The antidepressant efficacy of flurbiprofen in mice: Behavioral assessment

Research Article

Naktal M. Alberifki[1a]
Ahmed S. Naser[2b]

[1]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

[2]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

ORCİD-
[a]0000-0002-3491-5351
[b]0000-0003-1618-0678

Correspondence
Ahmed Salah Naser
ahmadphd0@gmail.com

Article info
Submission: 31-10-2023
Accepted: 16-03-2024
Online First: 25-04-2024
Publication: 30-04-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

ABSTRACT

Flurbiprofen is a nonsteroidal anti-inflammatory medication (NSAID). The psychological effect of nonsteroidal anti-inflammatory drugs (NSAIDs) is a source of contention based on clinical and experimental evidence. As a result, the goal of our study was evaluated the antidepressant effects of various flurbiprofen doses in male mice. We evaluated the effect of oral administration of flurbiprofen at 10 , 20 , and 40 mg/kg in the tail suspension and forced swimming tests after 1 h of treatment. Fluoxetine (10 mg/kg, i.p.) was used as a positive control. Flurbiprofen at 40 mg/kg showed a significant antidepressant effect, which was revealed by a significant decrease in immobility time compared with the control group, with the group administered flurbiprofen 10 mg/kg, and with the group given flurbiprofen 20 mg/kg in the tail suspension test. Flurbiprofen at 40 mg/kg showed an antidepressant effect, which was revealed by a significant decrease in immobility time compared with the control group and with the group given flurbiprofen 10 mg/kg. Flurbiprofen at 20 mg/kg had a minimal antidepressant effect in the swimming forced test, which was reflected by a non-significant decrease in immobility time compared with the control group. In conclusion, our results showed that relatively high therapeutic doses of flurbiprofen might have an antidepressant effect in mice model and we recommended for conducting other in vivo studies to clarify the variation in dose response.

Keywords: Antidepressant; flurbiprofen; forced swimming test; tail suspension test

INTRODUCTION

Depression is a serious and prevalent mental illness that can impair daily functioning and quality of life. According to the World Health Organization, depression will soon overtake heart disease as the second most common cause of the disease burden. Only 60–70% of depressed patients experience remission with antidepressant therapy, despite the fact that effective treatments like serotonin selective reuptake inhibitors (SSRIs) have improved the safety and tolerability of antidepressant medications (Rush et al., 2006). Recently, neuroimmune disorders that cause depression have received considerable attention. Numerous preclinical and clinical studies have examined the possible antidepressant effects of various anti-inflammatory medications. However, the outcomes of these trials vary widely (Bay-Richter and Wegener, 2022). Evidence suggests that the pathogenesis of depression involves inflammatory processes (the cytokine hypothesis of depression). First, proinflammatory cytokine administration causes depressive symptoms (Schiepers et al., 2005; Young et al., 2014). Second, proinflammatory cytokines can induce animal behaviors that are strikingly similar to those observed in depressed individuals (Dantzer et al., 1999; Miller and O'Callaghan, 2005). Third, proinflammatory

How to cite this article
Alberifki, NM., Naser, AS., (2024). The antidepressant efficacy of flurbiprofen in mice: Behavioral assessment. *Journal of Advances in VetBio Science and Techniques, 9*(1), 59-64. https://doi.org/10.31797/vetbio.1383152

cytokines increase the HPA axis' hyperactivity, which is frequently seen in depressive disorders(Yang et al., 2014; Zhou et al., 2022). Fourth, some cytokines impair serotonergic neurotransmission, which also happens to be a factor in depression (Dunn et al., 1999; Höglund et al., 2019; Wilson et al., 2002). Low dosages of flurbiprofen may reduce anxiety in male mice; however, the anti-anxiety activity does not manifest statistically significant with chronic or repeated treatment of flurbiprofen.(Albrefkani and Naser, 2023) . A recent study demonstrates that flurbiprofen exhibits significant dose-dependent anticonvulsant activity in mice (Naser and Alberifkani, 2023) . Another study found that flurbiprofen has a wide range of efficacy and is an effective painkiller for peripheral and abdominal pain , furthermore the study indicated the synergistic effect of flurbiprofen and alpha-lipoic acid as analgesic ,the synergistic effect of flurbiprofen and alpha-lipoic acid may have clinical benefits, including the reduction of flurbiprofen dose when used jointly (Alberifki and Naser, 2023). In the current study, we used tail suspension and forced swim tests to clarify how flurbiprofen affected depressive-like behavior in murine models.

