International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2020.27.32.

Research Article
Ciprofloxacin Produces Memory Deficits in Male Mice

Ahmad H. Alhowail

Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, 51452 Al-Qassim, Kingdom of Saudi Arabia

Abstract

Background and Objective: Ciprofloxacin is an antibiotic that is widely used to treat bacterial infections, but it is known to cause side-effects, including cognitive impairment. However, the associated mechanisms are still elusive. The objective of the present study was to examine the effect of ciprofloxacin on cognitive impairment using a mouse model. **Material and Methods:** Male mice (weight = 18-30 g) were divided into control and treatment groups (n = 8/group). The mice in the treatment group were administered 15 mg mL^{-1} of ciprofloxacin orally through their drinking water for 7 days. The dosage was comparable with that of the recommended clinical dosage for patients with bacterial infections. The memory of the mice was tested using 3 hippocampal-dependent tests: the Y-maze, novel object recognition (NOR) and the elevated plus maze (EPM) test. These tests were performed for 3 days, starting from day 4, during the 7 day treatment. **Results:** The mice treated with ciprofloxacin exhibited a decline in memory function in both the Y-maze and EPM tests. However, the decline was statistically significant ($p \leq 0.05$) only in the EPM test. The results of the NOR test showed no difference between the control and ciprofloxacin-treated mice. **Conclusion:** The present study indicated that ciprofloxacin administration causes a decline in the memory function of mice, as measured by the EPM test. Further research is needed to characterize the pathway of memory impairment and the potential mechanisms underlying the cognitive deficits.

Key words: Ciprofloxacin, memory impairment, behavioral tests, cognitive impairment, antimicrobial agents, EPM test, fluoroquinolones

Citation: Ahmad H. Alhowail, 2020. Ciprofloxacin produces memory deficits in male mice. Int. J. Pharmacol., 16: 27-32.

Corresponding Author: Ahmad H. Alhowail, Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, 51452 Al-Qassim, Kingdom of Saudi Arabia

Competing Interest: The author has declared that no competing interest exists.

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

INTRODUCTION

Antibiotics are widely used against bacterial infections in inpatients and outpatients and approximately 262.5 million courses of antibiotics were used[1] in the United States in 2011. Drug-induced cognitive dysfunction has usually been reported as a side-effect of neuropsychiatric medications[2,3]. Different types of antibiotics are used for different bacterial infections and multiple studies have shown that the antimicrobial drugs have a potential risk of inducing various side-effects, including cognitive impairments[4,5]. The cognitive impairments associated with antibiotic use is reported to induce transient changes that occur while taking the medication[6], but the exact etiology of cognitive dysfunction associated with antibiotics is not yet understood.

Ciprofloxacin belongs to the class of antibiotics called fluoroquinolones, which are broadly used in the treatment of bacterial infections. Fluoroquinolones, in general, have many side-effects, including adverse effects on the central nervous system (CNS) and the cardiovascular system[4,7]. A number of studies have reported that some fluoroquinolone medications can cause ventricular tachycardia, ventricular fibrillation and sudden cardiac death[8,9]. They have also been reported to affect CNS function[10], which can result in cognitive impairment. A study by Segev *et al.*[11] proposed that the quinolone class of antibiotics can potentially inhibit y-aminobutyric acid (GABA) receptors, increasing CNS excitation. Therefore, the current study evaluated the effects of ciprofloxacin on memory function in mice using a dose that is comparable to the clinically recommended dose for humans.

The results of the present study will advance new knowledge by demonstrating the role of ciprofloxacin on the induction of cognitive impairment in a mouse model. The present research focused on the relationship between the antimicrobial agents and cognitive impairment using a dosage of ciprofloxacin comparable to the recommended clinical dosage for human patients. The cognitive impairment induced by the model was evaluated using three behavioral tests: the Y-maze, novel object recognition (NOR) and elevated plus maze (EPM) test.

MATERIALS AND METHODS

This study was conducted in the month of July, 2019.

Animals: Sixteen male mice (10-11 weeks-old and weighing 18-30 g) were housed individually and were maintained in a controlled and pathogen-free environment (25°C) in the animal house of Qassim University, on a 12:12 h light:dark cycle (the light period started at 6:00 am), with free access to water and a standard chow diet. The animals were observed daily and behavioral tests were conducted during the light period. The mice were divided into 2 groups consisting of 8 animals/group: A ciprofloxacin-treated group and an untreated control group. This research was given ethical approved by the research unit of the College of Pharmacy, Qassim University (ID number 2019-CP-7).

