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Original Article

Assessment of anxiolytic-like effects of acute and chronic treatment of flurbiprofen in murine

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Abstract

Background: Non-steroidal anti-inflammatory drugs are commonly used medications with atypical pharmacological effects. This aims to evaluate the anxiolytic-like effects of flurbiprofen in rodent models.

Methods: In vivo experimental trial was conducted from October 2022 to January 2023 at the college of veterinary medicine, university of Mosul, Iraq. The effect of flurbiprofen was assessed in mice exposed to the elevated plus maze (EPM), light-dark box test (LDT), and open-field test (OFT). Fifty male mice were divided into two groups of twenty-five, weighing 30–35 g, for acute and chronic treatment. Each group was subdivided into five subgroups: distilled water was administered to the control group; the positive control was injected with 10 mg/kg diazepam; and the flurbiprofen groups were administered orally at 10, 20, and 40 mg/kg. Each subgroup was subjected to EPM, LDT, and OFT one hour after administration. The second group was also subdivided like the first group. It was treated for 15 days constantly and subjected to anxiety tests on the 16th day.

Results: Acute treatment with 20 mg/kg flurbiprofen revealed an anxiolytic effect, with increased time spent in the open arm of the EPM test, increased time spent in the LDB test, and increased time spent in the central area in the OFT compared to the control group. Chronic administration of flurbiprofen was ineffective in producing an anxiolytic effect.

Conclusion: The low doses of flurbiprofen may eliminate the anxiety effect in experimental mice; however, the anti-anxiety effect does not appear significantly after repeated or chronic administration of flurbiprofen.

Keywords: Flurbiprofen, Anxiety, Elevated Plus Maze Open Field Test, Mice, Iraq

Background

Anxiety and depression are recognized as psychiatric diseases worldwide [1]. These two mental disorders are linked to physiological, cognitive, behavioral, and psychological alterations in individuals classified as having negative emotional experiences [2]. Depression and anxiety have detrimental impacts on human and animal life, causing significant functional loss, and depression and anxiety disorders are frequently detected together [3-4]. The etiopathogenesis of both diseases has not yet been clarified, and antidepressant drugs from the selective serotonin reuptake inhibitor (SSRI) group are the first-choice treatment [5]. Alprazolam and diazepam appeared beneficial in lowering both the frequency and intensity of panic attacks. The use of benzodiazepines for treating panic disorders has been supported by previous research [6]. A low dose of a nonselective beta blocker, propranolol, administered on the morning of day-case surgery, significantly treated patients' worry [7-8]. In latest years, substantial attention has been paid to neuroimmune processes associated with depression. Numerous preclinical and clinical experiments have been shown to investigate the possible antidepressant and anxiolytic benefits of various anti-inflammatory medications [9]. Flurbiprofen produces anti-inflammatory properties by inhibiting pro-inflammatory cytokines by inhibiting cyclooxygenase 1 and cyclooxygenase 2 [10]. We aimed to assess the anxiolytic properties of acute and chronic flurbiprofen treatment in murine models.

Methods

Study design

An experimental study was designed to assess the effect of flurbiprofen in mice exposed to the elevated plus maze (EPM), light-dark box test (LDT), and open-field tests (OFT). The

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study was conducted in an isolated room in the college of veterinary medicine, university of Mosul, Iraq, from October 2022 to January 2023.

Animals

Male Swiss albino mice weighing 25–32g were purchased from the laboratory animal house of the Faculty of Veterinary Medicine of the University of Mosul in Iraq. Mice were housed at a temperature of 20 ± 2°C with a 12/12 hours light, dark cycle and given water and food ad libitum. The procedures used were in accordance with European legislation on the use and care of laboratory animals (EU Directive 2010/63/EU) and authorized by the Department of Physiology, Biochemistry, and Pharmacology (Ref: 2022-08-15/1470). Every effort was made to reduce the number of animals used and their suffering.

Dosage preparation

Different flurbiprofen doses (10, 20, and 40 mg/kg) were chosen. Flurbiprofen was dissolved in distilled water and given orally at a 10 mL/kg dose by a gavage needle. Diazepam (10mg/kg) was dissolved in normal saline and given intraperitoneally in a dose volume of 10ml/kg.

