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Aripiprazole mitigates lipopolysaccharide-induced memory impairments in rats

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ABSTRACT: Aripiprazole (APZ) is an atypical class of antipsychotics prescribed to patients with schizophrenia, bipolar disorder, and treatment-resistant depression. Preclinical and clinical research have shown that APZ is helpful for cognitive performance in people with schizophrenia who exhibit negative symptoms. An endotoxin extract from gram-negative bacteria lipopolysaccharide (LPS) was reported to induce memory deficits and used as a model for explaining Alzheimer's disease (AD). This research aimed to determine that APZ could mitigate cognitive deficits with LPS-induced neurotoxicity in rats. Two doses of APZ (1 or 2 mg/kg) were administrated orally for 28 days to groups of rats. Four doses (1 mg/kg) of LPS were injected peripherally to induce neurotoxic. The cognitive characteristics were tested using the Y-maze and the elevated plus maze (EPM). In order to assess the levels of acetylcholine (ACh), the brain tissues were collected. The results highlighted that the transfer latency (TL) time in EPM was prolonged after LPS induction and also it reduced the novel arm performance of the Y-maze test. The APZ administration reduced the TL in EPM and improved the number of novel arm entries and also it extended the animals' time spent in the novel arm. Further, the higher dose of APZ (2 mg/kg, p.o.) improved the cholinergic activities in the brain by increasing the ACh levels after LPS induction. These results are suggested that APZ is one of the therapeutic avenues for cognitive deficits related to schizophrenia and other neuroinflammatory-related neurodegenerative insults.

KEYWORDS: Aripiprazole; Neurodegeneration; Dementia; Elevated plus-maze; Y-Maze; Acetylcholine.

1. INTRODUCTION

Dementia is a condition marked by a decrease in cognitive function, which includes memory, speaking, and problem-solving skills [1]. Psychotic disorders and dementia are two distinct clinical entities that share certain similarities in their clinical presentation, including cognitive impairment and behavioral changes. Moreover, psychotic disorders are characterized by delusions, hallucinations, disorganized thinking, and abnormal behavior, while dementia is marked by a progressive decline in cognitive function, memory impairment, and personality changes [2]. More number of literature reveals a possible connection between the two situations, the nature of this relationship is not yet fully understood. According to a study, those with schizophrenia had a higher risk of dementia, whereas another found that people with dementia had a higher risk of psychosis than those without the condition [3,4]. Additionally, a meta-analysis found that those with dementia have a higher risk of developing psychosis [5]. One potential explanation for this relationship is that the two conditions may share common underlying neurobiological mechanisms. Both conditions have been associated with abnormalities in the dopamine neurotransmitter system [6,7]. According to studies, defects in the dopaminergic system may contribute a role in the emergence of both dementia and psychosis [8]. Overall, the relationship between psychotic disorders and dementia remains an area of active research, and additional research is required to comprehend the nature of this link and the underlying mechanisms.

Antipsychotic medications are commonly used to manage symptoms associated with schizophrenia as well as bipolar disorder. However, a list of mounting reports supported that these medications may also be advantageous for these patients' cognitive impairment. According to several studies, antipsychotic drugs may help people with schizophrenia's cognitive abilities, especially in the areas of attention, working

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memory, and executive function [9,10]. Another study discovered that antipsychotic drug therapy led to improvements in cognitive function in bipolar disorder patients [11]. The mechanisms underlying these cognitive benefits are not fully understood, but some research suggested that antipsychotic medications may improve cognitive function by modulating neurotransmitter systems in the brain, such as dopamine and glutamate [9]. Overall, while antipsychotic medications are primarily used to manage symptoms of psychosis, they may also have potential benefits for cognitive impairment in patients with psychotic disorders. However, more investigation are required to discover the most effective treatment plans for cognitive impairment in this patient population, as well as the mechanisms underpinning these advantages.

Aripiprazole (APZ) is the 2nd generation antipsychotic medication with a distinct mode of action. It is a selective serotonergic 5-HT_{2A} receptor antagonist while functioning as an agonist at 5-HT_{1A} as well as dopamine D_2 receptors. APZ is a successful therapy for several mental diseases, including schizophrenia, bipolar disorder, and severe depression, due to its complex pharmacological profile [12]. Cognitive functions like executive function, memory, and attention are all improved by APZ. In persons with schizophrenia, APZ may improve cognitive performance by modifying the prefrontal cortex's dopamine pathway. By adjusting the dopamine system in this area, APZ may enhance cognitive performance in people with schizophrenia. In addition, APZ has been shown to improve cognitive flexibility and social cognition in patients with autism [13]. In further, the present objects were motivated to understand the neuroprotective effects of APZ on LPS-induced memory impairment in rats, and spatial memory was analyzed to target various cognitive-related parameters by using EPM and Y-maze tests, and also ACh levels were measured in brain tissues to support APZ activity on cholinergic system.

