS-induced mice brains: The results of ciproxifan on COX-2 activities in LPS-challenged mice brains is shown in Fig. 3. Elevation of COX-2 activities results in increasing the production of prostaglandins that leads to inflammation. As compared among groups, significant differences were shown for COX-2 levels (F(3,20) = 26.60, p<0.001) by analyzing with one-way ANOVA. Further, LPS-induction with 4 doses showed a significant elevation in COX-2 levels (724.7±23.94 pg mg^{-1} protein, p<0.001) in the brain as paralleled to control animals (487.4±21.18 pg mg^{-1} protein). However, pre-treatment with

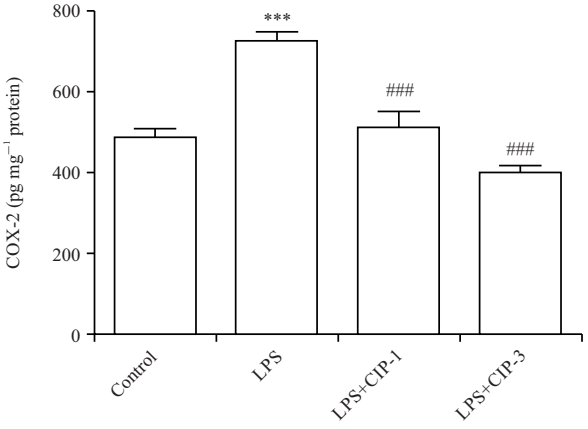

Fig. 3: Effect of ciproxifan (CIP) on cyclooxygenase-2 (COX-2) levels in lipopolysaccharide (LPS)-induced mice brains
Results are expressed by Mean±SEM (n = 6), one-way ANOVA (F(3,20) = 26.60, p<0.001) followed by Tukey-Kramer multiple comparisons test, ***p<0.001 as compared to the control group and ###p<0.001 as compared to the LPS-induced group

both doses of ciproxifan (1 and 3 mg kg^{-1}, p.o.) considerably attenuated COX-2 activity (512.1±39.70 pg mg^{-1} protein and 400.6±15.73 pg mg^{-1} protein, respectively, p<0.001) in LPS-challenged mice. In both ciproxifan treatment groups, the COX-2 levels were similar as referred to the control animals.

Ciproxifan improved MRCC activities in LPS-induced mice brains: The effect of ciproxifan treatment on MRCC-I, II and IV activities in LPS-challenged brain is depicted in Fig. 4. Reduction in the level of MRCC activities associates the mitochondrial dysfunctions. The one-way ANOVA analysis of MRCC-I activities resulted that there were significant differences (F(3,20) = 22.60, p<0.001) among the groups in Fig. 4a. Additionally, when compared to control (250.1±14.10 pg mg^{-1} protein), LPS injections caused a substantial decrease (199.6±7.42 pg mg^{-1} protein, p<0.05) in MRCC-I activity in mice brains. However, pre-treatment of ciproxifan at 1 mg kg^{-1} (301.7±11.73 pg mg^{-1} protein) and 3 mg kg^{-1} (328.9±13.72 pg mg^{-1} protein) diminished (p<0.001) the LPS-induced reduction of MRCC-I activities in the brain homogenates.

Reference from Fig. 4b, regarding MRCC-II activities, when compared among all the treated groups, significant changes (F(3,20) = 5.762, p<0.01) were noted in mice brains. Furthermore, when compared to control groups (2.03±0.15 ng mg^{-1} protein), administration of LPS induced a substantial decrease (1.44±0.09 ng mg^{-1} protein, p<0.05) in MRCC-II activity in the brain. Interestingly, there were significant improvements in MRCC-II activities with the administration of both doses of ciproxifan (2.21±0.17 ng mg^{-1}

