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Teriflunomide mitigates pain-depression dyad in mice: Modulation of PI3K-mediated defensive signaling

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ABSTRACT

The pain-depression dyad refers to a complex and often bidirectional relationship between physical pain and depression. It suggests that these two experiences can interact and exacerbate each other, leading to a cycle of worsening symptoms. Both pain and depression involve changes in brain chemistry and neural pathways. The phosphoinositide 3-kinase (PI3K) signaling pathway often contributes to mood stabilization and in alleviating pain. Teriflunomide is an active metabolite of leflunomide that positively impacts brain functioning. Thus, the current study was designed to investigate the potential of teriflunomide against reserpine-induced pain-depression dyad and also examined the role of PI3K in the observed effect of teriflunomide. Reserpine (0.5 mg/Kg; i.p) treatment for 3 consecutive days significantly induced the pain-depression dyad along with memory impairment in mice. Administration of reserpine significantly reduced the brain glutathione (GSH) level, uplifting the brain thiobarbituric acid reactive substance (TBARS) and serum tumor necrosis factor-alpha (TNF-α) level. Teriflunomide (10 and 20 mg/Kg) treatment significantly reverted the depressive, pain-inducing and memory-impairing effect of reserpine. Both doses of teriflunomide significantly uplifted the brain GSH level and downregulated the serum TNF-α level. Whereas, only a 20 mg/Kg dose of teriflunomide significantly reduced the brain TBARS level. Furthermore, the PI3K inhibitor (LY294002) treatment significantly alleviated the anti-depressant, anti-nociceptive and memory-improving effect of teriflunomide (20 mg/Kg). LY294002 treatment also reverted the teriflunomide-induced biochemical modulations (20 mg/Kg). LY294002 significantly reduced the brain GSH and uplifted the level of TBARS and serum TNF-α level. Conclusively, the results of the current study delineate that teriflunomide might exert its anti-depressant, anti-nociceptive and memory-improving effect by stimulating the PI3K-mediated protection against oxidative and inflammatory stress.

1. Introduction

Pain and depression are two widespread and harmful conditions that exert a substantial socioeconomic burden on society. Clinical observations have long acknowledged the simultaneous presence and interplay between pain and depression (Khan et al., 2020). Pain stands as a pivotal determinant in the development of depression, with their concurrent presence serving to exacerbate the intensity of both conditions (Sheng et al., 2017). Research employing clinical interviews with individuals experiencing chronic pain reveals that the prevalence rate for depression ranges from 30 % to 54 %. (Søndergaard et al., 2017). Individuals experiencing chronic pain have a threefold higher likelihood of meeting the diagnostic criteria for depression compared to those who do not have pain, and the risk of developing depression rises as the number of pain symptoms increases (Taloyan and Löfvander, 2014). Moreover, researchers have also suggested that Individuals who encounter depression during the early stages of their life may undergo cognitive impairment because of prolonged episodes of depression (Sarbu et al., 2024). The co-occurrence of pain and depression can be attributed to shared neurobiological pathways that involve various

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neurotransmitters, such as serotonin, norepinephrine, Substance-P. (Pinheiro et al., 2015; Rijavec and Novak Grubic, 2012). Moreover, there is supporting evidence indicating that the connection between pain and depression may stem from neuronal tissue damage, which includes the involvement of neuroinflammation and oxidative/nitrosative stress processes (Birmann et al., 2019). It has been well established that a reduced level of brain 5-hydroxytryptamine (5-HT) is a fundamental contributing factor to depression (Moncrieff et al., 2023). Evidence delineates that oxidative stress can result in an increased production of neurotoxic compounds through the oxidation of tryptophan, the precursor of 5-HT. Specific molecules like malondialdehyde, which are associated with strong proinflammatory characteristics, have been linked to depression and pain due to their involvement in pathways such as tryptophan/kynurenine interaction (Bakunina et al., 2015). Furthermore, reports have documented that the activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) cascade mediates the analgesic and anti-depressant effects (Guo et al., 2019; You et al., 2023). Stimulation of the PI3K pathway offers protection to neurons and the brain against ischemic and oxidative stress-induced damage (Uranga et al., 2013).

Pain represents a significant feature that frequently co-occurs in individuals with depressive disorder, commonly denoted as the pain-depression dyad. Those experiencing depressive disorders have been observed to articulate a diminished tolerance to pain (Hedgecock et al., 2019). The reserpine-induced pain-depression dyad is a well-accepted mouse model that exhibits behaviors indicative of both pain and depression (Liu et al., 2014a). It has been documented that reserpine-induced increased pain perception in depression might be attributed to the increased level of Substance P in addition to monoamine depletion (Xu et al., 2013; Liu et al., 2014b). Xu et al found that reserpine treatment exaggerated pain response and uplifted the level of Substance P in comparison to the control group (Xu et al., 2013). Moreover, the depletion of monoamines induced by reserpine is attributed to its blocking action on H^+-coupled vesicular monoamine transporters-2 (Singh et al., 2021a). Besides the pain-depression dyad, reserpine also has the potential to induce memory impairment and promote oxidative stress in rodents. This memory impairment effect of reserpine could be due to its monoaminergic depletory action (Tseng et al., 2015). Monoamines play a crucial role in various cognitive functions, including memory (Takano, 2018).