2. RESULTS

2.1 Effect of APZ and LPS administrations on body weights (BW) of the rats

The graph contains the following four groups: control, LPS-induced, APZ1+LPS, and APZ2+LPS. Figure 1 shows how the APZ and LPS treatments affected the experimental animals' BW. On days 1, 7, 14, 21, and 28, the BW was recorded. The outcomes of the two-way ANOVA analysis suggest that no statistically significant variations in body weight were detected between the treatment groups and the days of measurement.

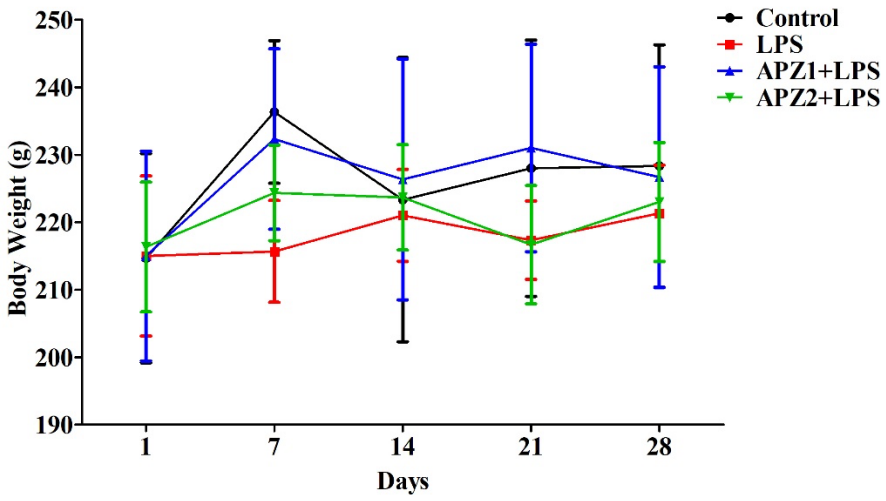

Figure 1. Effect of APZ and LPS on BW (mean ± SEM, n=6).
Two-way ANOVA [F(12,100)=0.1036 for interaction between treatments and days; F(3,100)=0.4854 for between treatments; F(4,100)=0.4764 for between days;] comparisons test was utilized. The p-values for these comparisons were all greater than 0.05, there were no alterations in BW considered between the treatment and days.

2.2 Effect of APZ and LPS administrations on the survival of the rats

Figure 2 shows the effect of APZ and LPS on the survival rate of rats during the treatment period. The rats displayed a 100% survival rate after receiving APZ (1 or 2 mg/kg) for 28 days continuously and LPS (1 mg/kg) for 4 days. The survival rate was observed on days 1, 7, 14, 21, and 28.

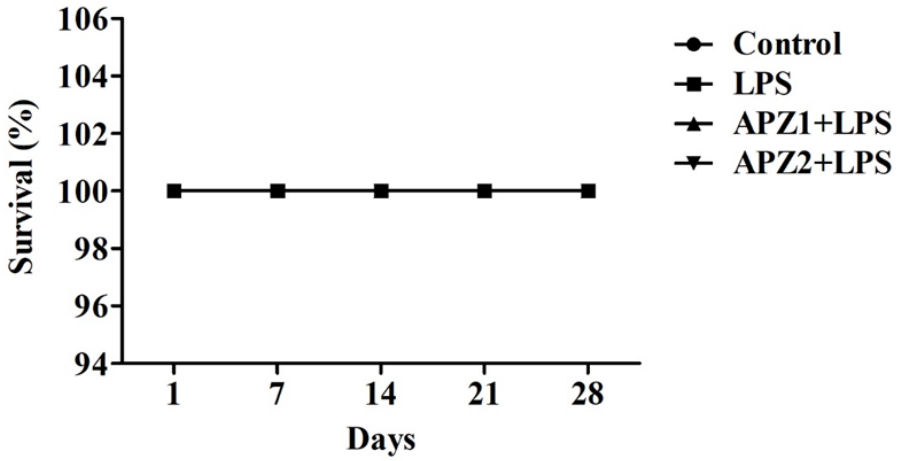

Figure 2. Effect of APZ and LPS on the survival rate of animals. There was no death recorded during the entire treatment.