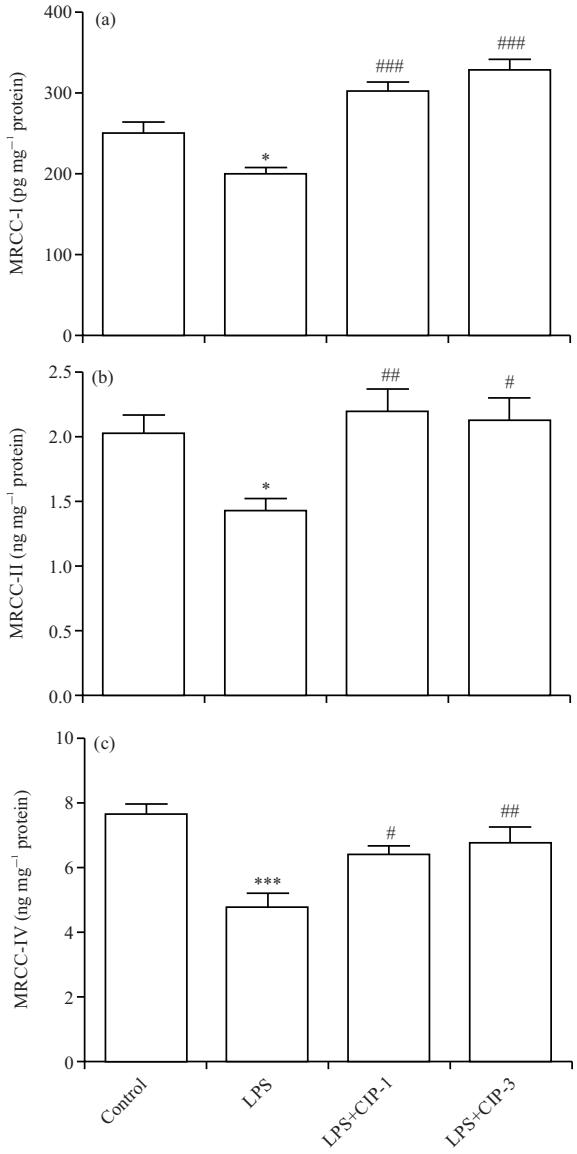

Fig. 4(a-c): Effect of ciproxifan (CIP) on mitochondrial respiratory chain complexes (MRCC) activities in lipopolysaccharide (LPS)-induced mice brains, (a) MRCC-I, (b) MRCC-II and (c) MRCC-IV activities
Results are expressed by Mean±SEM (n = 6), one-way ANOVA (F(3,20) = 22.60, p<0.001 for MRCC-I, F(3,20) = 5.762, p<0.01for MRCC-II, F(3,20) = 9.534, p<0.001 for MRCC-IV) followed by Tukey-Kramer multiple comparisons test, *p<0.05 and ***p<0.001 as compared to the control group, #p<0.05 and ###p<0.001 as compared to the LPS-induced group

protein, p<0.01 for 1 mg kg^{-1} and 2.14±0.17 ng mg^{-1} protein, p<0.05 for 3 mg kg^{-1}, p.o.) in LPS-induced mice brains.

Additionally, it was referred in Fig. 4c that there were significant variations among the groups for brain MRCC-IV activities (F(3,20) = 9.534, p<0.001) in mice. In continuous as compared to the control mice (7.66±0.31 ng mg^{-1} protein),

the LPS-induced animals showed a decline in MRCC-IV activities (4.78±0.42 ng mg^{-1} protein, $p<0.001$) in brain tissues. However, 30 days pre-treatment with both doses of ciproxifan (1 and 3 mg kg^{-1}, p.o.) reversed the MRCC-IV levels (6.40±0.26 ng mg^{-1} protein, $p<0.05$ and 6.75±0.51 ng mg^{-1} protein, $p<0.01$, correspondingly) in a dose-depended manner in mice brains. Regarding the level of brain MRCC-I, II and IV, activities with the treatment of ciproxifan 1 and 3 mg kg^{-1} in LPS-induction as identical to control animals.

