International Journal of Pharmacology

ISSN 1811-7775

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OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.321.330

Research Article
Neuroprotective Effect of Clobenpropit in Lipopolysaccharides-induced Mice via Enhancing Cholinergic Transmission

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Abstract

Background and Objective: Brain histamine and its receptors play a vital role in memory functions and also histamine H_3 receptor antagonist/inverse agonists have been proposed to treat cognitive disorders. This study examines the potential of clobenpropit, a selective antagonist on histamine H_3 receptors on Lipopolysaccharides(LPS)-induced cognitive impairment and neuronal cholinergic deficits in a mouse model. **Materials and Methods:** Consecutively 4 doses of LPS (250 μg kg^{-1}, i.p.) were injected on days 22, 23, 24 and 25 of drug treatment in groups of mice to induce cognitive impairment. Clobenpropit (1 and 3 mg kg^{-1}, p.o.) was administrated continuously for 30 days and cognitive behaviours were assessed using Elevated Plus Maze (EPM), Novel Object Recognition (NOR) and Y-maze. Brain tissues were collected for the estimation of Acetylcholine (ACh) and Acetylcholinesterase (AChE) levels. **Results:** Clobenpropit reversed the behavioural deficits induced by LPS in behavioural models. Reduction of transfer latency in EPM test, higher exploration time with a novel object as well as improvement of discrimination index in NOR test and enhancement of novel arm performance, as well as the total number of both sessions entries in Y-maze test, were observed in LPS-induced mice. Besides, it reversed the LPS-induced cholinergic deficits in the brain by elevating the ACh levels and reducing AChE activities. **Conclusion:** Clobenpropit highlights to induce neuroprotection against LPS-induced cognitive impairments through facilitating cholinergic activity in mouse brain.

Key words: Clobenpropit, lipopolysaccharides, memory, histamine, dementia, acetylcholine, acetylcholinesterase

Citation: Mani, V., M. Arfeen, H.M. Ali, A.M.H. Abdel-Moneim and A. Alhowail, 2022. Neuroprotective effect of clobenpropit in lipopolysaccharides-induced mice via enhancing cholinergic transmission. Int. J. Pharmacol., 18: 321-330.

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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

Reference to the World Alzheimer Report, globally more than 50 million people live with dementia and it is estimated that the number will be reached around 152 million by 2050[1]. Because of the high prevalence, Alzheimer's Disease (AD) and other dementias are considered a major burden in the global healthcare system. AD is one of the neurodegenerative disorders characterized clinically by progressive memory deficits and impaired cognitive functions. Pathologically, the aggregation of plaques due to excessive production of β-amyloid (Aβ) and formation of Neurofibrillary Tangles (NFTs) from phosphorylation of tau protein are lead to neuronal degeneration in AD[2]. Particularly, understanding of exact vulnerability for cognitive deficits resulting in dementia is challengeable, there are several mechanisms including cholinergic deficiency, oxidative stress and neuroinflammation, which are proposed as responsible for memory and learning impairments[3]. Among mechanisms, the cholinergic hypothesis with lowering Acetylcholine (ACh) levels was commonly identified in neurodegenerative diseases and other ageing-related memory deficits[4]. Furthermore, the Acetylcholinesterase (AChE) enzyme involves catalyzes the cleavage of ACh in the synaptic cleft and alters cortical cholinergic neurotransmission. In AD, the excess of Aβ and NFTs causes gradual loss of limbic and neocortical cholinergic innervation. Additionally, treatment with selective cholinesterase inhibitors resulted in an elevation of ACh levels at cholinergic synapses is an effective therapy for the management of AD and dementia[5].

Lipopolysaccharide (LPS) is an isolated component from the cell wall of Gram-negative bacteria. Experimentally it induces neuronal toxicity by stimulating neuroinflammatory responses, memory impairment, cholinergic deficiency and oxidative stress in the brain system. Impairment of memory by LPS is mainly associated with neuroinflammation, which is associated with stimulation of microglial cells, the release of various inflammatory mediators such as cytokines in the brain and the degeneration of brain cholinergic neurons[6]. In an animal model, the intraperitoneal (i.p.) administration of LPS leads to the production of neuroinflammatory cytokines including TNF-α, IL-1 α, IL-1β and IL-6 in the mouse brain hippocampus, which plays a key role in memory functions in the brain[7]. Furthermore, in cholinergic neurons, LPS could reduce ACh generation by impairing choline acetyltransferase function and weakening cholinergic communication between neurons[8]. So, LPS-induced neuronal toxicity could be a suitable model for exploring the underlying mechanism related to memory deficits and cholinergic dysfunction.