2.3 APZ protected LPS-induced memory deficits in EPM test

In Figure 3, a common behavioral test for rodents used to measure memory and cognitive retention, the EPM test, demonstrates the influence of APZ on the TL of rats that are LPS-induced. A higher TL indicated an increase in cognitive impairment. The TL is "the time it takes a rat to move from the maze's open arms to its closed arms"[14,15].

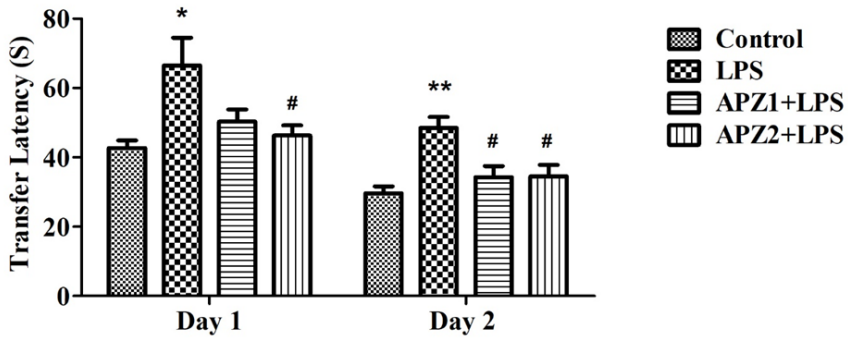

Figure 3. Effect of APZ on day 1 and day 2 TL (s) of LPS-induced rats (mean ± SEM, n=6) using EPM test.
One-way ANOVA [$F(3,20)=4.931$, $p<0.05$ for day 1; and $F(3,20)=7.662$, $p<0.01$ for day 2] comparisons test was utilized.
*$p<0.05$ and **$p<0.01$ vs control group.
#$p<0.05$ vs LPS-induced group.

On the first day, applying one-way ANOVA analysis [$p<0.05$, $F(3,20)=4.931$] showed considerable changes in TL time between experimental groups. Moreover, there was a substantial increase in cognitive impairment in the LPS-induced group associated to the control group (42.67±2.231S), as indicated by prolonged its TL value ($p< 0.05$, 66.50±7.995S). Additionally, the administration of APZ significantly reduced the cognitive impairment brought on by LPS, as shown by the fact that the p-value for the comparison between the APZ2+LPS ($p<0.05$, 46.33±2.894S) and LPS-induced groups.

In continuous, the second day's retention of TL varied significantly [$p<0.01$, $F(3,20)=7.662$] between the groups, according to one-way ANOVA analysis. Further, when considered with the control group

(26.67±1.961S) on the second day, the LPS-induced group has a higher TL value ($p<0.01$, 48.50±3.128S), which showed that LPS-induced cognitive impairment. Additionally, the TL values for the APZ1+LPS ($p<0.05$, 34.33±3.169S) and APZ2+LPS ($p<0.05$, 34.50±3.314S) groups against the LPS-induced group, demonstrated that the administration of APZ significantly reduced the cognitive impairment brought on by LPS on day 2 as well.

2.4. APZ did not alter the number of known arms entries (NKAE) in LPS-induced rats during the Y-maze test session

The results from Figure 4A explain how APZ affects the NKAE of rats individually in the Y-maze. The control group, LPS-induced group, APZ1+LPS, and treatment affect the NKAE made by rats ($p<0.05$, 3.17±0.477) of the Y-maze as paralleled to the control rats (5.50±0.428). The findings revealed how LPS affects NKAE. The graph's statistical findings imply that LPS treatment affects the number of times that rats enter the known arms of the Y-maze. Parallel to the LPS-induced rats, both the treatment groups APZ1+LPS and APZ2+LPS did not alter significantly the NKAE during the test session.

2.5. APZ improved the number of novel arm entries (NNAE) in LPS-induced rats during the Y-maze test session

Using a Y-maze, Figure 4B, illustrates the impact of APZ on the NNAE of LPS-induced rats. It was noted that the treatment of APZ and LPS successfully altered the NNAE [$p<0.01$, $F(3,20)=6.418$] by animals, using One-way ANOVA analysis. The LPS-induced group has a lower number of entries ($p<0.01$, 2.00±0.447) as referred to the control rats (4.50±0.428). However, the NNAE was significantly increased in APZ1+LPS and APZ2+LPS groups ($p<0.05$, 3.83±0.307, and 4.00±0.516, respectively). Therefore, it is believed that the differences between these groups have a lower likelihood of occurring by chance alone than the difference between the LPS-induced and control groups.