Drug administration: Ciprofloxacin was dissolved in the drinking water at a concentration of 15 mg mL^{-1} and was administered to the mice in treatment group (n = 8). The dosage was within the normal range that is used as a therapeutic dose for patients with bacterial infections[12]. All the mice in both the ciprofloxacin treated and untreated control groups were subjected to behavioral tests on day 4 of ciprofloxacin treatment. The duration of the treatment was 7 days.

Assessment of spatial memory using the Y-maze: The Y-maze test is used to assess the ability of animals to discriminate places they have already explored and their propensity to explore new places[13]. The Y-maze was made of wood and had the following dimensions: 35×7×10 cm. The arms were at a 120° angle to the other arms and were painted brown to make it easier to observe the mice during the experiment. The Y-maze apparatus was placed on the floor and a light was placed on top of each arm to ensure an equal distribution of light. A camera was used to record all the test sessions. The Y-maze tests evaluated the spatial memory of the mice by providing different cues (circles, triangles and later an X) at the end of each arm.

This test consisted of 2 sessions: a training session and a testing session. During the training session, each mouse was allowed to explore 2 arms freely: The start arm, in which it was placed and the familiar arm, which was located at either the left or the right side of the start arm. This session lasted for 15 min. The testing session began 3 h after the training session. During the testing session, the mouse was placed in the same arm (the start arm) as in the training session for 5 min. The mouse was allowed to explore the entire maze, including the new arm (the novel arm). The testing session was video-recorded. The number of entries and the time spent in the novel arm were scored and analyzed[14]. The mouse was considered to have entered an arm when more than half of its body was in it.

Novel object recognition test (NOR): The novel object recognition (NOR) test is a behavioral test that evaluates memory function[15]. The test apparatus had a dimension of 35×35×35 cm and was made up of wood. Three objects were used in the test: two teacups and a novel object (a rectangular box similar in size to the tea cups). The mouse was placed in the apparatus and was allowed to explore the tea cups for 15 min, after which it was returned to its cage for 3 h. The mouse was then placed in the apparatus again for a 5 min testing session, with one of the tea cups replaced with the novel object. The time spent by the mouse near the novel object was recorded, calculated and scored and the results were analyzed[16].

EPM test: The EPM is another apparatus that has been used for behavioral assays and to measure memory function. The EPM was purchased from Medicraft (India). The maze was composed of 2 open and 2 closed arms. The length of each open and closed arm was 30 cm and the width was 5 cm. The height of the walls of the closed arms was 20 cm. The central area between the 4 arms was 5 cm^2. The maze was placed on a stand that elevated it about 30 cm from the floor.

Each mouse was placed in an open arm at the start of the training session. The mouse was allowed to explore the apparatus for 5 min and was then returned to its cage. After 3 h, each mouse was placed in the same location where it was placed during the training session. The time spent in the open arms was measured using a stopwatch. The animal was considered to have entered a closed arm when all its legs were in the closed arm. A video camera was placed above the maze to record each session. The total time spent by the mouse in the open arms was scored and analyzed[17].

Statistical analysis: The independent-sample Student's t-test was used to analyze the differences between the ciprofloxacin-treated and untreated control mice groups. The difference with a $p \leq 0.05$ was considered to be statistically significant. The results are presented as group Mean±SEM and were analyzed using Graphpad Prism 5 software.

RESULTS

Mortality: There was no incidence of death among the mice that received ciprofloxacin (Fig. 1); 100% of the ciprofloxacin-treated mice were alive for the 7 days (Fig. 1).