DISCUSSION

Current results provided scientific evidence that ciproxifan, an antagonist of HH_3R has neuroprotective effects on LPS-induced neuronal inflammation and mitochondrial dysfunctions in mouse experimental models. The majority of findings supported ciproxifan neuroprotective effects by reducing neuroinflammation and mitochondrial toxicity as evidenced by decreased Cyclooxygenase-2 (COX-2) and pro-inflammatory cytokines while improving anti-inflammatory cytokines and Mitochondrial Respiratory Chain Complexes (MRCC) activities in LPS-induced mouse brains. Thus, by modulating COX activity and cytokine levels in the brain, peripheral injection of LPS has been linked to the production of neuroinflammation and mitochondrial dysfunction[11-13]. Corresponding to these results, current findings also validated the generation of neuroinflammation in the mouse brain by increasing COX-2, IL-6 and TNF-α production, while decreasing TGF-1 and IL-10 levels with LPS administration. Additionally, MRCC (I, II and IV) activities also declined accompanied by LPS toxicities. MRCC (I, II and IV) activities also decreased, which was associated with LPS toxicity.

Considering the significance of neuroinflammation in nervous system illnesses in conjunction with oxidative stress, which also plays a key role in memory and learning impairments in AD[3]. Hence, anti-inflammatory medications appear to offer some advantages. Furthermore, cytokines and interleukins increased inflammatory processes that trigger inflammatory pathways in neurodegenerative diseases such as AD, multiple sclerosis, PD and amyotrophic lateral sclerosis[14]. Neuronal inflammation is also associated with intracellular mechanisms in neuronal degeneration, such as protein degradation, mitochondrial dysfunction, axonal transport abnormalities and the apoptotic process[14,15]. According to a recent discovery, HH_3Rs are abundant not only in neurons but also in astrocytes and microglia. HH_3R antagonists have also shown anti-inflammatory effects in some experimental models due to their role in the modulation of neurotransmitters[4,16].

Elevation of pro-inflammatory cytokines including IL-6, TNF-α, IL-1α and IL-1β mediate the inflammatory responses in the nervous system of human and experimental rodent models that have been reported in AD and dementia[17]. Evidence suggested that inflammation in neuronal might induce the production and aggregation of Aβ protein in AD and a therapeutic approach against neuroinflammation is a key area to slow the progression of AD[18]. In the experiment, systemic administration of LPS induced COX enzymes (COX-I and II), iNOS and pro-inflammatory cytokines including TNF-α, IL-1, IL-2, IFN-γ and IL-6 by a single and multi doses[19]. Similarly, the present results demonstrated the elevation of IL-6, TNF-α and COX-2 levels with four doses of LPS (250 μg kg^{-1}, i.p.) injections. Previous research has also revealed that the administration of the same doses of LPS generated the production of COX-2, TNF-α and IL-6 levels in brain tissues[11,12]. Furthermore, peritoneal injection of LPS resulted in the systemic elevation of IL-6 and TNF-α which can enter the CNS via the bloodstream[20]. Hence, in the inflammatory pathway, an inducible enzyme COX-2 is activated by cytokines and mitogens. The activation of cytokines including TNF-α, IL-1β and IL-6 mediated the expression of COX-2 levels[21]. Interestingly, in current results, groups of mice treated with both doses of ciproxifan (1 and 3 mg kg^{-1}, p.o.) significantly reduced the IL-6 and COX-2 levels in the LPS-induced brain. Also, attenuation of LPS-induced brain TNF-α levels has resulted in only at a high dose of ciproxifan (3 mg kg^{-1}, p.o.). Concurrently, the 15 days treatment with similar selected doses of ciproxifan (1 and 3 mg kg^{-1}, p.o.) reduced inflammation using an APP transgenic animal model (B6.129-Tg (APPSw) 40Btla/J) by decreasing the level of pro-inflammatory cytokines IL-1α, IL-1β and IL-6 and both COX enzymes (COX-1 and 2) in the brain[4].