Till now, the effective management of the pain-depression dyad in a clinical setting remains a complex task (Roughan et al., 2021). Addressing the pain-depression dyad can be challenging. Treating one condition may positively impact the other, but it's often necessary to address both concurrently (Maallo et al., 2021). In the early days, serotonin noradrenaline reuptake inhibitors (duloxetine) and selective serotonin reuptake inhibitors (paroxetine and sertraline) are frequently prescribed as antidepressant medications for managing concurrent

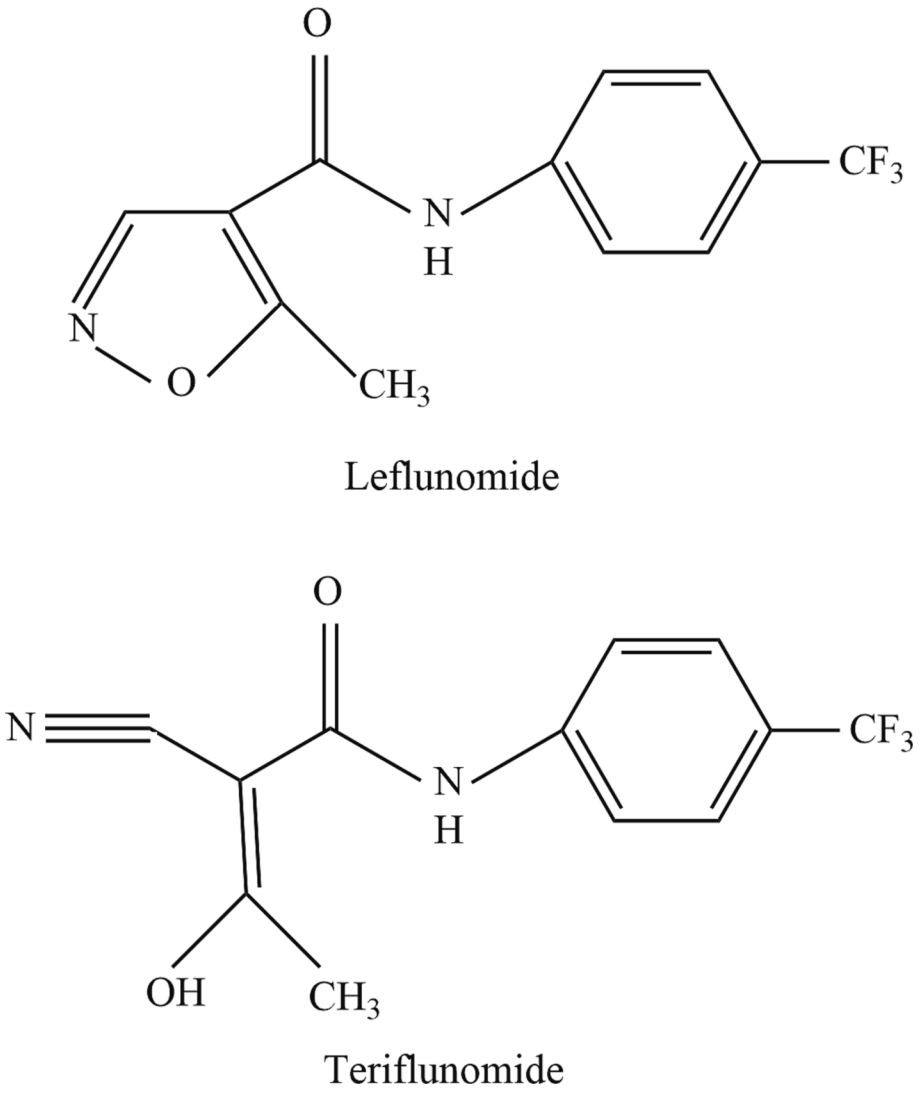

Fig. 1. Chemical structure of leflunomide and teriflunomide.

chronic pain and depression (IsHak et al., 2018). However, other clinical reports have consistently shown that the pain-relieving effects of antidepressants can be distinguished from their antidepressant properties (Li, 2015). Addressing pain and depression simultaneously in individuals with comorbidities is crucial. By focusing on the shared underlying mechanisms of both pain and depression, we can enhance our ability to effectively manage these concurrent conditions. Teriflunomide (TFN) is an active metabolite of leflunomide, which is primarily utilized to treat rheumatoid arthritis (Lycke et al., 2023) (Fig. 1). Reports have shown that teriflunomide has a positive impact on brain functioning, preserving neuronal activity and protecting mitochondria in brain slices exposed to oxidative stress. Consequently, teriflunomide has been approved by the U.S. Food and Drug Administration (September 12, 2012) as a once-daily pill for managing relapsing forms of multiple sclerosis (Bar-Or et al., 2014). Scientific evidence indicates that depression can arise from the disturbance of homeostatic mechanisms governing synaptic plasticity. This disruption leads to the destabilization and reduction of synaptic connections within the circuits regulating mood and emotion (Duman and Aghajanian, 2012). The alterations in synaptic plasticity elicited by depression are linked to modifications in both the structure and functionality of the hippocampus (Liu et al., 2017). Furthermore, chronic pain has also been reported to induce profound changes in hippocampal plasticity (Grilli, 2017). In the presence of oxidative stress, teriflunomide has been reported to preserve hippocampal neuronal functionality and avert oxidative stress-induced morphological and motility changes in hippocampal mitochondria (Malla et al., 2022). In light of teriflunomide's potential in mitigating oxidative stress-induced alterations in hippocampal neuronal functioning, the present investigation was designed to evaluate its potential against the reserpine-induced pain-depression dyad, with a specific emphasis on oxidative stress. Moreover, the role of PI3K was also studied in the observed effect of teriflunomide.