MATERIALS AND METHODS

Experimental animals

All experiments were conducted on eight- to ten-week-old male Swiss albino mice (*Mus musculus*) in plastic cages measuring 32 × 18 × 24 cm, which were housed in groups of 4-5 in a cage with free access to food and water. Mice were purchased from the Laboratory Animal House of the University of Mosul College of Veterinary Medicine. They were kept in a climate-controlled room with a constant temperature of 23±2 °C and a 12-hour light/dark cycle.

Ethical approval

The University of Mosul College of Veterinary Medicine's animal care and use committees approved all animal use and procedures in accordance with the National Institutes of Health's standards (Date: 15/3/2022, Number: UM.VET.2022.056).

Drugs

Flurbiprofen (fortine 100mg film-coated tablet, Bilim Pharmaceuticals Industry/ Turkey) was orally administered after dilution in distilled water. Fluoxetine (APO fluoxetine 20mg capsule Bristol Laboratories Limited / UK) was diluted in distilled water and administered intraperitoneally (i.p.) at a volume of administration of 2 mL/kg body weight. An oral dose of flurbiprofen was administered 60 minutes before the test.

Study design

Twenty-five mice were allocated into five groups, as below:

1st group was given distilled water orally (negative control).

2nd group was administered fluoxetine at 10 mg/kg intraperitoneally (positive control).

3rd group was administered flurbiprofen at 10 mg/kg orally.

4th group was administered flurbiprofen at 20 mg/kg orally.

5th group was administered flurbiprofen at 40 mg/kg orally.

An hour after the treatment of the five groups, the following two experiments were performed:

Tail suspension test

The tail suspension test, which has been previously described by (Umemura et al., 2017; Ueno et al.,2022; Onouchi et al., 2014), was used to examine depression-like behavior. White acrylic walls (20 × 40 × 60 cm) with one open side that allowed for video recording of the animals made up the test apparatus. Using adhesive tape placed approximately 1 cm from the tip of the mouse's tail, each mouse was suspended by the tail 60 cm above the chamber floor. A video camera records the subsequent behavior for 5 minutes. The following parameters were later determined by analyzing the behavior: total time spent immobile. The total time that each mouse was immobile was measured in seconds, and the percentage of total time per

minute was calculated. The term "immobile time" was used in this test to describe the time when the animals were motionless for less than one second.

Forced swim test

The forced swim test was also used to look for signs of depression. The device was a cylinder (20 cm in height by 10 cm in diameter). Cylinder was positioned in the middle of the device, which was made up of a square area enclosed by white acrylic walls measuring 20 × 40 × 60 cm, one of which was open to record the behavior. Based on previous studies (Abbas et al., 2015; Matsuda et al. 2016), the cylinder was filled with water (23 °C) to a depth of 7.5 cm. Five minutes of video were recorded while the mice were inside the cylinder. The total duration of immobility was one of the parameters determined by analyzing mouse behavior. Each mouse's immobility time was measured in seconds and expressed as a percentage of the total time. The term "immobile period" was used in this test to describe the time when the animals were motionless for less than one second.

$$\% \text{ Immobility Time} = \frac{\text{Total time (300sec)} - \text{Immobility time}}{\text{Total time (300sec)}} \times 100$$

Statistical analysis

Data were analyzed statistically and expressed as mean ±standard error of the mean (SEM). All data were subjected to one-way ANOVA, followed by a post-hoc LSD test for multiple comparisons. Probability *p≤ 0.05 was statistically significant, and this was done using the Statistical Package for Social Sciences program version 16.

RESULTS

Flurbiprofen at 40 mg/kg showed a significant antidepressant effect, which was revealed by a significant decrease in immobility time (48.60±11.05) compared with the control group (155.20±22.00), with the group administered flurbiprofen 10 mg/kg (193.40±13.73), and with the group given flurbiprofen 20 mg/kg (189.40±12.58) in the tail suspension test (Table 1).

Table 1. The antidepressant effect of acute administration of flurbiprofen by tail suspension test

Groups	Immobility time (sec) in tail suspension test	Percentage of immobility time
Control	155.20±22.00	52%
Fluoxetine10mg/kg	65.80±12.11*	22%
Flu10mg/kg	193.40±13.73a	64%
Flu20mg/kg	189.40±12.58a	63%
Flu40mg/kg	48.60±11.05*bc	16%

Values are referred to mean ± SE of five male mice/group. * Referred to significantly dissimilar from the control values, p ≤ 0.05. a Referred to significantly dissimilar from the values of the fluoxetine 10 mg/kg group, p ≤ 0.05. b Referred to significantly dissimilar from the values of the Flu 10mg group ,p ≤ 0.05. c Referred to significantly dissimilar from the values of the Flu 20 mg group ,p ≤ 0.05.