The histamine H_3 receptors are distributed in the cortex, basal ganglia, globus pallidus and hippocampus in the brain. They are playing an attractive target for various CNS-related disorders including dementia by regulating the presynaptic release of histamine as an autoreceptor and other cognitive-related neurotransmitters including acetylcholine, dopamine, serotonin and norepinephrine as a heteroreceptor[9]. Clobenpropit acts as a selective antagonist on histamine H_3 receptors, has been reported to improve memory function in numerous behavioural tasks and altered the levels of the neurotransmitter in the animal models. Using the step-through passive avoidance test, the administration of clobenpropit successively reversed the scopolamine-induced learning deficit in mice by activation of the noradrenergic system[10]. Additionally, a bilateral intrahippocampal injection of clobenpropit ameliorates spatial memory deficits induced by MK-801 an eight-arm radial maze task of rats by altering various neurotransmitters levels[11]. Therefore, the current study aimed to explore the neuroprotective effects of clobenpropit on LPS-induced memory deficit and cholinergic dysfunction in mice models.

MATERIALS AND METHODS

Study area: The study was carried out at Pharmacology Research Laboratory, Department of Pharmacology and Toxicology, Qassim University, Saudi Arabia from December, 2020-April, 2021.

Drugs and chemicals: Fine chemicals such as clobenpropit hydrobromide from Cayman Chemical (Ann Arbor, Michigan, USA) and lipopolysaccharides from Sigma-Aldrich Co (St. Louis, MO, USA) were procured. ELISA kits for mouse acetylcholine and acetylcholinesterase were purchased from Cloud-Clone Corp., USA. All other chemicals and solvents were obtained from local suppliers and were used of analytical grade.

Animals: A total number of 24 adult male ICR mice with ages between 8-12 weeks (25-35 g) were obtained from the animal facility, Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Saudi Arabia. Animals were allotted randomly into 4 different groups and each group consists of 6 animals. Three mice were housed in each polypropylene cage and they were allowed food and water *ad libitum* during the whole experimental procedure. All the animals were acclimatized minimum of 7 days in standard laboratory conditions before beginning the experiments. The

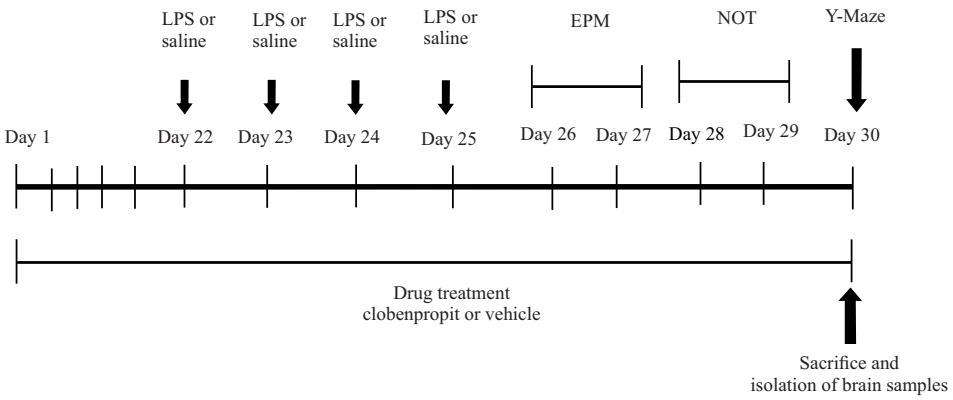

Fig. 1: Timeline administration of drug, behavioural assessments and isolation of brain samples

experimental protocols of the present study were approved by Institutional Animal Ethical Committee, College of Pharmacy, Qassim University, Saudi Arabia (Approval ID 2020 -CP-7) and the maintenance of animals was followed as per the procedures from National Research Council (USA) guide for the care and use of laboratory animals.

Experimental design: Twenty-four mice were randomly divided into 4 groups (n = 6) and respectively treated with vehicle or clobenpropit. The 1st group was a control and treated only with vehicle (normal saline: 10 mL kg^{-1}, p.o./day) for 30 days and injected four doses of normal saline (10 mL kg^{-1}, i.p.,) on days 22, 23, 24 and 25 of treatment schedule (Fig. 1). The 2nd group (LPS) was considered LPS-induced that treated with vehicle (normal saline;10 mL kg^{-1}, p.o./day) for 30 days and injected four doses of lipopolysaccharides (LPS; 250 µg kg^{-1}, i.p.) on days 22, 23, 24 and 25 of treatment. The neurotoxic dose of LPS in the rodent model was followed according to the previous reports[12,13]. The 3rd (LPS+CLO_1) and 4th (LPS+CLO_3) groups were considered treatment groups administered orally with clobenpropit (1 or 3 mg kg^{-1}/day, respectively) for thirty days and injected four doses of LPS (250 µg kg^{-1}, i.p.) as LPS-induced group.