2. Materials and methods

2.1. Laboratory animals

This study employed Swiss albino mice of both genders, with body weights ranging from 20 to 30 g. They were acclimatized in the central animal house of Chandigarh University. The mice were given a pellet chow diet and allowed to freely access water. The experiments were conducted within the hours of 09:30 to 17:30 in a semi-soundproof laboratory setting. The experimental protocol received proper approval from the Institutional Animal Ethics Committee (Reg. no. CU/2022/IAEC/7/07). The experiments were conducted following the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals, which operates under the Ministry of Environment, Forest and Climate Change, Government of India.

2.2. Drugs and reagents

Reserpine (CAS. No. 50555) was bought from Sisco Research Laboratories Pvt. Ltd, Maharashtra, India. Mouse TNF-α kit (CAS. No. KB2145) was procured from Krishgen Biosystems, Maharashtra, India. PI3K inhibitor, LY294002 was purchased from Sigma-Aldrich, USA. Teriflunomide (CAS. No. 550028) and imipramine (CAS. No. SSH0677) were procured from Tokyo Chemical Industry (TCI) Pvt. Ltd, Hyderabad, Telangana, India. All additional reagents and chemicals meeting the requirement for analytical standards were obtained from authorized suppliers. All reagents employed in the study were prepared freshly prior to usage.

2.3. Experimental design

Reserpine was administered to mice via intraperitoneal injection (i.p) at a dosage of 0.5 mg/Kg for three consecutive days to induce a pain-depression dyad (Singh et al., 2021b) (Fig. 2). The animals were categorized into six groups: Group 1 served as the vehicle control (0.1 % CMC; p.o), Group 2 animals underwent treatment with 0.1 % CMC (p.o) + reserpine (0.5 mg/Kg; i.p). Group 3 received a combination of imipramine (3 mg/Kg i.p) + reserpine. Animals in Group 4 were subjected to teriflunomide treatment (10 mg/Kg; p.o) + reserpine. Group 5 animals were administered teriflunomide (20 mg/Kg; p.o) + reserpine. Group 6 received the PI3K inhibitor (LY294002) at a dose of 25 μg/kg along with teriflunomide (20 mg/Kg; p.o) and reserpine. The 0.1 % CMC, teriflunomide (10 and 20 mg/Kg, p.o) and imipramine (15 mg/Kg; i.p) were given 30 min prior to reserpine treatment for the first three days. Thereafter 0.1 % CMC, teriflunomide and imipramine were given alone for the rest of 2 days in the respective groups. In the mechanistic group, LY294002 was given 30 min before administering teriflunomide (20 mg/Kg, p.o) treatment. The magnitude of depression and pain was assessed on the 4th and 6th days by using various models such as forced swim test (FST), tail suspension test (TST), tail-flick test (TFT), hot water allodynia, and Eddy's hot plate test. The Eddy's hot plate test, Hot allodynia, FST and TST were conducted with a 1-hour interval between them. After an additional 2 h, the MWM test was performed. The sequence of tests conducted was: Eddy's hot plate test > Hot allodynia > FST > TST > MWM. On day 6 after behavioral analysis, the animals were anesthetized using ketamine (50 mg/Kg i.p), and blood samples were taken for the assessment of serum cytokine analysis. The animals were sacrificed and brain tissues were harvested for biochemical analysis (Fig. 2).

2.4. Behavioral model

2.4.1. Forced swim test

This experiment was conducted by using the procedure explained by Porsolt (Porsolt et al., 1977). In this examination, the equipment comprises a see-through cylindrical vessel measuring 25 cm in height and 10 cm in diameter. The glass container was filled with water up to such a level that mice did not escape the water container. The temperature of water was consistently held at 25 °C. After the completion of each trial, the water in the container was replaced with fresh water to avoid any bias related to olfaction. Throughout the experiment, each animal was forced to swim for 6 min. During this period the immobility time was recorded (Porsolt et al., 1977).

2.4.2. Tail suspension test

In this test, an animal was suspended at a height of 50 cm from the ground by using adhesive tape. The tape was affixed approximately 1 cm away from the tail's tip. The period of immobility was measured over 4 min out of a total of 6 min. The first 2 min of the test were for the adaptation of mice to the suspension. An animal was considered immobile if it did not show any paw movement (no struggle period) (Liu et al., 2014b).