2.6. APZ attenuated the percentage of time spent in the novel arm (TSNA%) in LPS-induced rats during the Y-maze test session

As seen in Figure 4C, APZ affects the TSNA% during the test session of LPS-induced rats using the Y-maze. The statistical analysis revealed the alterations [$p<0.01$, $F(3,20)=6.029$] in the TSNA% during the test session when analyzing between the groups. Further, the LPS-induced group has a lower proportion of time spent in the novel arm ($p<0.05$, 8.22±1.08%) as parallel to the control (13.94±1.47%). These suggested that the LPS induction had a significant impact on the TSNA% and that the rats exposed to LPS may have suffered cognitive deficits. Moreover, the APZ2+LPS group has a higher TSNA% ($p<0.01$, 14.00±1.007%) as referred to LPS-induced. These results highlighted the LPS-treated rats' cognitive behavior deficits were significantly protected by the APZ therapy.

2.7. APZ increased the total number of entries (TNE) in LPS-induced rats during Y-maze the trial session

The results show in Figure. 4D, a significant variance [$p<0.001$, $F(3,20)=25.01$] in the TNE during the trial session among the treated groups. In continuous, the LPS-induced group presented a dropping in the TNE into two known arms ($p<0.001$, 3.50±0.764) as compared to the control group (8.00±0.365). In addition, the higher TNE in both treated groups such as APZ1+LPS and APZ2+LPS ($p<0.001$, 9.67±0.843 and 10.83±0.477, respectively) when referred to the LPS-induced rats, these were supported the improvement of maze performance by APZ treatment in LPS induction.

2.8. APZ augmented TNE in LPS-induced rats during the Y-maze test session

On the other hand in Figure 4E, highlights the effect of APZ and LPS administration on the TNE in test sessions of animals' Y-maze performance. The one-way ANOVA analysis between all treated groups showed an alteration [$p<0.01$, $F(3,20)=7.937$] in the TNE performing the test session. Additionally, the LPS-induced group ($p<0.001$, 5.167±0.833) showed a considerable impact on the TNE during the test session as compared to the control group (10.00±0.365). Also for the treated groups APZ1+LPS and APZ2+LPS presented an increase in the TNE ($p<0.05$, 8.167±0.749 and 8.667±0.843, respectively) as matched to the LPS-induced group. These suggested that the LPS-induced rats' cognitive behavior was significantly improved by the APZ therapy.