Table 2. The antidepressant effect of acute administration of flurbiprofen by forced swim test

Groups	Immobility time (sec) in forced swim test	Percentage of immobility time
Control	144.20±12.85	48%
Fluoxetine10mg/kg	73.20±4.14*	25%
Flu10mg/kg	94.20±3.76*	31%
Flu20mg/kg	118.40±17.56 a	39%
Flu40mg/kg	67.80±8.23*b	23%

Values are referred to mean ± SE of five male mice/group. * Referred to significantly dissimilar from the control values, p ≤ 0.05. a Referred to significantly dissimilar from the values of the fluoxetine 10 mg/kg group, p ≤ 0.05. b Referred to significantly dissimilar from the values of the Flu 10mg group ,p ≤ 0.05.

Flurbiprofen at 40 mg/kg showed an antidepressant effect, which was revealed by a significant decrease in immobility time (67.80±8.23) compared with the control group

(144.20±12.85) and with the group given flurbiprofen 10 mg/kg (94.20±3.76). Flurbiprofen at 20 mg/kg had a minimal antidepressant effect in the swimming forced test, which was reflected by a non-significant decrease in immobility time (118.40±17.56) compared with the control group (144.20±12.85) (Table 2).

DISCUSSION

In this study, we assessed the antidepressant effects of single doses of flurbiprofen (a non-selective reversible COX inhibitor) in a mouse model. Our results revealed a different perception about the doses of flurbiprofen, with the low dose failing to demonstrate an antidepressant effect, while the high dose produced a notable antidepressant effect in the two depression models. When comparing the results of our study with other studies on the same topic, our results were consistent with a study conducted on mice injected with colon-26 adenocarcinoma cells and administered ibuprofen at 10 mg/kg through drinking water. Ibuprofen showed an antidepressant effect in the forced swimming test (Norden et al,. 2015). Other researchers have reported that ibuprofen enhanced the performance of mice in the passive avoidance trial while also reducing anxiety and antidepressant behaviors. However, ibuprofen did not improve spatial memory in the Morris water maze experiment or recognition ability in the novel object recognition test (Salmani et al., 2021). Another study was conducted to determine the effect of repeated administration for seven days of ketoprofen in mice, where ketoprofen showed an antidepressant effect represented by increasing the time of immobility in the forced swimming test, and this study suggested that the reason for this activity is the effect on the serotonin pathway, either receptors or metabolism (Răducanu et al., 2012). Other researchers mention that the co-administration of flurbiprofen and fluoxetine in mice did not has a synergistic effect against depression (Alboni et al., 2018). Anti-inflammatory drugs exert their therapeutic effects in part by regulating cytokine formation. The observation that depressed people have higher plasma levels of certain cytokines than healthy controls lends support to the "cytokine hypothesis" of depression (Warner-Schmidt et al., 2011). In rodent models, p11, a member of the S100 protein family, is a critical molecule of depressive-like states and antidepressant reactions, p11, also known as S100A10, is a tiny acidic protein that reacts with unique serotonin receptors to start regulating trafficking and control cell-surface localization. (Svenningsson et al., 2006; Warner-Schmidt et al., 2010, 2009). This activity alters the firing rate of the cells, resulting in significant behavioral outcomes. In classic behavioral paradigms such as the tail suspension and forced swim tests, p11 knockout (KO) mice exhibit a depressive-like phenotype, whereas p11 overexpression mice exhibit antidepressant-like responses (Warner-Schmidt et al., 2010). According to a study conducted in mice, tumor necrosis factor-alpha (TNFα) and interferon act on p11 to mediate their antidepressant activity. IFN interacts directly with IFN binding sites on the p11 promoter to increase p11 levels (Warner-Schmidt et al., 2011). Our study has some limitations such as the number of animals that have been used, the regime of dosing of animals, and finally, the use of only two tests for assaying antidepressant effects.

CONCLUSION

In conclusion, we conclude from our study that a single dose of flurbiprofen 40 mg/kg decreased immobility time in both the tail suspension test and the forced swim test, so we demonstrate that this dose can generate an antidepressant effect in mice model.

ACKNOWLEDGMENT

We appreciate the support from the College of Veterinary Medicine at the University of Mosul in helping us prepare the workplace and the materials needed to complete this work.

Financial support: The authors did not take any support payments from any institution in conducting the study.

Conflict of interest: The authors declare that we have no competing interest.

Ethical statement or informed consent: The University of Mosul College of Veterinary Medicine's animal care and use committees approved all animal use and procedures in accordance with the National Institutes of Health's standards (Date: 15/3/2022, Number: UM.VET.2022.056).