Trials design

At the start of the trials, the mice were randomly separated into two experimental groups, each involving five subgroups of five mice:

1-First group: for acute treatment, which was subjected to anxiety tests after one hour of administration, which was classified into:
Group 1 was treated with distilled water as a negative control.
Group 2 was treated intraperitoneally with diazepam (10 mg/kg).
Group 3 was orally administered flurbiprofen at a dose of 10 mg/kg.
Group 4 was orally administered flurbiprofen (20 mg/kg).
Group 5 was orally administered flurbiprofen (40 mg/kg).
2-Second group: chronic treatment, which was subjected to anxiety tests after 15 days of continuous administration, was classified as follows:
Group 1 was treated with distilled water as a negative control.
Group 2 was treated intraperitoneally with diazepam (10 mg/kg).
Group 3 was orally administered flurbiprofen at a dose of 10 mg/kg.
Group 4 was orally administered flurbiprofen (20 mg/kg).
Group 5 was orally administered flurbiprofen (40 mg/kg).

Anxiety tests

Anxiety in mice was assessed using the elevated plus maze (EPM) test. It had two open arms (35×5 cm2), two opposite closed arms (35×5 cm2), and a small middle square (5×5 cm2) between arms. The maze was placed 50 cm above the ground in a dim area. Each mouse was placed in the center of the elevated plus maze with its head facing the open arm.
A video camera was used to record the mice's free exploration for 5 min [11-12].

The following were recorded within 5 minutes:
1-Time spent in open arms.
2- Time spent in the closed arm.
Also, anxiety behavior in mice was assessed using dark and lightbox tests. The trial used two compartments for video recordings: a light side (42 × 30 × 20 cm3; white walls and highly lit with a 100 W bulb) and a dark side (42 × 30 × 20 cm3; opaque black walls and dark), with an opening (6 × 6 cm2) between the two sections and a mobile phone stand situated 50 cm overhead the box [13-14].
The mouse was located on the dark side with its head to the light side and allowed to discover for 5 minutes and record the following:
1. Period of stay on the dark side.
2-Periods of Staying on the bright side.
Then, an open-field test was conducted, which can be used to detect anxiety in mice. The floor of the transparent acrylic box (72×72×36 cm3) was divided into 16 equal-sized squares (18×18 cm2) [15]. The center was four squares, whereas the periphery was 12 squares along the walls.
A video camera was used to record the following.
1- Period of stay in the middle of the field.
2- Period of stay in the vicinity of the field.
The devices were rinsed after each mouse to remove the odor of the previous mice.

Statistical analysis

The elevated plus maze, light-dark box test, and open-field test parameters were analyzed by one-way analysis of variance. Statistical significance was set at $P \leq 0.05$. Data are expressed as mean ± standard error of the mean (SEM). Data were analyzed using SPSS for Windows - Version 16.

Results

Time spent on the closed arm

Flurbiprofen, one hour after administration of 20 mg/kg, caused an anxiolytic effect, represented by a significant increase in the time spent in the open arm and a significant decrease in the time spent in the closed arm compared to the control group in the elevated maze test (figure 1A and figure 1B).

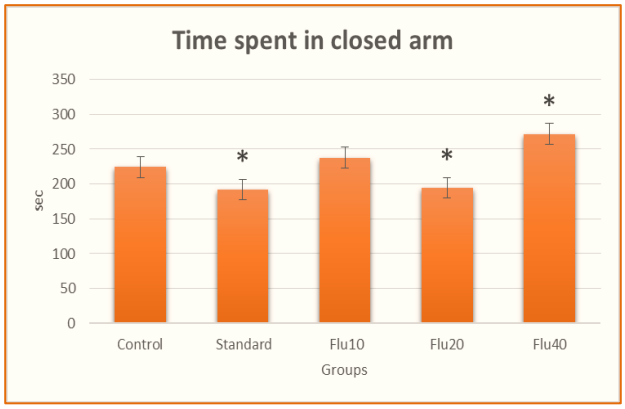

Fig. 1A. Effects of acute oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam(standard) (10 mg/kg) on time spent in the closed arms for 5 minutes in the elevated plus maze. Data are expressed as mean ±SEM (5 mice/group)
* Significantly different from control data ($P \leq 0.05$).

The light/dark box test revealed anti-anxiety effects as a significant increase in the time spent by mice dosed with flurbiprofen at a dose of 20 mg/kg body weight on the bright side and a significant decrease in the time spent on the dark side compared to the control group (figure 1C and figure 1D).