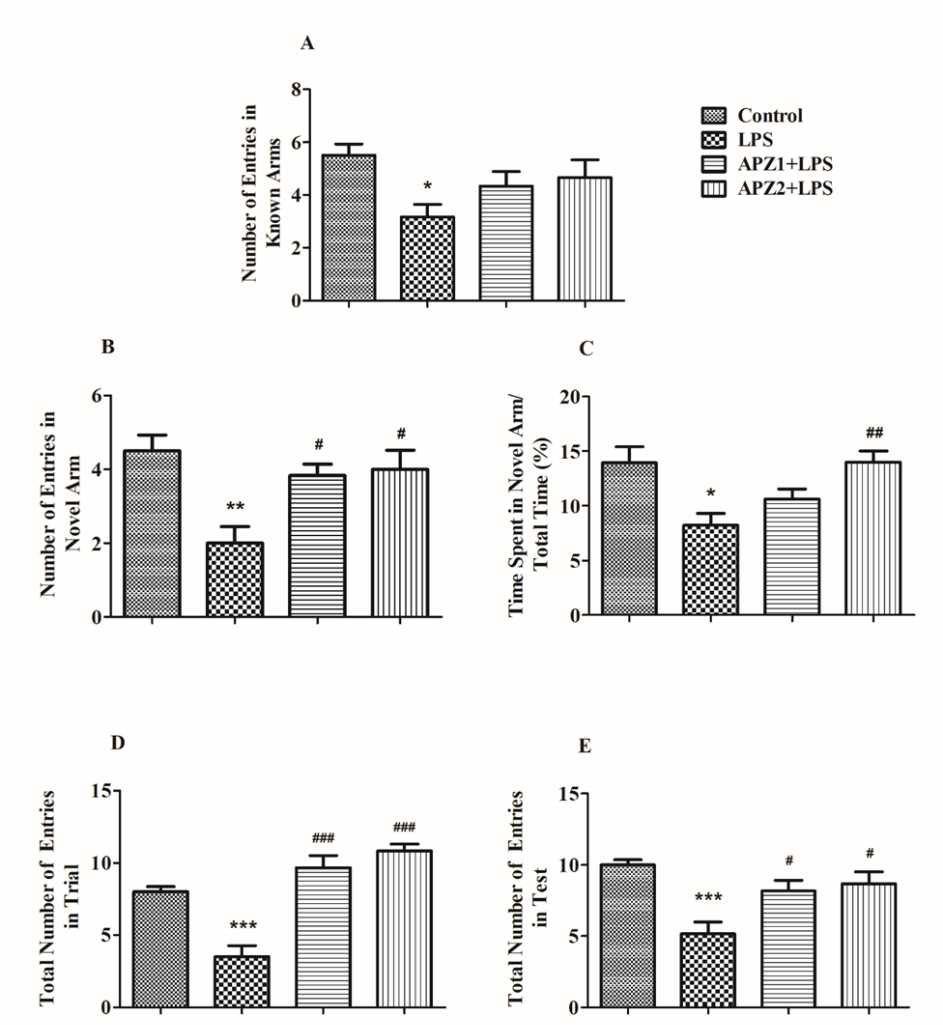

Figure 4. Effect of APZ on (A) the NKAE in the test, (B) the NNAE in the test, (C) the TSNA% in the test, (D) the TNE in the trial, and (E) the TNE in the test of LPS-induced rats using Y-Maze test (mean ± SEM, n=6).
One-way ANOVA [$F(3,20)=3.206$, $p<0.05$ for the NKAE in the test; $F(3,20)=6.418$, $p<0.01$ for the NNAE in the test; $F(3,20)=6.029$, $p<0.01$ for the TSNA% in the test; $F(3,20)=25.01$, $p<0.001$ for TNE in the trial; and $F(3,20)=7.937$, $p<0.01$ for TNE in the test] comparisons test was utilized.
*$p<0.05$, **$p<0.01$, and ***$p<0.001$ vs control group.
#$p<0.05$, ##$p<0.01$, and ###$p<0.001$ vs LPS-induced group.

2.9. APZ elevated LPS-induced rats' brain ACh levels

Figure 5 shows the impact of APZ on ACh levels in rat brains exposed to LPS. After treatment with targeted groups, there was a significant change in brain ACh levels [$F(3,20)=5.778$, $p<0.01$] noted. It was demonstrated that LPS induction decreased the brain ACh levels ($p<0.05$, 204.6±10.35 pg/mg protein) in rats as considered to the control rats (263.2±15.18 pg/mg protein). When focused on LPS-induced levels, the APZ2+LPS treatments significantly raised ACh levels ($p<0.05$, 253.3±13.06 pg/mg protein). In contrast, there

were no appreciable differences between the ACh levels in brain homogenate after treatment with APZ1+LPS and LPS (216.8±6.471 pg/mg protein).

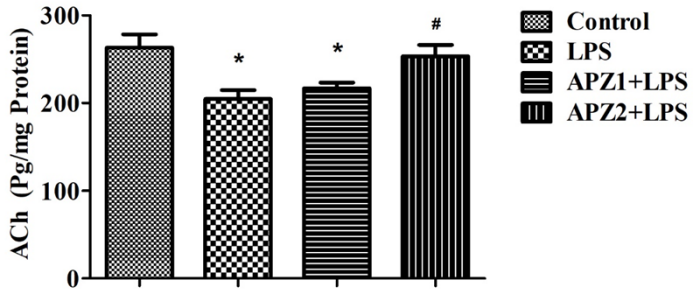

Figure 5. Effect of APZ on brain ACh levels in LPS-induced rats (mean ± SEM, n=6). One-way ANOVA [F(3,20)=5.778, p<0.01] comparisons test was utilized.
*p<0.05 vs control group.
#p<0.05 vs LPS-induced group.

3. DISCUSSION

Neurodegeneration especially dementia is a major public health issue that impacts millions of individuals worldwide. Even though it affects a high number population and results in high financial strain, there is no solution for dementia currently, and available therapies only provide symptomatic alleviations [16]. As a result, more study into the basic processes of neurodegeneration and the creation of more effective therapies is urgently needed. Neuroinflammation is one of the key mechanisms in neurodegenerative illnesses including AD, which is a major cause of dementia [17]. LPS is an endotoxin that induces various levels of cognitive deficits and neurotoxicities by resulting from the induction of neuroinflammation, which is parallel to AD [18]. Early research suggested that new-generation antipsychotics including APZ may help people with schizophrenia by improving their both positive and negative symptoms, cognitive performance, depressive behaviors, and anxiety issues [19,20]. The current study investigated the potential therapeutic advantages of APZ on LPS-induced substatıal cognitive functions in rats. The results demonstrate that LPS significantly increased cognitive impairment, while the administration of APZ significantly reduced it and improved cholinergic functions. These results might imply that APZ can help lessen cognitive impairment brought on by neuroinflammation.