Behavioral performance in the Y-maze: There were no significant group differences in number of entries into or the

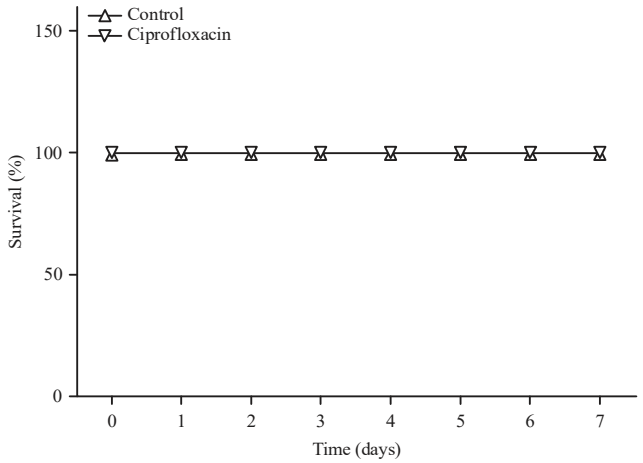

Fig. 1: Survival rate of the mice throughout of the duration of the study

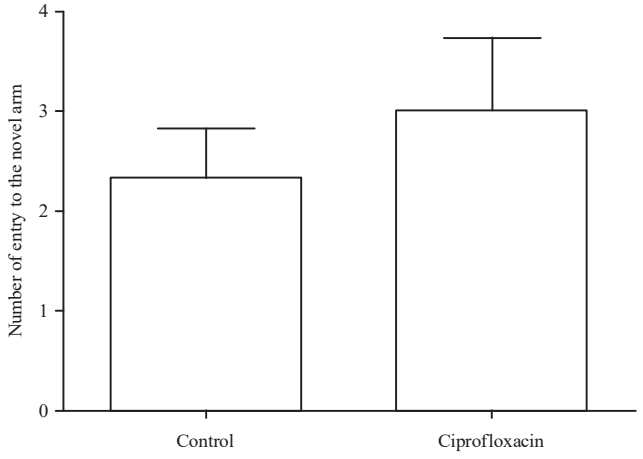

Fig. 2: Number of entries into the novel arm of the Y-maze test

Ciprofloxacin-treated mice exhibited a slight cognitive deficit in terms of their number of entries into the novel arm, compared to the untreated control mice, n = 8/group, $p<0.05$ was considered a statistical significant difference

total time spent in the novel arm (Fig. 2, 3). Moreover, the mice in both groups chose to enter the novel arm at the beginning of the Y-maze test session. These results indicated the ciprofloxacin group of mice had partially impaired memory function, as the increased likelihood of these mice to choose to the familiar arms, versus the novel arm, indicated impaired memory. In addition, the mice also did not to stay a longer time in the novel arm, which is a sign of memory impairment in the Y-maze test.

Effects of ciprofloxacin treatment on the NOR test: The NOR test showed no significant differences between the

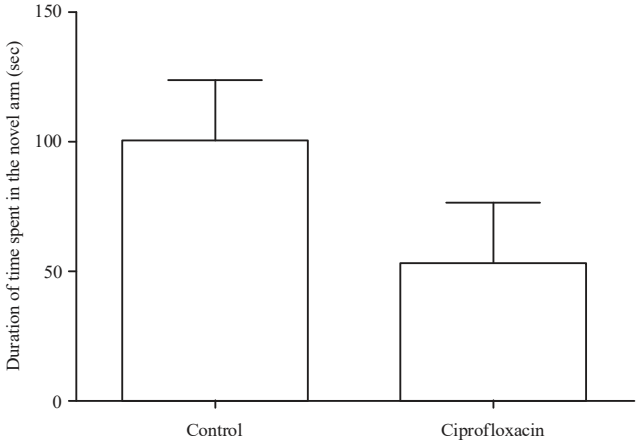

Fig. 3: Duration of time spent on the novel arm of the Y-maze test

Ciprofloxacin-treated mice exhibited a slight cognitive deficit in terms of the total time they spent in the novel arm, compared to the untreated control mice, n = 8/group, p<0.05 was considered a statistically significant difference