In this present study, the LPS-induced neuroinflammation has been further characterized by decreasing the levels of anti-inflammatory cytokines such as IL-10 and TGF-β_1 in the mouse brain. Similarly, previous reports also supported the dysregulation of anti-inflammatory activities by reduction of both IL-10 and TGF-β_1 levels followed by LPS (250 μg kg^{-1}, i.p.) induction[11,12]. Presently, ciproxifan in the doses 1 and 3 mg kg^{-1} attenuated LPS-induced decrease the levels of brain IL-10 and TGF-β_1 in mice suggesting the anti-inflammatory activity. From the results of previous study, using APP transgenic mice, treatment of ciproxifan (1 and 3 mg kg^{-1}, p.o.) significantly elevated the levels of TGF-β_1[4].

The administration of LPS also leads to the breakdown of mitochondrial membrane potential by declining the enzymatic complexes (I, II and IV) activities in these current results. In eukaryotic cells, mitochondria act as a power station

by producing ATP through the oxidation of glucose and other sugars. A series of large protein complexes in the inner mitochondrial membrane transfer electrons from NADH which is created in the citric acid cycle in the mitochondrial matrix, to oxygen, creating a transmembrane electrochemical gradient by pumping protons across the membrane[22]. Among, complex-I, also known as NADH-ubiquinone oxidoreductase, couples electron transport from NADH to quinone with proton translocation across the inner mitochondrial membrane[23]. As part of the respiratory chain's complex II, Succinate Dehydrogenase (SDH) connects the tricarboxylic acid cycle with mitochondrial oxidative phosphorylation[24]. Cytochrome c oxidase refers to a complex IV enzyme that regulates oxidative phosphorylation and also initiates the end step of the electron transport chain reaction in mitochondria[25]. Furthermore, mitochondria can play a role in inflammatory reactions in a variety of ways. For example, mitochondria are responsible for a considerable portion of the cellular energy demand during the immune response[26]. An acute systemic inflammation by a single dose of LPS injection (250 g/mouse, i.p.) resulted in mitochondrial damage manifested as a significant drop in membrane potential and loss of mitochondrial redox function[27]. In addition, in mitochondrial isolation from the hippocampus and prefrontal cortex of rats treated with Intracerebroventricular (ICV) infusion of LPS, the activity of complex enzymes (I, II, IV and V) was shown to be reduced[8]. In LPS-challenged mice, treatment with both dosages of ciproxifan (1 and 3 mg kg^{-1}, p.o.) dramatically restored mitochondrial damage by enhancing MRCC (I, II and IV) activities. As a result, current findings suggested that ciproxifan had a region-specific potential in the LPS-induced neuroinflammatory mouse model.

The aforementioned findings could support ciproxifan neuroprotective efficacy in reversing LPS-induced inflammatory insults by antagonizing pre-synaptic HH_3Rs in the brain. In addition, the study observed the effects of ciproxifan on neuroinflammation-induced mitochondrial dysfunction. The current preliminary pre-clinical findings may support ciproxifan's potential for preventing neuroinflammatory-related neuronal diseases, such as neurodegeneration. The current research has evidenced with few selected cytokines, besides there are several factors are involved in the induction of inflammation in the brain system. Regarding the reversal of mitochondrial dysfunction, the antioxidant system potential of ciproxifan needs to be extended. The current study drew attention to the need for more research into the favourable effects of ciproxifan in the treatment of neurodegenerative illnesses.

CONCLUSION

Collectively, ciproxifan was showed its anti-inflammatory potential by attenuating LPS-induced elevation of COX-2 enzyme activities and pro-inflammatory cytokines (TNF-α and IL-6) levels in the brain. Further, it increased the anti-inflammatory cytokines ($TGF\text{-}\beta_1$ and IL-10) levels. Additionally, pre-treatment with ciproxifan improved the MRCC (I, II and IV) functions in LPS-challenged mice.