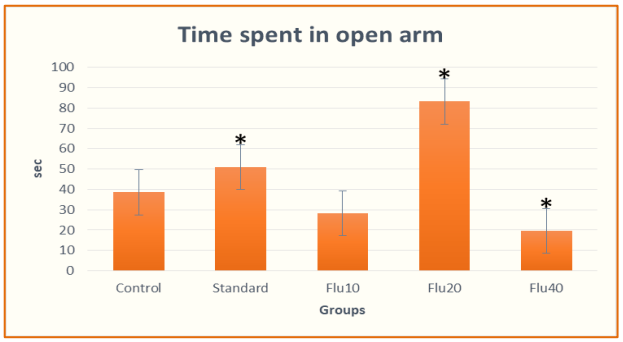

Fig. 1B. Effects of acute oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam (10 mg/kg) on time spent in the open arms for 5 minutes in the elevated plus maze. Data are expressed as mean ±SEM (5 mice/group)
* Significantly different from control data ($P \leq 0.05$).

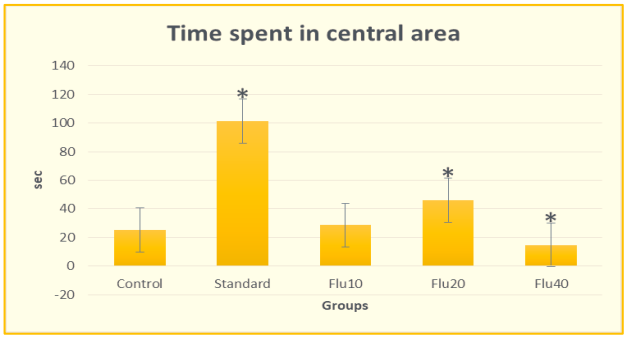

Fig. 1E. Effects of acute oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam (10 mg/kg) on time spent in the central area for 5 minutes in the open field. Data are expressed as mean ±SEM (5 mice/group)
* Significantly different from control data ($P \leq 0.05$).

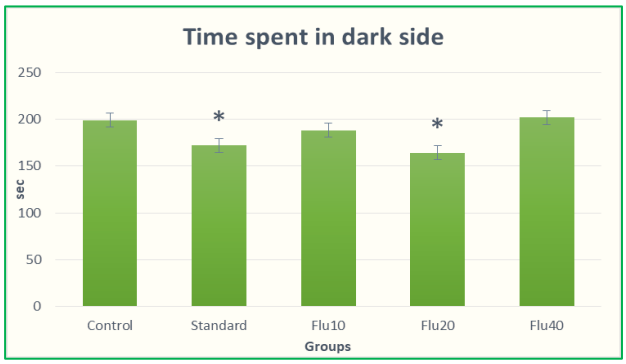

Fig. 1C. Effects of acute oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam (10 mg/kg) on time spent on the dark side for 5 minutes in the light/dark box. Data are expressed as mean ±SEM (5 mice/group) * Significantly different from control data ($P \leq 0.05$).

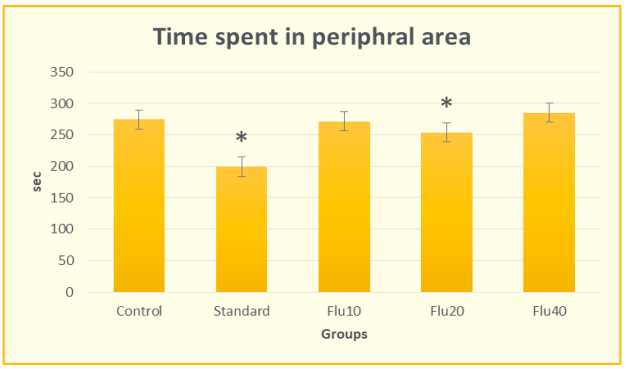

Fig. 1F. Effects of acute oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam (10 mg/kg) on time spent in the central area for 5 minutes in the open field. Data are expressed as mean ±SEM (5 mice/group)
* Significantly different from control data ($P \leq 0.05$).

The open field test revealed a significant increase in the time spent in the center and a significant decrease in the time spent in the periphery compared with the control group (figure 1E and figure 1F). Flurbiprofen at 10 and 40 mg/kg showed no anxiolytic effects.