2.4.3. Eddy's hot plate test

In the hot plate experiment, mice were positioned on a consistently heated plate set at 55 °C. Twenty seconds was the cutoff time for this test. Before administering a compound, the mouse's initial latency response (measured in seconds) to the thermal stimulus was checked, i.e., either lifting the hind paw or jumping response with all feet on the plate. The post-treatment latency responses were then assessed on the fourth and sixth days after the administration of the compound to the animals (Linciano et al., 2020).

2.4.4. Tail-flick test

For this test, water was maintained at 55 ± 1 °C and was used for recording the tail-flick response. During the test, each mouse received a terry cloth towel wrap before the tail was gently dipped into the hot water. At least 5 cm of the tail was dipped in the water to record the flick

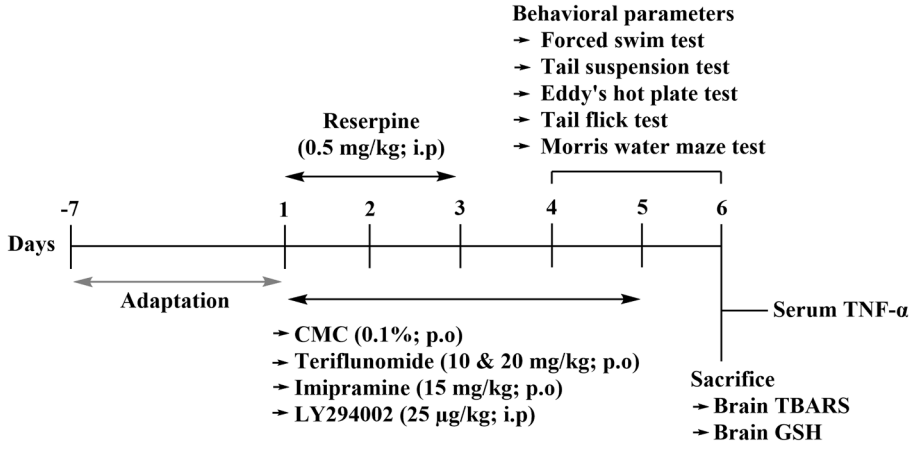

Fig. 2. Schematic description of the experimental design.

response. The cut-off time for this test was 40 sec. Any pharmacological treatment increasing latency to flick the tail in comparison to the disease control group delineates the analgesic potential of that agent (Latif et al., 2021).

2.4.5. Morris water maze test

The setup comprises a circular drum with a smooth surface, measuring 90 cm in diameter and 50 cm in height, along with a 9 cm-diameter circular platform inside the drum. The platform was positioned within one of the four quadrants of the drum. The circular platform was positioned 1 cm below the water's surface. During the test, the water temperature was maintained within the range of 23 to 25 °C to prevent any temperature-related bias. To conceal the platform a nontoxic white color was mixed with the water to make it translucent. An animal was placed into the drum from the direction opposite to the platform and then allowed to swim within the drum. The time taken to find the platform was recorded as a measure of memory. The animal's visit to the platform was deemed a success if it remained on the platform for a minimum of 15 sec. The maximum time of the test was 5 min. Before each experiment, all the mice underwent rigorous training for a minimum of five days to learn how to find a platform (Morris, 1981).

2.5. Biochemical estimations

2.5.1. Processing of brain samples for oxidative stress analysis

The homogenates of isolated brain samples were prepared in phosphate buffer (pH 7.4). 100 mg of minced brain tissue was homogenized in a 100 mL phosphate buffer (pH of 7.4). After homogenization, the blend underwent centrifugation at a speed of 3000 rpm for 15 min. The obtained supernatant was preserved for determining the levels of thiobarbituric acid reactive species (TBARS) and reduced glutathione (GSH).

2.5.2. Determination of brain TBARS level

In this test, the production of pink color as a result of the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) and other aldehydes is indicative of lipid peroxidation. In this procedure, 0.1 mL of brain homogenate supernatant was added to a 2 mL (1:1:1) mixture of thiobarbituric acid- trichloro acetic acid- hydrochloric acid. The test tube containing this mixture was subsequently introduced into the water bath and kept there for 15 min at a temperature of 100 °C. Following the cooling process, the mixture underwent centrifugation for 10 min at a speed of 1000 rotations per minute. The clear supernatant was collected and measured at 535 nm. The findings were quantified as µM/mg of tissue (Niehaus and Samuelsson, 1968).

2.5.3. Determination of brain GSH level

The Beutler et al. approach was used with little modification to estimate the GSH level in the mice brain (Beutler et al., 1963). In this test, the interaction of 5.5′- dithiobis (2-nitrobenzoic acid) (DTNB) with the sulphydryl group of GSH results in the production of the yellow color. In this procedure, 0.5 mL of supernatant, 2 mL of 0.3 M disodium hydrogen phosphate buffer (pH 8.4), and 0.25 mL of freshly prepared DTNB were added. The absorbance was measured at 412 nm for the resultant yellow-colored solution. The findings were reported as µM/mg of the tissue mass.

2.5.4. Estimation of serum TNF-α level

Serum samples underwent the TNF-α estimation using the procedure outlined in the ELISA kits provided by Krishgen Biosystems in Mumbai, India.