Behavioural tests

Elevated plus maze (EPM) test: The EPM was performed to evaluate the Transfer Latency (TL) of mice according to the procedure described previously[14]. The TL is defined as "the time taken (in seconds) by the mice to move from the open arm to either one of the closed arms with all its four feet"[14]. EPM consists of 4 equal-sized wooden arms 16×5 cm (length×width) and is elevated 25 cm above the ground. Two are closed-arm, both are surrounded by 12 cm high walls and arranged perpendicularly to two open arms on the opposite side. On training day (26th day of treatment), each mouse was placed away from the central platform at the end of an open arm. TL was recorded for each mouse and if any animal fails to enter into closed-arm within 90 sec, it was gently pushed into the closed arm and allowed to explore another two minutes. After 24 hrs (on the 27th day of treatment), TL was recorded another time as retention of learned–task memory.

Novel object recognition (NOR) test: The procedure of the NOR task was followed as described early with minor modifications[15]. An open wooden box (80×60×40 cm) and discriminating objects (familiar object: rectangle box and novel object: Cylindrical box, with same tall and firm) were used to achieve the NOR task. The experiments were performed in 3 different phases such as habituation (28th day of drug treatment), familiarization and test phases (29th day of drug treatment). During the habituation phase, each mouse was allowed to explore freely in the box without any objects for 5 min. The Familiarization Phase (FP) was conducted after 24 hrs (29th day of drug treatment) of the habituation phase. Each mouse was allowed to explore with two similar familiar objects (FO_1 and FO_2) for 5 min during the FP and the exploration times of FO_1 and FO_2 were noted. Total time spent by an animal directing its nose to an object at a distance $\leq$2 cm and touching it with the nose was referred to as exploration time[15]. Following the FP session, the Test Phase (TP) was performed after a 4 hrs inter-trial interval. During TP, again animals were allowed to explore with 2 discriminating objects, (a Familiar Object FO_1: Rectangle box and Novel Object NO: cylindrical box) and the period of this phase was allowed for 5 min. The exploring times of FO_1 and NO were recorded. To explain the discrimination ability of animals between FO_1 and NO during the TP phase was calculated Discrimination Index (DI):

$$DI = \frac{N-F}{N+E}$$

Where:
N = Exploration time of NO
F = Exploration time of the FO_1

Y-maze test: The Y-maze test was conducted according to the previous method with minor modifications[16]. The apparatus was made with 3 wooden arms at a 120° angle (35×5×10 cm) between each other. To differentiate the arm, a different pattern of the picture was posted at each arm end. During the training session (T_1, 30th day of treatment), the novel arm was blocked and each of the mice was allowed to explore freely into 2 familiar arms for 5 min. The number of entries into 2 familiar arms at the T_1 was recorded. After 4 hrs interval from T_1, the test session (T_2) was conducted. In T_2, each mouse was allowed to explore all the arms, including a novel arm for 5 min. The number of entries in known and novel arms and time spent in the known and novel arm were recorded during T_2. An animal was considered to have entered an arm if it entered 85% of its body[16]. The percentage of time spent in the novel arm was calculated as the total time spent in the novel arm divided by the time spent in all the arms during the test session.

Preparation of brain homogenate: End of the 30th day of treatment, end of the Y-maze task, all the mice were sacrificed by cervical dislocation. The whole fresh brain was collected from the skull of each mouse and the brain tissue was homogenized with phosphate-buffered saline (4°C, pH 7.4) using a homogenizer. The collected homogenate was then centrifuged for 10 min at 4000 rpm. The cloudy supernatant aliquot was transferred into 4 mL vials and stored at -80°C for the following biochemical assays.

Biochemical assays using brain homogenate: The total protein content of the brain homogenate was quantified following a biuret colorimetric method (Total protein kit, Crescent Diagnostics, Saudi Arabia). The levels of the neurotransmitter Acetylcholine (ACh) and its metabolizing enzyme Acetylcholinesterase (AChE) in brain homogenate were evaluated using Enzyme-Linked Immunosorbent Assay (ELISA) kits (Cloud-Clone Corp., USA). The absorbance was measured at 450 nm using a microplate reader (EL×800, BioTek Instruments, Inc.).

Statistical analysis: Results were indicated as Mean±Standard Error (SEM). One-way ANOVA test was used for comparisons between the groups and Tukey-Kramer post hoc test was used for calculating significance levels between the two groups (GraphPad version 9, GraphPad Software Inc., United States). Unpaired student's 't'-test was employed for comparison of corresponding groups between 2 different objects in NOR. $p<0.05$ was considered statistically significant.