SIGNIFICANCE STATEMENT

Overall, attained findings showed that the HH_3R antagonist ciproxifan could be a viable neuroprotective target against neuronal inflammation and mitochondrial damage in the brain when tested in a mouse model. The current findings highlighted that ciproxifan may have beneficial effects in decreasing inflammatory trigger protection and improving mitochondrial functions. The achieved results underline that ciproxifan would be a promising drug in the prevention of neuroinflammatory insults in various neurodegenerative diseases.

ACKNOWLEDGMENT

The authors gratefully acknowledge Qassim University, represented by the Deanship of Scientific Research on the financial support for this research under the number (5601-pharmacy-2019-2-2-I) during the academic year 1440AH/2019AD.

REFERENCES

1. Zhang, A., S. Hao, J. Bi, Y. Bao, X. Zhang, L. An and B. Jiang, 2009. Effects of catalpol on mitochondrial function and working memory in mice after lipopolysaccharide-induced acute systemic inflammation. Exp. Toxicol. Pathol., 61: 461-469.
2. Kamdi, S.P., A. Raval and K.T. Nakhate, 2021. Phloridzin attenuates lipopolysaccharide-induced cognitive impairment via antioxidant, anti-inflammatory and neuromodulatory activities. Cytokine, Vol. 139. 10.1016/j.cyto.2020.155408.