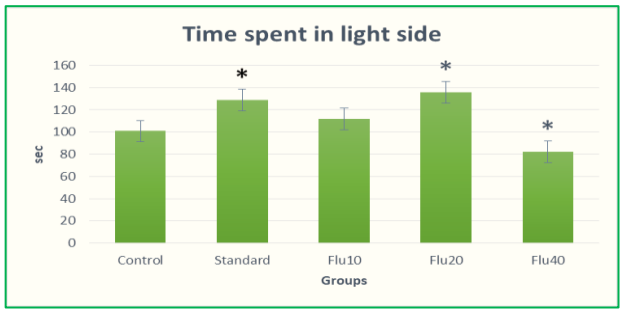

Fig. 1D. Effects of acute oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam (10 mg/kg) on time spent on the light side for 5 minutes in the light/dark box. Data are expressed as mean ±SEM (5 mice/group)
* Significantly different from control data ($P \leq 0.05$).

In the light-dark test, the time spent on the light side was significantly longer in the standard and flurbiprofen at 20mg/kg than in the control group. The time spent on the dark side was significantly shorter in the standard than in the control group (figure 2C and figure 2D).

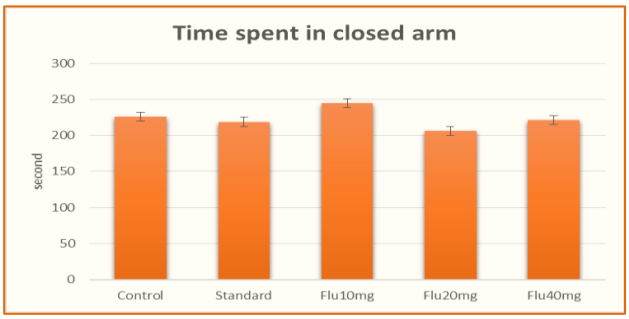

Fig. 2A. Effects of chronic oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam(standard) (10 mg/kg) on time spent in the closed arms for 5 minutes in the elevated plus maze. Data are expressed as mean ±SEM (5 mice/group) * Significantly different from control data ($P \leq 0.05$).

The results of chronic treatment with flurbiprofen varied. In contrast to acute treatment in the elevated plus maze test, the time spent in the open arms was significantly shorter in flurbiprofen at 10, 20, and 40mg/kg than in the control group, while the time spent in the open arm was longer in the standard group than in control group. The time spent in the closed arms showed no statistically significant difference between the groups (figure 2A and figure 2B).

In the open field test, the time spent in the Centre area was significantly longer in the standard group and flurbiprofen 20mg/kg than in the control group. The time spent in the Centre area was significantly shorter in flurbiprofen at 10 and 40 mg/kg than in the standard group. On the other hand, the time spent on the outer edges was significantly shorter in the standard group than that in the control group (figure 2E and figure 2F).

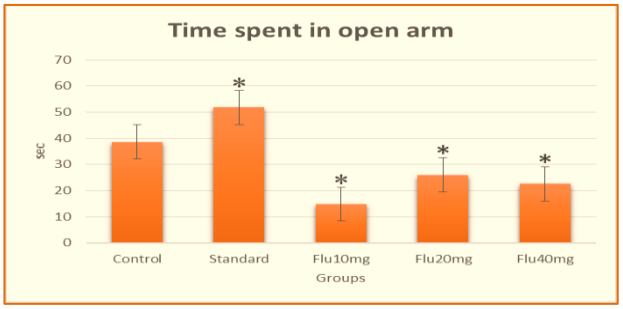

Fig. 2B. Effects of chronic oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam (10 mg/kg) on time spent in the open arms for 5 minutes in the elevated plus maze. Data are expressed as mean ±SEM (5 mice/group).

* Significantly different from control data ($P \leq 0.05$).

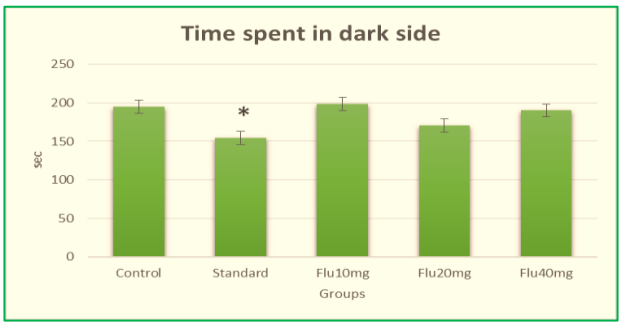

Fig. 2C. Effects of chronic oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam (10 mg/kg) on time dark side for 5 minutes in the light/dark box. Data are expressed as mean ±SEM (5 mice/group)

* Significantly different from control data ($P \leq 0.05$).