2.5.4.1. Standards stock preparation. A 0.5 ug/mL concentration was prepared by adding 1.8 µL of a standard solution of mouse TNF-α in 1998.2 µL of assay diluent (1X). Further to make a 1000 pg/mL middle stock solution, 2 µL of the initial standard (0.5 ug/mL) was diluted with 998 µL of the assay diluent (1X). The different concentrations of the standard (450 pg/mL, 225 pg/mL, 112.5 pg/mL, 56.25 pg/mL, 28.13 pg/mL, and 14.06 pg/mL) were prepared by diluting the middle stock solution.

2.5.4.2. Assay procedure. The plate was initially loaded with 100 µL/well of standards and samples, followed by sealing and incubating at 37C for 2 h. The plate was subjected to four wash cycles with wash buffer (1X), and any excess buffer was eliminated by firmly inverting the plate onto absorbent paper and tapping it. Liquid present on the outer bottom surface of the microtiter wells was removed to prevent any potential interference with the subsequent reading process. Additionally, each well received 100 µL of the diluted detection antibody solution, followed by sealing the plate and incubating it at 37C for 1 h. The plate underwent four washes using wash buffer at a 1X concentration. To this, 100 µL of a diluted Streptavidin-HRP solution was introduced into each well, followed by sealing the plate and incubating it at 37C for 30 min. Subsequently, the plate underwent four wash cycles using 1X wash buffer after the incubation period. Thereafter, 100 µL of the TMB substrate solution was introduced and incubated in the dark at 37 °C for 30 min. Consequently, the wells exhibiting a positive response displayed a blue color. The reaction was halted by introducing 100 µL of stop solution into each well. The blue color of the positive wells transformed into a vibrant yellow color. Finally, the absorbance measurement was conducted at 450 nm, and this was done within 30 min after halting the reaction.

2.6. Statistical analysis

The results were displayed in the form of mean ± standard deviation (SD). Statistical significance was determined using GraphPad Prism 7.00 software, employing two-way analysis of variance (ANOVA) for behavioral data and one-way analysis of variance (ANOVA) for biochemical measurements (GraphPad Software Inc. U.S.A.).

3. Results

3.1. Effect on immobility time in the FST

On both day 4 and day 6 of the FST, the administration of reserpine significantly extended the duration of immobility (Day 4: 185.600 ± 10.644 sec and Day 6: 171.400 ± 13.050 sec) when compared to the results observed in the control group (Day 4: 73.296 ± 12.155 sec and Day 6: 76.430 ± 17.430 sec) (Fig. 3A). Standard imipramine administration significantly alleviated the depressive effect of reserpine. A significant reduction in the immobility time was recorded with the imipramine treatment on day 4 and day 6 (Day 4: 95.200 ± 30.392 sec and Day 6: 86.600 ± 22.523 sec). Teriflunomide (10 and 20 mg/Kg) treatment significantly alleviated the depressive effect of reserpine. The observations of day 4 and day 6 showed that teriflunomide (10 mg/Kg) decreased the immobility time (Day 4: 111.400 ± 19.731 sec and Day 6: 98.600 ± 10.738 sec) when compared to the group treated with reserpine alone. Similarly, a 20 mg/kg dose of teriflunomide also reduced the immobility time (Day 4: 100.800 ± 10.060 sec and Day 6: 95.200 ± 4.207 sec) as compared to the reserpine-treated group. In this investigation, PI3K inhibitor, LY2934002 significantly alleviated the antidepressant effect of teriflunomide (20 mg/Kg). The observations of day 4 and day 6 showed that the LY294002 treatment significantly uplifted the duration of immobility (Day 4: 182.400 ± 24.276 sec and Day 6: 186.400 ± 28.395 sec) as compared to the teriflunomide (20 mg/Kg) treated group (Fig. 3A).

3.2. Effect on immobility time in the TST

The reserpine administration resulted in a significant increase in the immobility time on the fourth and sixth day of assessment (Day 4: 240.000 ± 7.906 sec and Day 6: 171.000 ± 22.902 sec) when compared to the control group (Day 4: 93.000 ± 15.604 sec and Day 6: 93.000 ± 15.556 sec) (Fig. 3B). The administration of imipramine at a dosage of 15 mg/Kg significantly reduced the immobility time on the fourth and sixth day (Day 4: 104.400 ± 17.387 sec and Day 6: 97.600 ± 14.450 sec) when compared to the group treated with reserpine. Both doses of teriflunomide (10 and 20 mg/Kg) treatment significantly abolished the depression-inducing effect of reserpine. Teriflunomide (10 mg/kg) significantly reduced the duration of immobility on the 4th and 6th day of observation (Day 4: 134.600 ± 16.227 sec and Day 6: 121.400 ± 9.965 sec) as compared to the group treated with reserpine. Similarly, a 20 mg/kg dose of teriflunomide also reduced the immobility time (Day 4: 124.000 ± 10.724 sec and Day 6: 110.800 ± 8.438 sec) as compared to the reserpine-treated group. Furthermore, LY294002 significantly abolished the anti-depressant effect of teriflunomide (20 mg/Kg). The outcome of this group showed that LY294002 pretreatment significantly increased the immobility time on both days of observation (Day 4: 215.200 ± 14.498 sec and Day 6: 200.800 ± 22.208 sec) when compared to the alone teriflunomide-treated group (Fig. 3B).