REFERENCES

Abbas, M.G, Hirotaka, S., Shingo, S., Mari, H., Tsuyoshi, M., & Takeshi, S. (2015). Comprehensive Behavioral Analysis of Male Ox1r−/− Mice Showed Implication of Orexin Receptor-1 in Mood, Anxiety, and Social Behavior. *Frontiers in Behavioral Neuroscience*, 9: 324. https://doi.org/10.3389/fnbeh.2015.00324

Alberifki, N. M, & Ahmed, S. N. (2023). Flurbiprofen: Determination of Safety Profile, Analgesic Effect, and Interaction with Lipoic Acid in Murine. *Iraqi Journal of Veterinary Sciences*, 37 (2): 447–52. https://doi.org/10.33899/ijvs.2023.136615.2599

Alboni, S., Cristina, B., Giacomo, C., Fabio, T., & Nicoletta, B. (2018). Neither All Anti-Inflammatory Drugs nor All Doses Are Effective in Accelerating the Antidepressant-like Effect of Fluoxetine in an Animal Model of Depression. *Journal of Affective Disorders*, 235: 124–28. https://doi.org/10.1016/j.jad.2018.04.063

Albrefkani, N., & Ahmed, N. (2023). Evaluation of the Anticonvulsant Properties of Flurbiprofen in Pilocarpine-Induced Convulsions in Mice. *Baghdad Journal of Biochemistry and Applied Biological Sciences*,4(1):1-9. https://doi.org/10.47419/bjbabs.v4i01.182

Bay-Richter, C., & Gregers, W. (2022). Antidepressant Effects of NSAIDs in Rodent Models of Depression - A Systematic Review. *Frontiers in Pharmacology*, 13. https://doi.org/10.3389/fphar.2022.909981

Dantzer, R., Emmanuelle, E. W., Ljubisa, V., & Raz, Y. (1999). Cytokines, Stress, and Depression. *Cytokines, Stress, and Depression*, 317–29. https://doi.org/10.1007/978-0-585-37970-8_17

Dunn, A. J., Jianping, W., & Tetsuya, A. (1999). Effects of Cytokines on Cerebral Neurotransmission. *Cytokines, Stress, and Depression*, 117–27. https://doi.org/10.1007/978-0-585-37970-8_8

Höglund, E., Øyvind, Ø., & Svante, W. (2019). Tryptophan Metabolic Pathways and Brain Serotonergic Activity: A Comparative Review. *Frontiers in Endocrinology*, 158. https://doi.org/10.3389/fendo.2019.00158

Matsuda, I., Hirotaka, S., Nobuyuki, Y., Tsuyoshi, M., &Atsu, A. (2016). Comprehensive Behavioral Phenotyping of a New Semaphorin 3 F Mutant Mouse. *Molecular Brain*, 9 (1): 1–13. https://doi.org/10.1186/s13041-016-0196-4

Miller, D. B., & James, P. O. (2005). Depression, Cytokines, and Glial Function. *Metabolism*, 54 (5): 33–38. https://doi.org/10.1016/j.metabol.2005.01.011

Naser, A. S., & Albrefkani, N. (2023). Assessment of Anxiolytic-like Effects of Acute and Chronic Treatment of Flurbiprofen in Murine. *Journal of Ideas in Health*, 6 (1): 814–19. https://doi.org/10.47108/jidhealth.Vol6.Iss1.268

Norden, D. M., Donna, O. M., Sabahattin, B., Raymond, D. D., Peter, J., Jonathan, P. G., &Loren, E. W. (2015). Ibuprofen Ameliorates Fatigue-and Depressive-like Behavior in Tumor-Bearing Mice. *Life Sciences*, 143: 65–70. https://doi.org/10.1016/j.lfs.2015.10.020

Onouchi, T., Katsunori, K., Kazuyoshi, S., Atsushi, S., Ron, S., Chiho, S., Masafumi, K., Keizo, T., Ryuji, N., & Akiko, I. (2014). Targeted Deletion of the C-Terminus of the Mouse Adenomatous Polyposis Coli Tumor Suppressor Results in Neurologic Phenotypes Related to Schizophrenia. *Molecular Brain*, 7 (1): 1–14. https://doi.org/10.1186/1756-6606-7-21

Răducanu, I., Ana, S., Gabriel, P., CRISTINA, I., ANDREEA, A., &Ion, F. (2012). Assessing Depressive Effect of Ketoprofen and Its Mechanism of Action Using the Forced Swimming Test in Mice. *Farmacia*, 60 (5): 759–66.