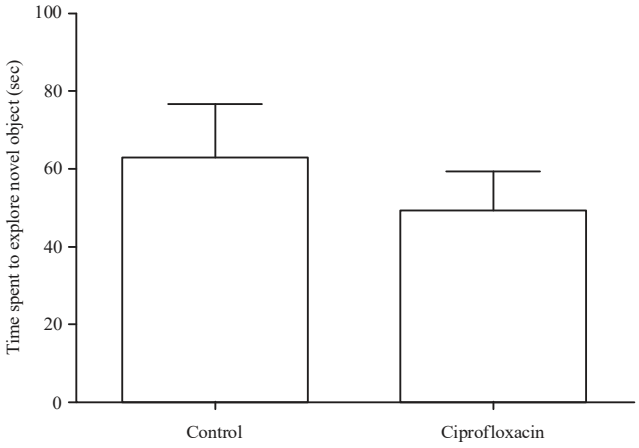

Fig. 4: Novel object recognition (NOR) behavioral test

Results showed no statistically significant difference between the ciprofloxacin-treated and untreated control groups (n = 8/group)

ciprofloxacin group and the control group during the training or testing sessions. The result suggested that there was no memory impairment by the ciprofloxacin treatment in mice (Fig. 4).

Effect of ciprofloxacin on the EPM test: The total time spent in the open arms and the transfer latency on the 4th day of treatment was significantly higher ($p \leq 0.05$) in the mice treated with ciprofloxacin compared to that in the untreated control mice on the EPM test. Thus, the results indicated that the antibiotic treatment may have resulted in the memory impairment in mice (Fig. 5).

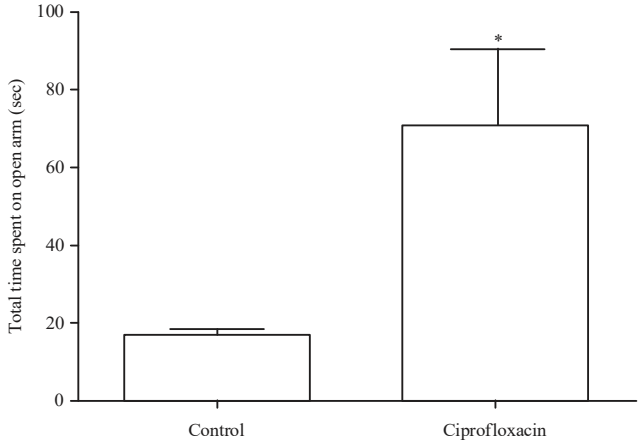

Fig. 5: Elevated plus maze (EPM) behavioral test

*Ciprofloxacin-treated mice spent significantly more time in the open arms of the EPM (p = 0.04) compared to the untreated control group (n = 8/group), indicating the role of ciprofloxacin in memory impairment

DISCUSSION

The objective of this study was to evaluate the effects of the antibiotic ciprofloxacin on memory function using multiple behavioral tests in a mouse model. The major findings of the study were, as expected, that ciprofloxacin has deleterious effects on memory in mouse models, including memory impairments assessed by the Y-maze and EPM tests.

Research has shown that ciprofloxacin is effective against bacterial infections and improves the survival rate of patients, compared to other antibiotics, . Although, studies have identified beneficial effects of ciprofloxacin in bacterial infection control[18,19], it is reported to produce cardiotoxicity and CNS side-effects, which potentially affect brain function, thereby causing cognitive impairment[4,5]. There was no incidence of death in animals receiving ciprofloxacin and these animals were in good condition, even though ciprofloxacin is reported to have cardiotoxic effects[20] and to alter brain function[21].

The Y-maze was chosen to evaluate the spatial working memory of mice in the treatment group because it is a hippocampal-dependent task[13]. The Y-maze results revealed that the mice administered ciprofloxacin were able to distinguish the novel arm from the familiar arm or start arms. Therefore, the results for the ciprofloxacin group did not differ from the untreated control group, indicating that the administered dose of ciprofloxacin did not affect the memory ability of the mice to complete this task.

The current study also used the NOR test to evaluate memory function[15] following ciprofloxacin treatment. The

animals' performance during this task was not impaired, suggesting that ciprofloxacin did not have an effect on the animals' memory. It is noteworthy that working and spatial memory are hippocampal-dependent, whereas the NOR tasks depend on the dorsal hippocampus[22].

In contrast to the Y-Maze and NOR test results, the results of the EPM test suggest that ciprofloxacin treatment did cause cognitive impairment. Further, the animals' performance on the Y-Maze and NOR tests indicated that the performance deficit on the EPM test was not due to lethargy. The data also suggest that ciprofloxacin treatment might disrupt the memory either by directly altering the brain neurotransmission or indirectly by affecting the heart, which may in turn alter the amount of blood supplied to the brain.