RESULTS

Clobenpropit shortened the transfer latency (TL) of LPS-challenged mice in the elevated plus-maze (EPM) test: Using EPM test, on retention trail, shorter TL time is considered as an improvement of memory capacity in rodents. The effect of clobenpropit on TL of LPS-induced mice is shown in Fig. 2. When compared among all the groups, differences were noticed for the TL ($F(3,20) = 29.38$, $p<0.001$) using one-way ANOVA analysis. Multiple *post hoc* analyses of comparison between the selected groups showed LPS treatment considerably ($p<0.001$) increase the TL duration as compared to the control group. It is indicating that induction of LPS resulted in cognitive impairment in mice. However, 30 days of pre-treatment with both doses of clobenpropit

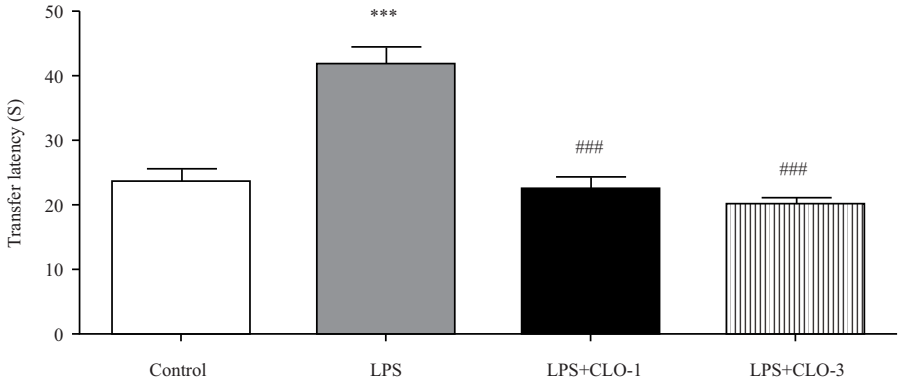

Fig. 2: Effect of clobenpropit (CLO) on transfer latency of lipopolysaccharides (LPS)-induced mice using elevated plus-maze
The results are expressed by Mean±SEM (n = 6). One-way ANOVA [$F(3,20) = 29.38$, $p<0.001$] followed by Tukey-Kramer multiple comparisons test, ***$p<0.001$ as compared to the control group, ###$p<0.001$ as compared to the LPS-induced group

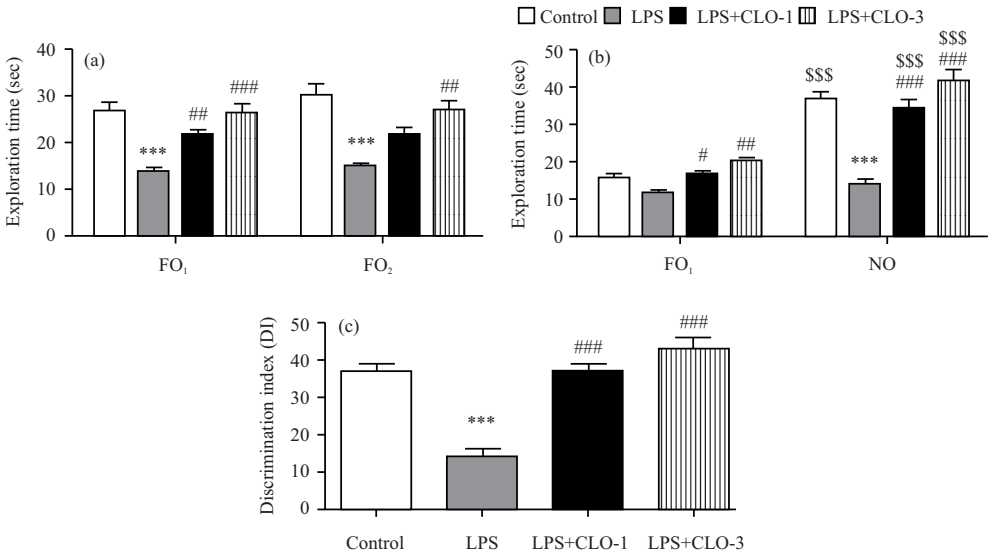

Fig 3(a-c): Effect of clobenpropit (CLO) on lipopolysaccharides(LPS)-induced cognitive impairment in mice using novel object recognition test, (a) Exploration time of two familiar objects (FO_1 and FO_2) during the familiarization phase (FP), (b) Exploration time of familiar (FO_1) and novel (NO) objects during the test phase (TP) and (c) Discrimination index of LPS-induced mice

The results are expressed by Mean±SEM (n = 6). One-way ANOVA [F(3,20) = 14.68, p<0.001 for FO_1 and F(3,20) = 9.658, p<0.001 for FO_2 during T_1, F(3,20) = 10.07, p<0.001 for FO_1 and F(3,20) = 40.80, p<0.001 for NO during T_2, F(3,20) = 29.90, p<0.001 for discrimination index] followed by Tukey-Kramer multiple comparisons test for comparisons of within the groups. The student's unpaired 't' test was used to comparisons of corresponding each group of exploration time. $^{\$\$\$}$p<0.001 as compared to the corresponding group of FO_1, ***p<0.001 as compared to the control group, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p<0.001 as compared to LPS-induced group

(1 and 3 mg kg^{-1}, p.o.) significantly (p<0.001) reduced TL duration as compared to LPS-induced groups. Moreover, the reversal of TL duration is comparable to the control group.