3.3. Effect on paw withdrawal latency in the Eddys hot plate test

In this study, the reserpine treatment significantly induced nociception in comparison to the control group (Fig. 4A). The group that received reserpine exhibited a significant reduction in latency time during Eddy's hot plate test (Day 4: 4.680 ± 0.753 sec and Day 6: 4.480 ± 0.756 sec) when compared to the control group (Day 4: 10.002 ± 0.953 sec and Day 6: 9.902 ± 0.770 sec) on both days of assessment. The standard drug imipramine significantly increased the latency time on day 4 and day 6 (Day 4: 8.296 ± 0.541 sec and Day 6: 8.552 ± 0.431 sec) when compared to the reserpine group. At 10 mg/Kg dose, teriflunomide exhibited a significant improvement in latency time on day 4 (5.580 ± 0.760 sec) and day 6 (6.380 ± 0.746 sec) when compared to the group treated with reserpine. Similarly, the higher dose of teriflunomide (20 mg/Kg) also alleviated the nociceptive effect of reserpine. Teriflunomide (20 mg/Kg) significantly increased the latency time on day 4 and day 6 (Day 4: 6.760 ± 0.868 sec and Day 6: 6.880 ± 0.482 sec) when compared to the group treated with reserpine. Pretreatment with PI3K inhibitor, LY294002 significantly abolished the anti-nociceptive effect of teriflunomide. The outcome of this group showed that the LY294002 treatment significantly reduced the latency time (Day 4: 4.060 ± 0.802 sec and Day 6: 3.960 ± 0.915 sec) when compared to the group treated with teriflunomide (20 mg/Kg) (Fig. 4A).

3.4. Effect on tail withdrawal latency in tail flick test

The reserpine-treated group significantly decreased the tail-flick time (Day 4: 3.304 ± 0.330 sec and Day 6: 2.828 ± 0.358 sec) when compared to the control group (Day 4: 9.460 ± 0.737 sec and Day 6: 9.480 ± 0.870 sec) on both days of evaluation (Fig. 4B). On both days of assessment, the standard imipramine treatment significantly increased

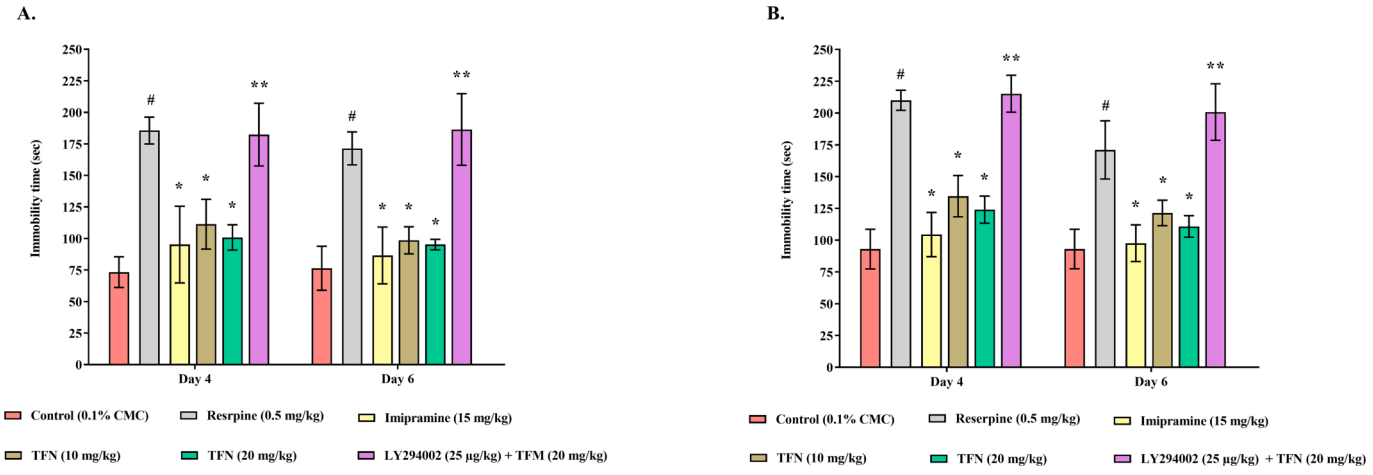

Fig. 3. Effect of various pharmacological interventions on immobility time in (A) FST and (B) TST. Two-way analysis of variance (ANOVA) followed by Tukey's test was used to establish the statistical significance. #: $p < 0.05$ vs control, *: $p < 0.05$ vs reserpine and **: $p < 0.05$ vs TFN (20 mg/Kg). [TFN, teriflunomide].