The ciprofloxacin-treated mice spent a significantly longer time in the open arms of the elevated plus maze and their freezing time was significantly longer than that of the control mice, indicating that ciprofloxacin administration resulted in an anxiety-like behavior. Anxiety is a natural response that promotes adaptive survival through escape from potential danger. However, extreme anxiety may disrupt regular brain function, causing alterations in the behavioral activity necessary for adaptation. The amygdala plays a vital role in the expression of anxiety and fear and the medial prefrontal cortex is important in the regulation of the amygdala-mediated expression of fear[23,24]. The results of the current study were supported by published studies, which show that ciprofloxacin has the potential to block the GABA receptor[25], which is a part of a major inhibitory system in the brain[26]. This inhibition of GABA receptors could increase brain activity, leading to the anxiety-like behavior.

Strength of the present study was that, to the best of the authors' knowledge, this is the first study to reveal a direct effect between ciprofloxacin antibiotic treatment and learning and memory function in mouse models. The dosage of ciprofloxacin used in the present study was comparable to clinically recommended therapeutic dose for patients with infections. Therefore, the results of experimental studies provided an obvious parallel to behaviors that can be seen in human patients. The animals used in both groups in this study were from the same strain and were similar in age to eliminate the effects of age and strain differences on the study's outcomes. Moreover, the study used wild type mice and infected-free animals to evaluate the direct effects of ciprofloxacin treatment without interference with other associated factors, such as microbes and inflammation that could potential impair memory.

CONCLUSION

Ciprofloxacin improves the survival rate of patients by killing pathogenic bacteria. The current study evaluated the effects of ciprofloxacin administration on memory impairment in mice using different behavioral tests. The results revealed that ciprofloxacin treatment caused some degree of memory impairment. Further studies are needed to explore the nature (transient or persistent) of the memory deficits and to elucidate the associated mechanisms of memory impairment.

SIGNIFICANCE STATEMENT

The present study is unique in demonstrating direct effects between ciprofloxacin antibiotic treatment and learning and memory function in mouse models. The study showed that these behavioral mouse models can be used to study memory deficits comparable to those observed in human patients, using dosages of ciprofloxacin comparable to clinically recommended therapeutic dosages for human patients with infections. The findings will help researchers to pursue more refined studies of the side-effects of ciprofloxacin.

REFERENCES

1. Warstler, A. and J. Bean, 2016. Antimicrobial-induced cognitive side effects. Ment. Health Clinician, 6: 207-214.
2. Millan, M.J., Y. Agid, M. Brüne, E.T. Bullmore and C.S. Carter *et al.*, 2012. Cognitive dysfunction in psychiatric disorders: Characteristics, causes and the quest for improved therapy. Nat. Rev. Drug Discov., 11: 141-168.
3. Marvanova, M., 2016. Drug-induced cognitive impairment: Effect of cardiovascular agents. Ment. Health Clinician, 6: 201-206.
4. Golomb, B.A., H.J. Koslik and A.J. Redd, 2015. Fluoroquinolone-induced serious, persistent, multisymptom adverse effects. BMJ Case Rep., Vol. 2015. 10.1136/bcr-2015-209821.
5. Kogan, Y., N. Elias, A. Paz and M. Odeh, 2018. Acute delirium associated with levofloxacin. J. Clin. Med. Res., 10: 725-727.
6. Farrell, K.R. and L. Ganzini, 1995. Misdiagnosing delirium as depression in medically ill elderly patients. Arch. Internal Med., 155: 2459-2464.
7. Sarro, A.D. and G.D. Sarro, 2001. Adverse reactions to fluoroquinolones. An overview on mechanistic aspects. Curr. Med. Chem., 8: 371-384.