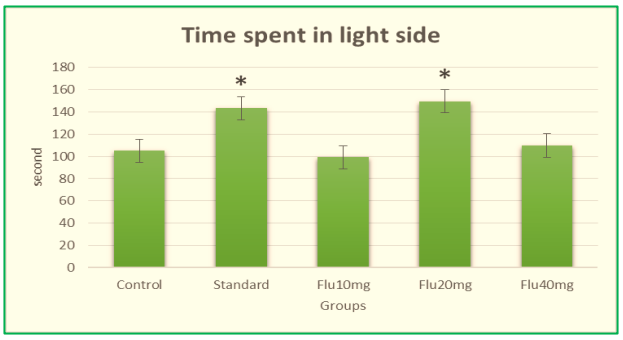

Fig. 2D. Effects of chronic oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam (10 mg/kg) on time light side for 5 minutes in the light/dark box. Data are expressed as mean ±SEM (5 mice/group)

* Significantly different from control data ($P \leq 0.05$).

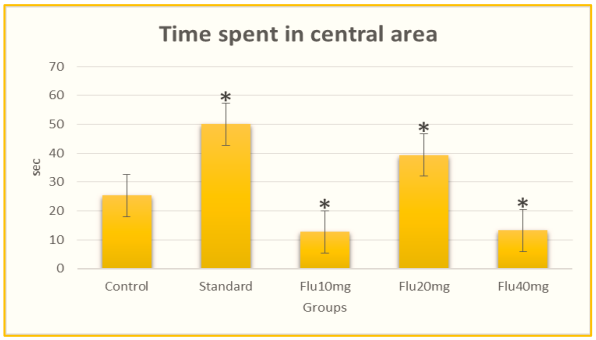

Fig. 2E. Effects of chronic oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam (10 mg/kg) on time spent in the central area for 5 minutes in the open field. Data are expressed as mean ±SEM (5 mice/group)

* Significantly different from control data ($P \leq 0.05$).

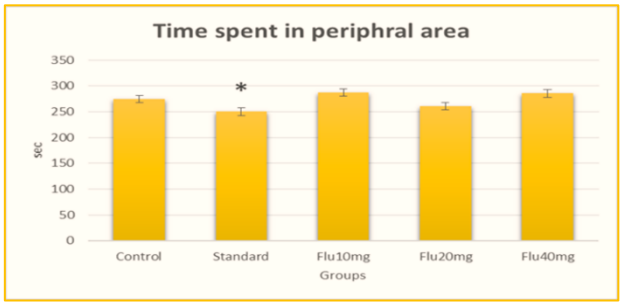

Fig. 2F. Effects of chronic oral administration of flurbiprofen (10,20, and 40 mg/kg) and diazepam (10 mg/kg) on time spent in the central area for 5 minutes in the open field. Data are expressed as mean ±SEM (5 mice/group)

* Significantly different from control data ($P \leq 0.05$).

Discussion

The present study showed that flurbiprofen has an anxiolytic-like effect, likely because of its anti-inflammatory properties [16]. Flurbiprofen at 20 mg/kg has an anxiolytic effect in acute treatment and, to a lesser extent, in chronic treatment.