Clobenpropit improved cognitive functions of LPS-challenged mice in the novel object recognition (NOR) test: Figure 3 represents the effect of clobenpropit on different cognitive parameters including exploration time of objects in both familiarization and test phases and discrimination ability of LPS-challenged mice in NOR test. In the Familiarization Phase (FP), using both similar objects (FO_1 and FO_2), significant differences were observed between all the groups in the exploration times of FO_1 (F(3,20) = 14.68, p<0.001) and FO_2 (F(3,20) = 9.658, p<0.001). When compare between both objects (FO_1 vs FO_2), similarity in exploration time was recorded between the same groups (Fig. 3a). Furthermore, treatment of mice with only LPS showed an extensive decrease (p<0.001) in exploration time of both objects FO_1 and FO_2, when matched with the corresponding control group. Improvement in exploration times was shown with drug treatment in LPS-challenged mice. Regarding FO_1, the dose at 1 mg kg^{-1} showed a significance level p<0.01 and at 3 mg kg^{-1} as p<0.001. For FO_2, p<0.01 was recorded at 3 mg kg^{-1} of clobenpropit.

In the Test Phase (TP), when 2 different objects (FO_1 and NO) were used, there was a significant difference (p<0.001) in exploration time between parallel groups when compared between 2 objects (FO_1 vs NO) (Fig. 3b). In the one-way ANOVA analysis among all the groups, there were notable differences in exploration time with FO_1 (F(3,20) = 10.07, p<0.001) and (F(3,20) = 40.80, p<0.001). When compared between control and LPS-induced groups, a significant reduction (p<0.001) was in the exploration time with NO but there were no changes with FO_1. However, substantial improvements in exploration time were noted with both doses (1 and 3 mg kg^{-1}, p.o.) of clobenpropit treatment. For further reference, p<0.001 (at both doses) as with NO and p<0.05 (at 1 mg kg^{-1}) and p<0.01 (at 3 mg kg^{-1}) as with FO_1.

The Discrimination Index (DI) is displayed in Fig. 3c, highlighting the ability of clobenpropit on discrimination between FO_1 and NO during session TP on LPS-induced mice. When compared among the groups, significant differences were found in DI (F(3,20) = 29.90, p<0.001) by analyzing with one-way ANOVA. LPS-treatment showed a significant reduction (p<0.001) in the DI value as compared to control. On the other hand, both doses (1 and 3 mg kg^{-1}, p.o.) of clobenpropit treatment considerably improved (p<0.001) the discrimination ability of LPS-challenged mice in the NOR task.