8. Anderson, M.E., A. Mazur, T. Yang and D.M. Roden, 2001. Potassium current antagonist properties and proarrhythmic consequences of quinolone antibiotics. J. Pharmacol. Exp. Ther., 296: 806-810.
9. Frothingham, R., 2001. Rates of torsades de pointes associated with ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin and moxifloxacin. Pharmacotherapy, 21: 1468-1472.
10. Farrington, J., A. Stoudemire and J. Tierney, 1995. The role of ciprofloxacin in a patient with delirium due to multiple etiologies. Gen. Hosp. Psychiatry, 17: 47-53.
11. Segev, S., M. Rehavi and E. Rubinstein, 1988. Quinolones, theophylline and diclofenac interactions with the gamma-aminobutyric acid receptor. Antimicrob. Agents Chemother., 32: 1624-1626.
12. Frosco, M.B., J.L. Melton, F.P. Stewart, B.A. Kulwich, L. Licata and J.F. Barrett, 1996. *In vivo* efficacies of levofloxacin and ciprofloxacin in acute murine hematogenous pyelonephritis induced by methicillin-susceptible and-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother., 40: 2529-2534.
13. Wolf, A., B. Bauer, E.L. Abner, T. Ashkenazy-Frolinger and A.M. Hartz, 2016. A comprehensive behavioral test battery to assess learning and memory in 129S6/Tg2576 mice. PloS One, Vol. 11, No. 1. 10.1371/journal.pone.0147733.
14. Valentim, A.M., P.O. Ribeiro, I.A.S. Olsson and L.M. Antunes, 2013. The memory stages of a spatial Y-maze task are not affected by a low dose of ketamine/midazolam. Eur. J. Pharmacol., 712: 39-47.
15. Lueptow, L.M., 2017. Novel object recognition test for the investigation of learning and memory in mice. J. Vis. Exp., Vol. 126. 10.3791/55718.
16. Antunes, M. and G. Biala, 2012. The novel object recognition memory: Neurobiology, test procedure and its modifications. Cognit. Process., 13: 93-110.
17. Komada, M., K. Takao and T. Miyakawa, 2008. Elevated plus maze for mice. J. Vis. Exp., Vol. 22. 10.3791/1088.
18. Hsieh, W.J., H.C. Lin, S.J. Hwang, M.C. Hou, F.Y. Lee, F.Y. Chang and S.D. Lee, 1998. The effect of ciprofloxacin in the prevention of bacterial infection in patients with cirrhosis after upper gastrointestinal bleeding. Am. J. Gastroenterol., 93: 962-966.
19. Dajcs, J.J., B.A. Thibodeaux, M.E. Marquart, D.O. Girgis, M. Traidej and R.J. O'Callaghan, 2004. Effectiveness of ciprofloxacin, levofloxacin, or moxifloxacin for treatment of experimental *Staphylococcus aureus* keratitis. Antimicrob. Agents Chemother., 48: 1948-1952.
20. Liu, X., J. Ma, L. Huang, W. Zhu, P. Yuan, R. Wan and K. Hong, 2017. Fluoroquinolones increase the risk of serious arrhythmias: A systematic review and meta-analysis. Medicine, Vol. 96, No. 44. 10.1097/MD.0000000000008273.
21. Ilgin, S., O.D. Can, O. Atli, U.I. Ucel, E. Sener and I. Guven, 2015. Ciprofloxacin-induced neurotoxicity: Evaluation of possible underlying mechanisms. Toxicol. Mech. Methods, 25: 374-381.
22. Cohen, S.J., A.H. Munchow, L.M. Rios, G. Zhang, H.N. Ásgeirsdóttir and R.W. Stackman Jr., 2013. The rodent hippocampus is essential for nonspatial object memory. Curr. Biol., 23: 1685-1690.
23. Ressler, K.J., 2010. Amygdala activity, fear and anxiety: Modulation by stress. Biol. Psychiatry, 67: 1117-1119.
24. Babaev, O., C.P. Chatain and D. Krueger-Burg, 2018. Inhibition in the amygdala anxiety circuitry. Exp. Mol. Med., Vol. 50. 10.1038/s12276-018-0063-8.
25. Kawakami, J., K. Yamamoto, A. Asanuma, K. Yanagisawa, Y. Sawada and T. Iga, 1997. Inhibitory effect of new quinolones on GABAA receptor-mediated response and its potentiation with felbinac in *Xenopus* oocytes injected with mouse-brain mRNA: Correlation with convulsive potency *in vivo*. Toxicol. Applied Pharmacol., 145: 246-254.
26. Obata, K., 2013. Synaptic inhibition and γ-aminobutyric acid in the mammalian central nervous system. Proc. Jap. Acad. Ser. B, 89: 139-156.