The inflammatory process, free radicals, and the level of inflammatory mediators are among the main etiology of depression and anxieties [16-17]. There are multiple studies on anxiety and depression in propionic acids from NSAIDs. One study revealed that ibuprofen failed to prevent brain disease in a lupus neuropsychiatric mouse model, in which chronic ibuprofen administration could not regularize the status of immunity, cognitive and behavioral actions, and brain mass in lupus-prone murine [18]. Another study revealed that ibuprofen could not counteract interferon-induced depression in a rat model, and locomotor activity was only impaired with a high dose of ibuprofen (75 mg/kg); thus, it was not evaluated further. After treatment with interferon, anxiety manifestations, and substantial alterations were observed throughout the splash test, and ibuprofen significantly reduced immobility time in the forced swim test. In contrast, grooming time increased when compared to the single doses of celecoxib and indomethacin, ibuprofen exhibited superior antidepressant properties when given with interferon [19]. Another study found that pre-treatment with meloxicam or ketoprofen-treated nociception increased PGE2 levels in the spinal cord and increased escape behavior time during forced swimming by 95% compared to the control group. Furthermore, it was found that plasma corticosterone levels were elevated by 97% in rats exposed to stress. In comparison, COX-inhibiting drugs reduced the plasma corticosterone level to 84% compared to the control group, which indicates the possibility of anxiolytic effects of non-steroidal anti-inflammatory drugs, and supports the use of (NSAIDs) for chronic pain caused by chronic depression and anxiety [20]. Another study revealed that the co-treatment of aspirin with fluoxetine could reverse the stress-induced escape deficit later a week of treatment and that the frequency of the antidepressant-like influence of aspirin was dose-dependent [21]. Our findings agree with a study in which mice were administered aluminum chloride to induce anxiety and tested in an open field. Blanchard et al. [22] initially discussed, an animal's hesitancy to move from one location to another or into the central region of the test reflects elevated anxiety levels in rodents to examine the effects of ibuprofen on anxiety, locomotion, and exploratory behavior in treated mice. In

ibuprofen-treated mice, the effects of aluminum chloride treatment were reversed, and there was a reduction in state anxiety. According to the findings of this study, ibuprofen may have a possible treatment effect in the treatment of anxiety associated with neurodegenerative disorders [23]. This study had some limitations. First, we did not use all of the trials for anxiety assessment. Second, this was an in vivo study. We did not do in vitro experiments that pinpoint the exact mechanism of action against anxiety, which merits further research.

Conclusion

In conclusion, the administration of 20 mg of flurbiprofen reversed the anxiety-induced condition in three anxiety tests after 1 h of treatment and after 15 days of treatment. The other doses of flurbiprofen were not effective as a treatment for anxiety. Therefore, neither all treatment regimens nor all doses of flurbiprofen are effective in producing the anxiolytic-like effect in a murine model.

Abbreviation

EPM: Elevated Plus Maze; LDT: Light-Dark Box Test; OFT: Open-Field Test; SSRI: Selective Serotonin Reuptake Inhibitor; NSAIDs: Non-Steroidal Anti-Inflammatory Drugs

Declaration

Acknowledgment

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Availability of data and materials

Data will be available by emailing ahmadphd0@gmail.com

Authors' contributions

Ahmed Salah Naser and Naktal Albrefkanie contributed equally in the study concept, design, writing, evaluating, proofreading. Approval of the final manuscript are shared by all authors.

Ethics approval and consent to participate

We conducted the research following the Declaration of Helsinki. The procedures used were in accordance with European legislation on the use and care of laboratory animals (EU Directive 2010/63/EU) and authorized by the Department of Physiology, Biochemistry, and Pharmacology, University of Mosul (2022-08-15/1470). Every effort was made to reduce the number of animals used and their suffering.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interest.

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References

1. Li W, Zhao N, Yan X, et al. The prevalence of depressive and anxiety symptoms and their associations with quality of life among clinically stable older patients with psychiatric disorders during the COVID-19 pandemic. Transl Psychiatry. 2021;11(1):1-8. DOI: 10.1038/s41398-021-01196-y
2. Steimer T. The biology of fear-and anxiety-related behaviors. Dialogues Clin Neurosci. 2002 Sep;4(3):231-49. doi: 10.31887/DCNS.2002.4.3/tsteimer.
3. Hussein RM, Mohamed WR, Omar HA. A neuroprotective role of kaempferol against chlorpyrifos-induced oxidative stress and memory deficits in rats via GSK3β-Nrf2 signaling pathway. Pestic Biochem Physiol. 2018; 152:29-37. DOI: 10.1016/j.pestbp.2018.08.008
4. Russo E, Leo A, Crupi R, et al. Everolimus improves memory and learning while worsening depressive and anxiety-like behavior in an animal model of depression. J Psychiatr Res. 2016; 78:1-10. DOI: 10.1016/j.jpsychires.2016.03.008
5. Montgomery SA, Judge R. Treatment of depression with associated anxiety: comparisons of tricyclic antidepressants and selective serotonin reuptake inhibitors. Acta Psychiatr Scand. 2000;101:9-16. DOI: 10.1111/j.1600-0447.2000.tb10943.x
6. Dunner DL, Ishiki D, Avery DH, Wilson LG, Hyde TS. Effect of alprazolam and diazepam on anxiety and panic attacks in panic disorder: a controlled study. J Clin Psychiatry. 1986 Sep;47(9):458-60. PMID: 2875064
7. Hayes PE, Schulz SC. Beta-blockers in anxiety disorders. J Affect Disord. 1987;13(2):119-130. DOI: 10.1016/0165-0327(87)90017-6
8. Mealy K, Ngeh N, Gillen P, Fitzpatrick G, Keane FB, Tanner A. Propranolol reduces the anxiety associated with day case surgery. Eur J surgery= Acta Chir. 1996;162(1):11-14. PMID: 8679756