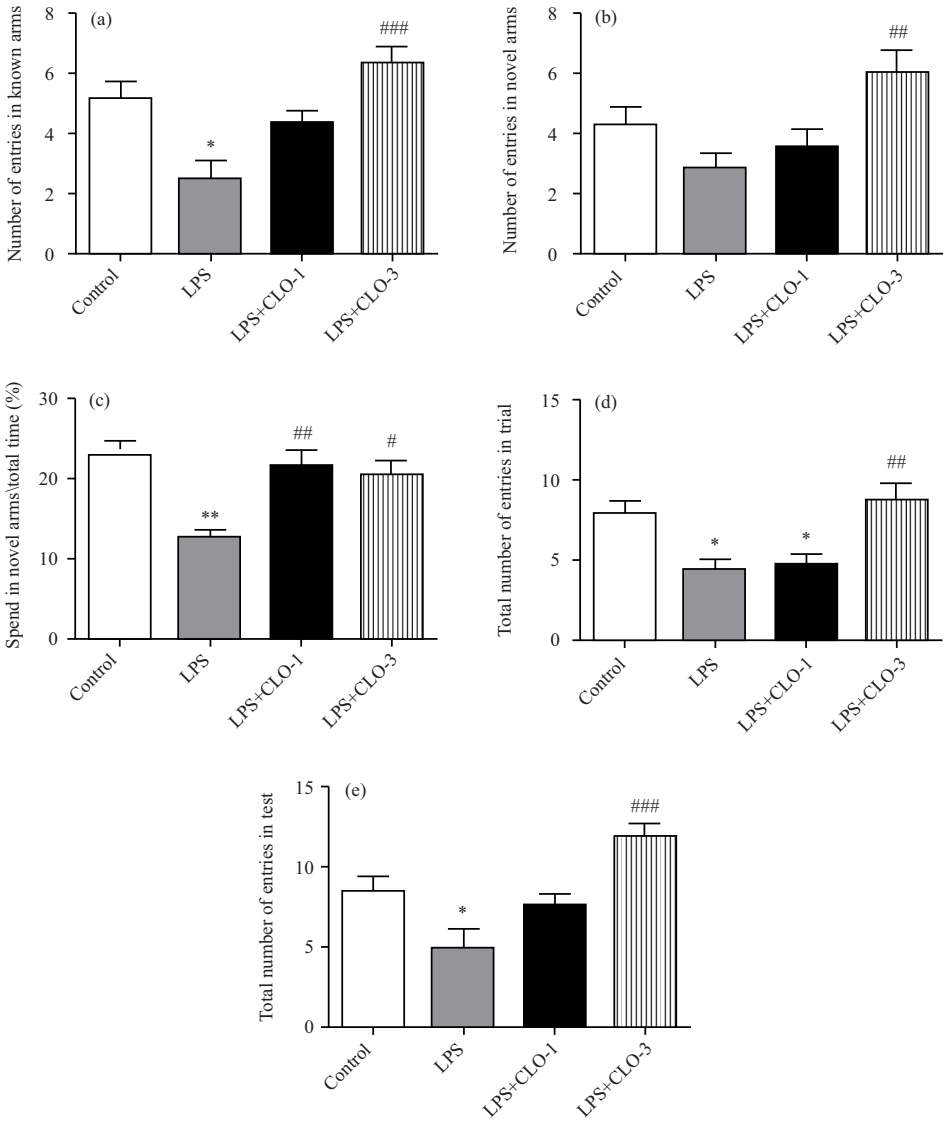

Fig. 4(a-e): Effect of clobenpropit (CLO) on lipopolysaccharides (LPS)-induced cognitive impairment in mice using Y-maze test, (a) The number of entries in known arms in test, (b) The number of entries in the novel arm in test, (c) Percentage of time spent in the novel arm in test, (d) The total number of entries in the trail and (e) The total number of entries

The results are expressed by Mean±SEM (n = 6). One-way ANOVA $F(3,20) = 8.438$, $p<0.01$ for the number of entries in known arm, $F(3,20) = 6.001$, $p<0.01$ for the number of entries in novel arms, $F(3,20) = 7.301$, $p<0.01$ for the percentage of time spend in novel arm, $F(3,20) = 7.991$, $p<0.01$ for the total number of entries in the trail, $F(3,20) = 11.07$, $p<0.001$ for the total number of entries in test] followed by Tukey-Kramer multiple comparisons test. *$p<0.05$ and **$p<0.01$ as compared to the control group, $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ as compared to the LPS-induced group

Clobenpropit improved cognitive functions of LPS-challenged mice in the Y-maze test: The effect of clobenpropit on Y-maze behavioural parameters in LPS-induced cognitive impairment mice is shown in Fig. 4. In focus, Fig. 4a-b are displayed results of the number of entries in the known arms and novel arms. Following one-way ANOVA, in the test session, significant variations have resulted among the groups in the number of entries in known arms ($F(3,20) = 8.438$, $p<0.01$) and a novel arm ($F(3,20) = 6.001$, $p<0.01$). Induced with LPS exhibited a significant decrease ($p<0.05$) in the number of known arms entries as compared to the corresponding control group, but there was no significant variation in the number of novel arm entires. Interestingly, the pre-treatment of a higher dose (3 mg kg^{-1}, p.o.) of clobenpropit indicated a comparable increase in the number of entries in known arms ($p<0.001$) and novel arm ($p<0.01$) when compared with respective LPS-induced group.

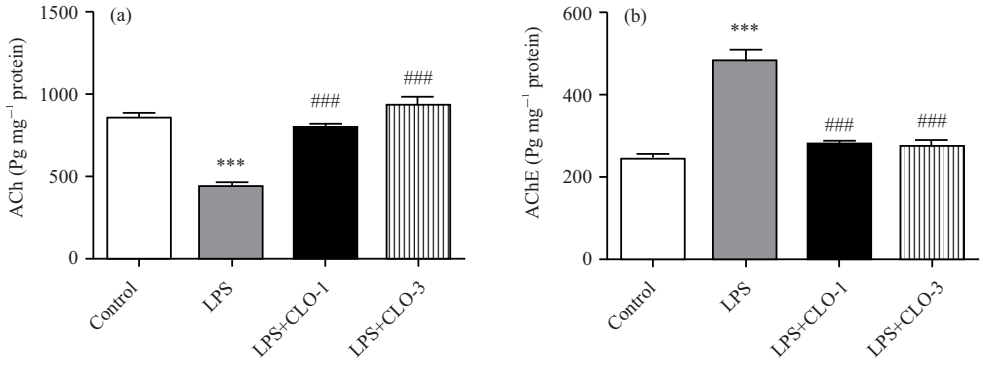

Fig. 5(a-b): Effect of clobenpropit (CLO) on lipopolysaccharides (LPS)-induced cholinergic deficits in mouse brain, (a) Acetylcholine (ACh) levels and (b) Acetylcholinesterase (AChE) activities