Rush, A. J., Helena, C. K., Harold, A. S., Maurizio, F., Madhukar, H. T., Ellen, F., Philip, T. N, Michael, E. T., Alan, J., G., &David, J. K. (2006). Report by the ACNP Task Force on Response and Remission in Major Depressive Disorder. *Neuropsychopharmacology*, 31 (9): 1841–53. https://doi.org/10.1038/sj.npp.1301131

Salmani, H., Mahmoud, H., Yousef, B., &Zahra, S. (2021). The Brain Consequences of Systemic Inflammation Were Not Fully Alleviated by Ibuprofen Treatment in Mice. *Pharmacological Reports* , 73 (1): 130–42. https://doi.org/10.1007/s43440-020-00141-y

Schiepers, O. J. G., Marieke, C., &Michael, M.(2005). Cytokines and Major Depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 29 (2): 201–17. https://doi.org/10.1016/j.pnpbp.2004 .11.003

Svenningsson, P., Karima, C., Ilan, R., Marc, F., Xiaoqun, Z., Malika, E., Jean-Marie, V., George, G. N., &Paul, G. (2006). Alterations in 5-HT1B Receptor Function by P11 in Depression-like States. *Science*, 311 (5757): 77–80. https://doi.org/10.1126/science.1117571

Ueno, H., Yu, T., Shinji, M., Kenta, W., Yosuke, M., Motoi, O., &Takeshi, I. (2022). Effect of Simultaneous Testing of Two Mice in the Tail Suspension Test and Forced Swim Test. *Scientific Reports* ,12 (1): 1–14. https://doi.org/10.1038/s41598-022-12986-9

Umemura, M., Tae, O., Ayako, M., Haruo, N., Keizo, T., Tsuyoshi, M., &Yuji, T. (2017). Comprehensive Behavioral Analysis of Activating Transcription Factor 5-Deficient Mice. *Frontiers in Behavioral Neuroscience*, 11: 125. https://doi.org/10.3389/fnbeh.2017.00125

Warner-Schmidt, J.L., Emily, Y. C., Xiaoqun, Z., John, J. M., Alexei, M., Per, S., & Paul, G. (2010). A Role for P11 in the Antidepressant Action of Brain-Derived Neurotrophic Factor. *Biological Psychiatry*, 68 (6): 528–35. https://doi.org/10.1016/j.biopsych.2010.04.029

Warner-Schmidt, J. L., Marc, F., Abigail, M., Emily, Y. C., Hongshi, Q., Per ,S., & Paul, G. (2009). Role of P11 in Cellular and Behavioral Effects of 5-HT4 Receptor Stimulation. *Journal of Neuroscience*, 29 (6): 1937–46. https://doi.org/10.1523/JNEUROSCI.5343-08.2009

Warner-Schmidt, J. L., Kimberly, E. V., Emily, Y. C., John, J. M., &Paul, G. (2011). Antidepressant Effects of Selective Serotonin Reuptake Inhibitors (SSRIs) Are Attenuated by Antiinflammatory Drugs in Mice and Humans. *Proceedings of the National Academy of Sciences*, 108 (22): 9262–67. https://doi.org/10.1073/pnas.1104836108

Wilson, C. J., Caleb, E. F., &Harvey, J. C. (2002). Cytokines and Cognition—the Case for a Head-to-toe Inflammatory Paradigm. *Journal of the American Geriatrics Society*, 50 (12): 2041–56. https://doi.org/10.1046/j.1532-5415.2002.50619.x

Yang, S., Hai-Yang, Y., Dan-Yu, K., Zhan-Qiang, M., Rong, Q., Qiang, F., &Shi-Ping, M. (2014). Antidepressant-like Effects of Salidroside on Olfactory Bulbectomy-Induced pro-Inflammatory Cytokine Production and Hyperactivity of HPA Axis in Rats. *Pharmacology Biochemistry and Behavior*, 124: 451–57. https://doi.org/10.1016/j.pbb.2014.07.015

Young, J. J., Davide, B., &Nunzio, P. (2014). A Review of the Relationship between Proinflammatory Cytokines and Major Depressive Disorder. *Journal of Affective Disorders*, 169: 15–20. https://doi.org/10.1016/j.jad.2014.07.032

Zhou, L., Tian, W., Yawen, Y., Mingan, L., Xiaohui, W. S., Yunjie, W., Ce, Z., &Fenghua, F. (2022). The Etiology of Poststroke-Depression: A Hypothesis Involving HPA Axis. *Biomedicine & Pharmacotherapy*, 151: 113146. https://doi.org/10.1016/j.biopha.2022.113146