9. Bay-Richter C, Wegener G. Antidepressant effects of NSAIDs in rodent models of depression—A systematic review. Front Pharmacol. 2022;13. DOI: 10.3389/fphar.2022.909981
10. Governa P, Cusi MG, Borgonetti V, et al. Beyond the biological effect of a chemically characterized poplar propolis: Antibacterial and antiviral activity and comparison with flurbiprofen in cytokines release by lps-stimulated human mononuclear cells. Biomedicines. 2019;7(4):73. DOI: 10.3390/biomedicines7040073
11. Miyakawa T, Leiter LM, Gerber DJ, et al. Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. Proc Natl Acad Sci. 2003;100(15):8987-8992. DOI: 10.1073/pnas.1432926100
12. Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav. 1996;54(1):21-30. DOI: 10.1016/0091-3057(95)02126-4
13. Bourin M, Hascoët M. The mouse light/dark box test. Eur J Pharmacol. 2003;463(1-3):55-65. DOI: 10.1016/s0014-2999(03)01274-3
14. Takao K, Miyakawa T. Light/dark Transition Test for Mice. JoVE. 1. 2006 Nov 13;(1):104. DOI: 10.3791/104.
15. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol. 2003;463(1-3):3-33. DOI: 10.1016/s0014-2999(03)01272-x
16. Salim S, Chugh G, Asghar M. Inflammation in anxiety. Adv Protein Chem Struct Biol. 2012; 88:1-25. DOI: 10.1016/B978-0-12-398314-5.00001-5
17. Sulakhiya K, Keshavlal GP, Bezbaruah BB, et al. Lipopolysaccharide induced anxiety-and depressive-like behaviour in mice are prevented by chronic pre-treatment of esculetin. Neurosci Lett. 2016;611:106-111. DOI: 10.1016/j.neulet.2015.11.031
18. Ballok DA, Ma X, Denburg JA, Arsenault L, Sakic B. Ibuprofen fails to prevent brain pathology in a model of neuropsychiatric lupus. J Rheumatol. 2006;33(11):2199-2213. PMCID: PMC1635783
19. Mesripour A, Shahnooshi S, Hajhashemi V. Celecoxib, ibuprofen, and indomethacin alleviate depression-like behavior induced by interferon-alfa in mice. J Complement Integr Med. 2020;17(1). DOI: 10.1515/jcim-2019-0016
20. Guevara C, Fernandez AC, Cardenas R, Suarez-Roca H. Reduction of spinal PGE2 concentrations prevents swim stress-induced thermal hyperalgesia. Neurosci Lett. 2015; 591:110-114. DOI: 10.1016/j.neulet.2015.02.035
21. Alboni S, Benatti C, Capone G, Tascedda F, Brunello N. Neither all anti-inflammatory drugs nor all doses are effective in accelerating the antidepressant-like effect of fluoxetine in an animal model of depression. J Affect Disord. 2018; 235:124-128. DOI: 10.1016/j.jad.2018.04.063
22. Blanchard DC, Griebel G, Blanchard RJ. Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. Neurosci Biobehav Rev. 2001;25(3):205-218. DOI: 10.1016/s0149-7634(01)00009-4
23. Jamil A, Mahboob A, Ahmed T. Ibuprofen targets neuronal pentraxins expresion and improves cognitive function in mouse model of AlCl3-induced neurotoxicity. Exp Ther Med. 2016;11(2):601-606. DOI: 10.3892/etm.2015.2928