A component of the cell wall is LPS immunostimulatory endotoxins bacteria classified as Gram-negative. The innate immune response to infections is triggered when a substantial bacterial Toll-like receptor 4 (TLR4) ligand is present [21]. Besides, LPS-induced neuroinflammation can cause a number of cellular alterations, including neurotoxicity, oxidative stress, and mitochondrial dysfunction, which may aid in the formation of neurodegenerative disorders [22]. In the rat model of LPS-induced cognitive impairment, intraperitoneal and intracerebroventricular LPS injections are the two most typical methods [18]. Studies have shown systemic administration of LPS-induced impairments in spatial memory, recognition memory, and learning ability followed by neuroinflammation [14]. Presently, followed four doses of LPS (1 mg/kg, i.p.) injections caused cognitive deficits by extending TL values in EPM and lowering the number of entries in Y-maze performance. Also, the same doses of LPS reduced the cholinergic functions by reducing the ACh levels in the rat's brain.

The potential therapeutic benefits of APZ were investigated, APZ with co-administration of mirtazapine reversed MK-801-related cognitive deficits in the NOR test and increased the neurotransmitters like serotonin and noradrenaline levels in the cortex, it is well known both of the neurotransmitters play in cognitive symptoms of schizophrenia patients [23]. In the more investigation (MWM) test, add-on therapy with cilostazol, APZ showed improvement in vascular dementia by shortening the escape latency [24]. In phencyclidine-induced memory deficits, APZ improved the exploratory preference of mice in the NOR test and these results supported the enhancement of recognition memory by APZ [25]. Further, APZ with fluoxetine improved the ethanol-induced depressant, anxiety, and memory impairment in various maze models such as MWM, forced swimming, and two-compartment exploratory tests in rats [26]. Moreover, combined therapy of APZ, olanzapine, and enrichment environment reversed tobacco smoke exposure-induced spatial memory deterioration by improving behaviors of escape latencies and crossed quadrants in the MWM test [27]. Presently, the study was performed with two maze models, such as EPM and Y-maze. In

both tests, the treatment of APZ resulted with an improvement in cognitive performance in targeted maze parameters on LPS-induced rats. In extensions, there was an enhancement in ACh levels with a higher dose of APZ (20 mg/kg, p.o.) in LPS-induced brain tissues.

The EPM is a behavioral test commonly used to assess cognitive impairment in rodents. However, the EPM has also been used in studies investigating the effects of inflammation on behavior. For example, studies have shown that the administration of LPS which induces a systemic inflammatory response, can lead to cognitive impairment in rodents as measured by the EPM [14,28]. In the context of the EPM test, the TL values on the initial training day denoted the animals' capacity for learning, while on the subsequent day, these TL values were indicative of their memory retention abilities. In general, the longer the TL values on each experimental day of the EPM test reflect the impairments of learning and memory behaviors, respectively [14,15]. Conferring to the current EPM data, the TL in the LPS-treated group was higher than that in the control group, providing proof of the cognitive impairment brought on by inflammation. After receiving APZ therapy, the TL returned to the level that was seen in the control group. Together, these findings showed that memory function was decreased in mice with inflammation caused by LPS and that this decreased function returned to normal following APZ treatment.

The second maze model, Y-maze is a wide model for assessing rodents' spatial memory tasks by looking at the animals' propensity to explore novel locations. It also extended to evaluate spatial recognition memory, anxiety-like behavior, and general exploratory behavior of animals [15]. Presently, according to the procedure, each of the animals was allowed to explore initially in a trial session followed by a test session with three hours interval period. The listed parameters include the NKAE as well as NNAE in the test session, the TSNA% in the test, and the TNE in the trial, as well as test sessions were recorded. Considering the NKAE into NNAE correlated to the alterations capability of rats in arm discrimination behavior [29]. The current findings demonstrated that LPS treatment decreased both known and novel arm entries, indicating a lesser capacity for LPS induction to change arm discrimination. Additionally, the decrease in NNAE highlights the animals' lack of spatial memory [15,29]. It's interesting to note that APZ treatment (1 and 2 mg/kg, p.o.) reversed the NNAE during the Y-maze test, evidencing that LPS-related memory impairment was attenuated.

The TSNA% against the total time, which considered time spent in all the arms and the centre of the Y-maze, was used to calculate the animals' coping behavior to the novel environment throughout the test session. The animal's increased anxiety activity served as a signal that the value of its coping behavior had decreased [30]. The LPS treatment demonstrated a lower value of coping behavior compared to the control group, referring that animals stay lower time in the novel environment, which was associated with anxiety-like conduct. To counteract the LPS-induced loss of coping strategy, oral administration of APZ (2 mg/kg) increased the amount of time rats spent in a novel environment. Another indication of the animals' curiosity behaviors in the Y-maze test was the TNE during trial and test sessions [31]. A group of rats exposed to LPS showed a lower capability of curiosity behaviors by lower the TNE in both sessions, besides both APZ doses had significantly more curious behavior results with a higher number of both session entries.