The results are expressed by Mean±SEM (n = 6). One-way ANOVA [F(3,20) = 50.17, p<0.001 for ACh; F(3,20) = 31.60, p<0.001 for AChE] followed by Tukey-Kramer multiple comparisons test. ***p<0.001 as compared to the control group; ###p<0.001 as compared to the LPS-induced group

The total time spent by the animal in the novel arm during the test session of the Y-maze test is shown in Fig. 4c. Comparison among the groups showed significant differences ($F(3,20) = 7.301$, $p<0.01$) in the percentage of time spent in the novel arm. LPS-induced mice exhibited shorter duration ($p<0.01$) time spent in the novel arm as compared to control mice. However, clobenpropit administration in LPS-challenged mice showed a comparable longer duration ($p<0.01$ with 1 mg kg^{-1}, $p<0.05$ with 3 mg kg^{-1}) in total time spent in the novel arms.

Furthermore, the total number of entries in trial and test sessions are plotted in Fig. 4d-e, correspondingly. There were considerable differences exhibited among the groups in the total number of entries in the trial ($F(3,20) = 7.991$, $p<0.01$) and test ($F(3,20) = 11.07$, $p<0.001$) sessions when used one-way ANOVA analysis. Induction of LPS demonstrated a significant decrease ($p<0.05$) in the total number of entries on the trial as well as test sessions. Nevertheless, significant increases in the total number of entries were recorded on the trial ($p<0.01$) as well as on test ($p<0.001$) sessions with clobenpropit (3 mg kg^{-1}, p.o.) pre-treatment in the LPS-induced mice group.

Clobenpropit elevated acetylcholine (ACh) and reduced acetylcholinesterase (AChE) levels in LPS-induced brain: The data in Fig. 5 displays the effect of concurrent thirty days of administration of clobenpropit on brain ACh and AChE levels in different groups of LPS treated mice. One-way ANOVA analysis performed among the groups showed significant differences [$F(3,20) = 50.17$, $p<0.001$] in brain homogenate ACh levels (Fig. 5a). When compared to control, it was found that LPS-induced mice exhibited significantly lower ($p<0.001$) ACh levels. Pre-treatment with clobenpropit (1 and 3 mg kg^{-1}, p.o.), however, significantly improved ($p<0.001$) the ACh levels in LPS- challenged mice brains.

Referring to results of AChE activities, when comparing among groups, differences in AChE levels ($F(3,20) = 31.60$, $p<0.001$) were found in brain homogenate (Fig. 5b). Treatment of LPS exhibited a significantly higher ($p<0.001$) in the brain AChE activity as compared to control. However, pre-treatment with both doses of clobenpropit (1 and 3 mg kg^{-1}, p.o.) significantly attenuated ($p<0.001$) the AChE activity in LPS-challenged mice brains.

DISCUSSION

The present results demonstrated that subsequent 4 intraperitoneal administrations of LPS resulted in cognitive deficits and parallel with cholinergic neuronal deficiency. The cognitive declines with LPS induction were evidenced by a significant shortening in Transfer Latency (TL) duration in Elevated Plus-Maze (EPM) test, lowering exploration time as well as discrimination ability in Novel Object Recognition (NOR) test and declined the mouse novel arms performance in Y-maze experiment. Also, LPS-challenge lowered the Acetylcholine (ACh) level by elevating the Acetylcholinesterase (AChE) activities. These obtained results are constant with our current previous reports which revealed that LPS-induction resulted in spatial cognitive decline using the Morris water maze task and cholinergic deficiency in rodents[12,13]. Interestingly, successive thirty days per administration of a histamine H_3 receptor antagonist clobenpropit attenuated LPS-induced neurobehavioural decline by reversing the memory impairment and increased cholinergic neuronal functions.

At present, several histamine H_3 receptor antagonists/inverse agonists are under clinical trials for the management of mild cognitive impairments, mild to moderate stage of Alzheimer's Disease (AD), types of dementia and several cognitive disorders[17]. The ability of the clobenpropit on enhancing memory deficits induced by LPS treatment in mice was evaluated using different maze models such as EPM, NOR and Y-maze. Regarding EPM assay measures the cognitive and memory processes like long-term spatial memory and is used to evaluate the duration of TL. Its principle is based on the natural aversion of testing animals, generally, rodents dislike staying in open and elevated spaces during exploration in EPM. So, shorter TL values from the EPM test support the improvement of spatial memory[3]. Remarkably, the duration of TL time was reduced by the treatment of clobenpropit (1 and 3 mg kg^{-1}, p.o.) in the LPS-induced mice supported the reversal of memory decline, which was induced by LPS toxicity.