3. Wang, W.Y., M.S. Tan, J.T. Yu and L. Tan, 2015. Role of pro-inflammatory cytokines released from microglia in Alzheimers disease. Ann. Transl. Med., Vol. 3. 10.3978/j. issn.2305-5839.2015.03.49.
4. Mani, V., S.M. Jaafar, N.S.M. Azahan, K. Ramasamy, S.M. Lim, L.C. Ming and A.B.A. Majeed, 2017. Ciproxifan improves cholinergic transmission, attenuates neuroinflammation and oxidative stress but does not reduce amyloid level in transgenic mice. Life Sci., 180: 23-35.
5. Heneka, M.T., M.J. Carson, J.E. Khoury, G.E. Landreth and F. Brosseron *et al*., 2015. Neuroinflammation in Alzheimer's disease. Lancet Neurol., 14: 388-405.
6. Joshi, R., D. Garabadu, G.R. Teja and S. Krishnamurthy, 2014. Silibinin ameliorates LPS-induced memory deficits in experimental animals. Neurobiol. Learn. Mem., 116: 117-131.
7. Fišar, Z., H. Hansíková, J. Křížová, R. Jirák and E. Kitzlerová *et al*., 2019. Activities of mitochondrial respiratory chain complexes in platelets of patients with Alzheimer's disease and depressive disorder. Mitochondrion, 48: 67-77.
8. Tripathi, A., P. Paliwal and S. Krishnamurthy, 2017. Piracetam attenuates LPS-induced neuroinflammation and cognitive impairment in rats. Cell. Mol. Neurobiol., 37: 1373-1386.
9. Gemkow, M.J., A.J. Davenport, S. Harich, B.A. Ellenbroek, A. Cesura and D. Hallett, 2009. The histamine H_3 receptor as a therapeutic drug target for CNS disorders. Drug Discovery Today, 14: 509-515.
10. Bardgett, M.E., N.N. Davis, P.J. Schultheis and M.S. Griffith, 2011. Ciproxifan, an H_3 receptor antagonist, alleviates hyperactivity and cognitive deficits in the APP_{Tg2576} mouse model of Alzheimer's disease. Neurobiol. Learn. Mem., 95: 64-72.
11. Azahan, N.S.M., V. Mani, K. Ramasamy, S.M. Lim and R.M.J. James *et al*., 2020. Mahanimbine-induced neuroprotection via cholinergic system and attenuated amyloidogenesis as well as neuroinflammation in lipopolysaccharides-induced mice. Pharmacogn. Mag., 16: 57-63.
12. Rahim, N.S., S.M. Lim, V. Mani, N.A.M.N. Hazalin, A.B.A. Majeed and K. Ramasamy, 2021. Virgin coconut oil-induced neuroprotection in lipopolysaccharide-challenged rats is mediated, in part, through cholinergic, anti-oxidative and anti-inflammatory pathways. J. Dietary Suppl., 18: 655-681.
13. Bullón, P., L. Román-Malo, F. Marín-Aguilar, J.M. Alvarez-Suarez, F. Giampieri, M. Battino and M.D. Cordero, 2015. Lipophilic antioxidants prevent lipopolysaccharide-induced mitochondrial dysfunction through mitochondrial biogenesis improvement. Pharmacol. Res., 1-8.
14. Chen, W.W., X. Zhang and W.J. Huang, 2016. Role of neuroinflammation in neurodegenerative diseases. Mol. Med. Rep., 13: 3391-3396.
15. Chen, H. and D.C. Chan, 2009. Mitochondrial dynamics-fusion, fission, movement and mitophagy-in neurodegenerative diseases. Hum. Mol. Genet., 18: R169-R176.
16. Wang, J., B. Liu, Y. Xu, H. Luan and C. Wang *et al*., 2022. Thioperamide attenuates neuroinflammation and cognitive impairments in Alzheimer's disease via inhibiting gliosis. Exp. Neurol., Vol. 347. 10.1016/j. expneurol.2021.113870.
17. Rojo, L.E., J.A. Fernández, A.A. Maccioni, J.M. Jimenez and R.B. Maccioni, 2008. Neuroinflammation: Implications for the pathogenesis and molecular diagnosis of Alzheimer's disease. Arch. Med. Res., 39: 1-16.
18. Minter, M.R., J.M. Taylor and P.J. Crack, 2016. The contribution of neuroinflammation to amyloid toxicity in Alzheimers disease. J. Neurochem., 136: 457-474.
19. Rubio-Perez, J.M. and J.M. Morillas-Ruiz, 2012. A review: Inflammatory process in Alzheimer's disease, role of cytokines. Sci. World J., Vol. 2012. 10.1100/2012/ 756357.
20. Perry, V.H., 2004. The influence of systemic inflammation on inflammation in the brain: Implications for chronic neurodegenerative disease. Brain Behav. Immun., 18: 407-413.
21. Consilvio, C., A.M. Vincent and E.L. Feldman, 2004. Neuroinflammation, COX-2 and ALS-a dual role?. Exp. Neurol., 187: 1-10.
22. Sousa, J.S., E. D'Imprima and J. Vonck, 2018. Mitochondrial Respiratory Chain Complexes. In: Membrane Protein Complexes: Structure and Function, Harris, J.R. and E.J. Boekema (Eds.)., Springer, Singapore, pp: 167-227.
23. Fiedorczuk, K. and L.A. Sazanov, 2018. Mammalian mitochondrial complex I structure and disease-causing mutations. Trends Cell Biol., 28: 835-867.
24. Cecchini, G., 2013. Respiratory complex II: Role in cellular physiology and disease. Biochim. Biophys. Acta Bioenerg., 1827: 541-542.
25. Li, Y., J.S. Park, J.H. Deng and Y. Bai, 2006. Cytochrome c oxidase subunit IV is essential for assembly and respiratory function of the enzyme complex. J. Bioenerg. Biomembr., 38: 283-291.
26. Karan, K.R., C. Trumpff, M.A. McGill, J.E. Thomas and G. Sturm *et al*., 2020. Mitochondrial respiratory capacity modulates LPS-induced inflammatory signatures in human blood. Brain Behav. Immun. Health, Vol. 5. 10.1016/j. bbih.2020.100080.
27. Noble, F., E. Rubira, M. Boulanouar, B. Palmier and M. Plotkine *et al*., 2007. Acute systemic inflammation induces central mitochondrial damage and mnesic deficit in adult Swiss mice. Neurosci. Lett., 424: 106-110.