In order to better understand the processes underlying the APZ's memory-improving effects, its impact on cholinergic transmission in LPS-induced rats was investigated. The central cholinergic neurons, which regulate signals across the cerebral cortex, maintain normal cognitive activities. Reduced learning, memory, and attention have all been linked to cholinergic neuron loss brought on by inflammatory susceptibility [32]. Early preclinical studies revealed that LPS treatment impact the release and production of neurotransmitters including ACh in the rodent's brain [33]. LPS induction resulted in a loss of cholinergic neurons in the basal forebrain and also altered the levels of ACh as well as its metabolic enzyme acetylcholinesterase (AChE) levels in the brain [14,34]. Recently, LPS administration reduced the release of ACh from the prefrontal cortex area and hippocampus in experimental rats [35]. Furthermore, the studies supported that memory deficits induced by LPS are considered a preclinical experimental model it parallels AD [18]. The present results also found the elevation of ACh levels in the rat's brain after four successful peritoneal injections. Remarkably, continuous twenty-eight days pre-administration of APZ (2 mg/kg, p.o.) enriched the cholinergic activity by elevating ACh levels in LPS-induced rats. This enrichment of cholinergic functions by APZ might be contributed to the reversal of cognitive parameters from both maze tests after LPS induction.

4. CONCLUSION

Overall, the study examined the effects of APZ on cognitive dysfunctions in rats induced by LPS. The results evidenced that cognitive impairment was significantly more prevalent in the LPS-induced rats. The administration of APZ significantly diminished the cognitive impairment brought on by LPS, resulting

in lowering the TL values in the EPM test and improving the novel arm performance in Y-maze tests. Furthermore, the improvement of cognitive function from APZ was supported by the elevation of cholinergic neuronal functions by increasing ACh levels in the LPS-induced rat brain. Hence, the study's findings imply that APZ might be useful in reducing cognitive impairment and improving cognitive function in neuroinflammatory-related neurodegeneration. Based on this preliminary study's results, it can be recommended that APZ can be further explored for developing treatments for cognitive dysfunction in neuroinflammatory-related neurodegenerative diseases including AD.

5. MATERIALS AND METHODS

5.1 Animals

Twenty-four male Sprague Dawley rats (age 3, 150–200 g) were utilized in this experimentation that was permitted by the Committee of Research Ethics, Deanship of Scientific Research, Qassim University (Project Number: 23-20-14; 12-1-2023). Each of the four groups comprised six rats, that were randomly assigned to experimental groups. Rats were housed in the College of Pharmacy's animal facility and temperature as well as humidity were maintained at 22±1 ^{o}C and 45 to 55 percent, respectively. The rats in this study were fed a regular diet and given free access to water.

5.2 Vehicle

The APZ was procured from Tabuk Pharmaceutical, Tabuk, Saudi Arabia. It was given to rats via oral gavage after being suspended in sodium carboxymethyl cellulose (0.5% w/v CMC). The LPS was acquired from Sigma-Aldrich, USA, and injected intraperitoneally to rats after being diluted in normal saline.

5.3 Experimental Design

The rats were separated into four groups and given the following treatments:

Control group: Received vehicle (CMC, 0.1 mL/100g, orally) for the 28 days of the research. They were also injected with normal saline (0.1 mL/100g, i.p.) on days 22, 23, 24, and 25.

LPS-induced group: Received vehicle (CMC, 0.1 mL/100g, orally) for the duration of the 28 days of the research. They were also injected with LPS (1 mg/kg, i.p.) on days 22, 23, 24, and 25.

APZ1+LPS group: For 28 days, APZ (1 mg/kg) was orally administered and also injected with LPS (1 mg/kg, i.p.) on days 22, 23, 24, and 25.

APZ2+LPS group: For 28 days, APZ (2 mg/kg) was orally administered and also injected with LPS (1 mg/kg, i.p.) on days 22, 23, 24, and 25.

The APZ doses (1 and 2 mg/kg) were selected by prior research [25], while the LPS dosages and timing schedules were chosen by recent publications [14,28].

The EPM and Y-maze tests were employed to measure cognitive functions. The EPM training and testing sessions were placed on days 26 and 27. On day 28, the Y-maze examination, training, and test sessions were held. Following the Y-maze test, the brain tissues were collected and preserved for bioassay of ACh (Figure 6).