Furthermore, the NOR task can be used to investigate different memories in rodents including short-term, intermediate-term, and long-term memory by manipulating the retention interval, or the time that animals must remember the sample items introduced during the familiarization process before moving on to the test phase[18]. The present results focused mainly on 2 different phases, such as the Familiarization Phase (FP) and the Test Phase (TP). In the TP session, a Familiar Object (FO) and a Novel Object (NO) were used to discriminate between both objects as working memory assessment, after a familiarization trial used with both similar objects (FO_1 and FO_2)[19]. It was proved that LPS treatment affects the ability of acquisition and discrimination in animals by lowering the exploration time of two Familiar Objects (FO_1 and FO_2) in FP session and Novel Objects (NO) in TP session, correspondingly. Markedly, pre-treatment of clobenpropit improved the exploration time in both sessions in LPS-induced mice. Additionally, in the TP session, the mice were spent more time with the NO as compared to the FO highlighting the retention capacity, discrimination ability between both objects and remembering the familiar object from FP[18,19]. Moreover, the higher DI value by clobenpropit treatment further supports the improvement of discrimination ability in the LPS-challenge.

Additionally, the present Y-maze results were also supporting the behavioural performance of clobenpropit treatment as parallel to the above two models. The parameters of the Y-maze test related to various behaviours like spatial recognition memory, general exploratory behaviour and anxiety-like behavior[16]. Our current results revealed that thirty days pre-treatment of clobenpropit improved the number of known and novel arms entries in LPS-induced mice supports the improvement of spatial memory. Additionally, prolongation of time spent by animals in a new environment is reflecting its higher coping behaviour, which also reflected the anxiolytic behaviour of the animals[20]. Present treatment of clobenpropit significantly reversed the LPS-induced loss of coping behaviour by extending the ratio of time spent in the novel arm and also the results supported its anxiolytic behaviour in the Y-maze test. Furthermore, increasing the total number of entries by treatment groups in both trial and test sessions demonstrated the increasing the curiosity behaviour of clobenpropit in LPS-induced mice[21].

The present enhancement of cognitive performance in all the maze models with clobenpropit treatment was accompanied by improving the cholinergic transmission, which resulted in elevating the ACh concentration in the LPS-challenged mouse brain. Among CNS neurotransmitters, ACh is one of the major neurotransmitters and it plays a vital role in central neuronal transmission, which supports hippocampus-mediated cognitive abilities including memory functions[5,22]. Early preclinical studies from our laboratory have been demonstrated that treatment of LPS altered the levels of ACh and AChE in the animal brain[12,13]. Both doses (1 and 3 mg kg^{-1}, p.o.) of clobenpropit significantly elevated the ACh levels and declined its hydrolyzing enzyme AChE activity in LPS-challenged mouse brain homogenate. In the line with the present results, our previous research finding resulted in the elevation of ACh levels in transgenic AD mouse (B6.129-Tg (APPSw)40BTLA/J mice6) brain through blocking histamine H_3 receptor by treating ciproxifan, a selective histamine H_3 receptor antagonist[3].

The current results might validate the possible efficacy of clobenpropit on the reversal of LPS-induced cognitive deficits by improving cholinergic transmission through antagonizing pre-synaptic histamine H_3 receptors in the cholinergic neuron. The LPS-induced neuronal toxicity is majorly demonstrated with inflammatory-related insult in neurons. So, the present preliminary pre-clinical results may direct the benefits of clobenpropit on the prevention of neuroinflammatory-related neuronal disorders. Challengingly, there is lacking scientific evidence to support the effect of clobenpropit on neuroinflammation. So, further studies need to be extended with exploring the potential of clobenpropit on various inflammatory target mechanisms including cytokine levels, cyclooxygenase activities, reactive oxygen species and secondary messengers.

CONCLUSION

Our results are supported the neuroprotective potential of clobenpropit against LPS-induced cognitive deficits in mice. Enhancement of various behavioural parameters was resulted using elevated plus-maze, novel object recognition and Y-maze tasks. The cholinergic activity was improved by elevating the acetylcholine level and reducing acetylcholinesterase activity also resulted in the LPS-challenged mouse brain.

SIGNIFICANCE STATEMENT

Our study aimed with exploring the potential of clobenpropit on LPS-induced memory deficits and cholinergic neuronal deficiencies in an experimental model. Present results have highlighted the possible beneficiary effects of clobenpropit via antagonizing histamine H_3 receptors on the reversal of LPS-induced memory deficits. The finding credit a therapeutic direction to study the benefits of histamine H_3 antagonists on neuroprotection in neuroinflammatory associated disorders.

ACKNOWLEDGMENT

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

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