5.4 Elevated Plus-Maze (EPM)

It is a behavioral paradigm that is considered to be a neutral behavioral model, and it is commonly used to evaluate the memory of rats [36]. It is built of wood, is raised above the ground by fifty centimeters, has a length of fifty centimeters and a width of ten centimeters, and the height of the closed arms is forty centimeters. The experiments were done in two parts with training taking place on day 26 and a test session on day 27. On the day of training, each rat was put into one of the open arms, and TL was noted as a learning capability. After twenty-four hours, the rat was kept in the same location as at the beginning of the experiment, and the TL time of each animal was determined and considered the retention capacity of memory. “The time it takes for a rat to use all four of its legs to enter any one of the closed arms” is referred to as TL [14,15].

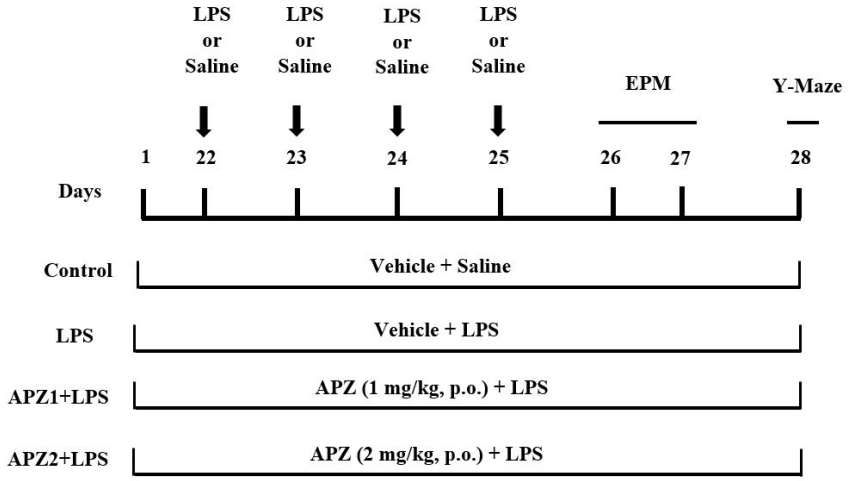

Figure 6. Timeline of the drug treatment and experiments.

5.5. Y-Maze

It is used to assess spatial memory by the rat's capacity to discern the difference between the unfamiliar (novel) and the known arms [14,15]. Y-maze is constructed of wood and each of its arms has the same size, 50 cm in length, 10 cm in width, and height 40 cm, and the angle between arms is 120˚. Each arm has pasted with a different picture at the end of the arm to make space for the rat to recognize and distinguish between them in the training and test sessions. It is included with both training and test sessions and that was performed on day 28 of drug treatment. In the training session, a total of three arms, two of them (known) were kept open while the third one was closed (novel). Each of the rats was given five minutes to acquaint themselves with the space in between the two opening arms before being removed. In the training session, the TNE into both know arms was counted. After three hours, the test session continued with the closed arm being opened for a period of five minutes in order to examine the subject's identification of the rat. The NKAE, NNAE, TSNA%, and TNE were measured [14,15].

5.6. Brain samples collection

At the end of the maze tests on the 28th day, all the rats were euthanized via cervical dislocation, with the administration of mild ether anesthesia. The brain tissues were isolated and put in ice-cold phosphate-buffered saline (pH of 7.4) at a temperature of 4 ^{o}C. Each of the brain homogenates was prepared by using a homogenizer followed by a centrifuge at a speed of 4000 rpm for ten minutes. The collected homogenates were kept at a temperature of -80 degrees Celsius for further examination. The BCA test technique was used in order to calculate the total amount of protein contained in each sample.

5.7. Analysis of ACh in brain homogenate

For a cholinergic parameter, the amount of ACh (catalog number: MBS262132) was measured. To analyze ACh levels in brain tissues, specific sandwich ELISA kits from MyBioSource (MyBioSource, Inc., USA) were employed. In the end, the intensity of color formed was measured by using a microplate reader (ELx800, BioTek Instruments, USA), and the absorbance was measured at 450 nm. A standard curve was plotted to estimate the specific quantity of ACh levels of each homogenate.

5.8. Statistical Analysis

Using GraphPad Prism, statistical analysis was carried out. BW comparisons were evaluated through a two-way ANOVA, incorporating treatments and days as factors, and subsequently, *post-hoc* analysis was performed using the Bonferroni test. Regarding other analyses, the one-way ANOVA was employed to compare all the groups, and to determine statistical significance between specific pairs of groups, a Tukey-Kramer *post-hoc* test was utilized. Statistical significance was concluded when the p-value was less than 0.05.

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