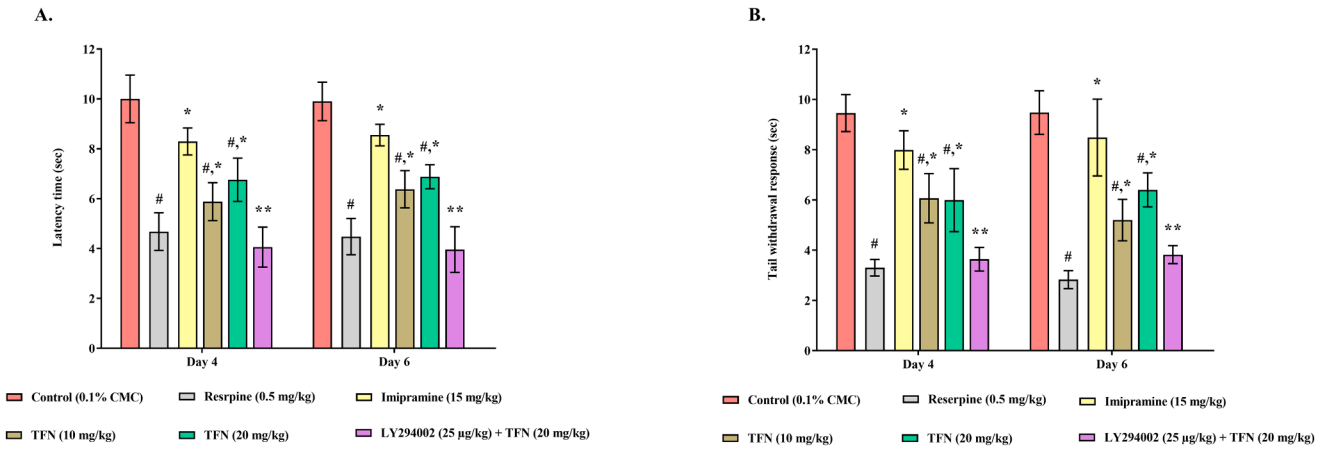

Fig. 4. Effect of various pharmacological interventions on (A) paw withdrawal response in Eddy's hot plate test and (B) tail withdrawal latency in the tail flick test. Two-way analysis of variance (ANOVA) followed by Tukey's test was used to establish the statistical significance. #: $p < 0.05$ vs control, *: $p < 0.05$ vs reserpine and **: $p < 0.05$ vs TFN (20 mg/Kg). [TFN, teriflunomide].

the tail-flick time (Day 4: 7.988 ± 0.766 sec and Day 6: 8.486 ± 1.528 sec) when compared to the group treated with reserpine. The low dose of teriflunomide (10 mg/Kg) significantly increased the tail-flick time on both days (Day 4: 6.070 ± 0.980 sec and Day 6: 5.200 ± 0.825 sec) when compared to the group treated with reserpine. The higher dose of teriflunomide also significantly increased the tail-flick time on both days of evaluation (Day 4: 5.992 ± 1.255 sec and Day 6: 6.400 ± 0.678 sec). The 20 mg/Kg dose of teriflunomide showed a prominent anti-nociceptive effect. The administration of LY294002, a PI3K inhibitor, significantly reversed the analgesic effect of teriflunomide (20 mg/Kg). The group treated with LY294002 showed a significant reduction in tail-flick time (Day 4: 3.640 ± 0.471 sec and Day 6: 3.822 ± 0.357 sec) when compared to the group treated solely with teriflunomide (20 mg/Kg) (Fig. 4B).

3.5. Effect on latency to find the platform in the MWM test

The reserpine treatment had a significant negative impact on spatial memory. On 4th and 6th day, a significant increase in latency time to locate the platform was observed in the group that received reserpine treatment (Day 4: 115.400 ± 25.696 sec and Day 6: 105.400 ± 16.456 sec)when compared to the control group (Day 4: 20.400 ± 10.714 sec and Day 6: 24.600 ± 8.849 sec) (Fig. 5). At a dose of 15 mg/Kg, imipramine significantly decreased the time to locate the platform (Day 4:

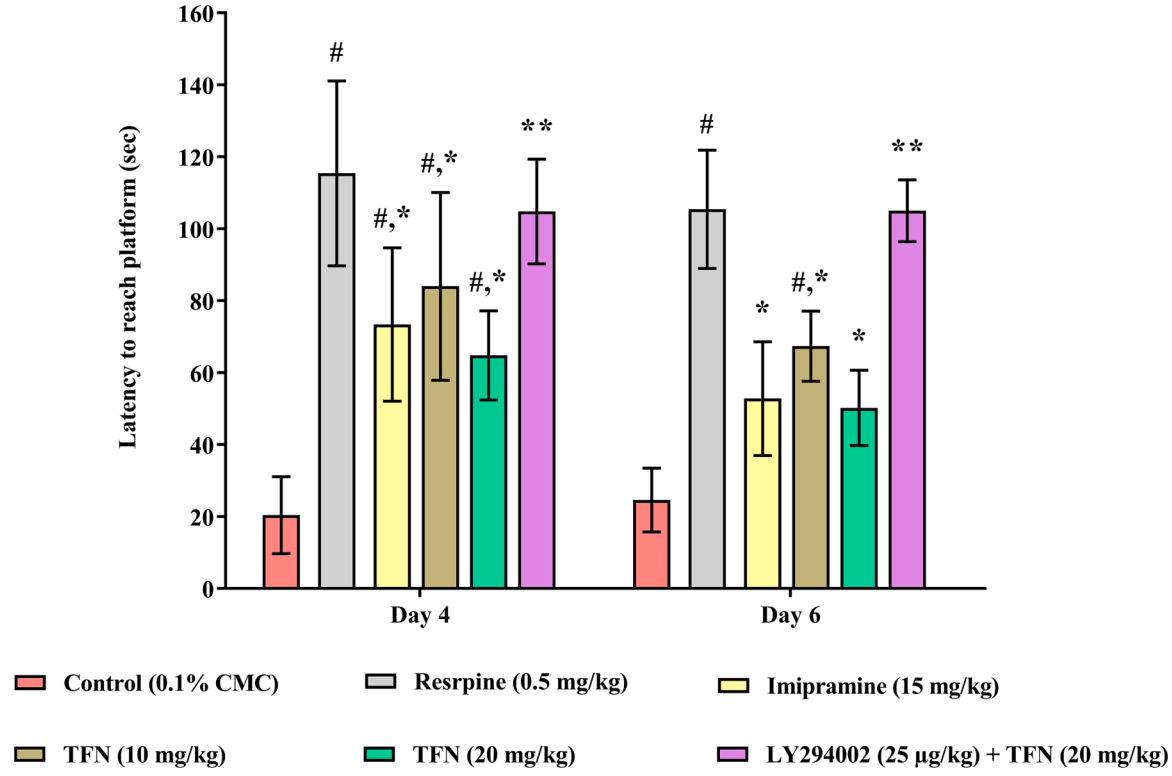

Fig. 5. Effect of various pharmacological interventions on latency to reach the platform on the MWM test. Two-way analysis of variance (ANOVA) followed by Tukey's test was used to establish the statistical significance. #: $p < 0.05$ vs control, *: $p < 0.05$ vs reserpine and **: $p < 0.05$ vs TFN (20 mg/Kg). [TFN, teriflunomide].

73.400 ± 21.314 sec and Day 6: 52.800 ± 15.786 sec) on both days when compared to the reserpine group. The administration of teriflunomide at a dosage of 10 mg/Kg significantly decreased the time to find the platform on the fourth and sixth day of assessment (Day 4: 84.000 ± 26.077 sec and Day 6: 64.400 ± 9.737 sec) when compared to the group treated with reserpine. However, on the fourth day, the effect of a 10 and 20 mg/kg dose of teriflunomide was also significant from the effect noted in the control group. While the higher dose of teriflunomide (20 mg/Kg) effectively eliminated the detrimental impact of reserpine on spatial memory on day 6. Teriflunomide (20 mg/Kg) significantly decreased the time to locate the platform on both day 4 and day 6 (Day 4: 64.800 ± 12.398 sec and Day 6: 50.200 ± 10.450 sec) when compared to the group treated with reserpine. In this study, the spatial memory-enhancing effect of teriflunomide (20 mg/Kg) was counteracted by a PI3K inhibitor, LY294002. LY294002 significantly increased the time (Day 4: 104.800 ± 14.567 sec and Day 6: 105.000 ± 8.573 sec) to find the platform on day 4 and day 6 of the evaluation (Fig. 5).

3.6. Effect on the brain GSH level

In the present study, reserpine treatment significantly reduced brain GSH levels (0.506 ± 0.073 μM/mg) when compared to the control group (1.079 ± 0.068 μM/mg) (Fig. 6A). Administration of imipramine significantly reverted the GSH-lowering effect of reserpine. Imipramine significantly upregulated the brain GSH level (0.870 ± 0.199 μM/mg) as compared to the reserpine group. Pretreatment with teriflunomide (10 mg/Kg) significantly reverted the GSH lowering effect of reserpine. Teriflunomide (10 mg/Kg) significantly increased the level of GSH in the brain (0.819 ± 0.071 μM/mg) as compared to the reserpine group. The higher dose of teriflunomide (20 mg/Kg) also reverted the GSH lowering effect of reserpine. However, the effect of the 20 mg/Kg dose of teriflunomide was more prominent (0.966 ± 0.068 μM/mg) as compared to the 10 mg/Kg dose of teriflunomide. The outcome of teriflunomide (20 mg/Kg) + LY294002 showed that LY294002 treatment significantly abolished the GSH upregulating effect of teriflunomide (20 mg/Kg). A significant reduction in the brain GSH level was observed with the LY294002 treatment (0.588 ± 0.134 μM/mg) (Fig. 6A).

3.7. Effect on the brain TBARS level

In the present study, outcomes showed that reserpine treatment significantly uplifted the level of TBARS in the brain (0.512 ± 0.126 nM/mg) as compared to the control group (0.212 ± 0.052 nM/mg) (Fig. 6B). The standard drug imipramine significantly reduced the level of TBARS (0.285 ± 0.081 nM/mg) when compared to the reserpine-treated group. Whereas, the test drug teriflunomide (10 mg/Kg) had no significant effect on the TBARS level (0.441 ± 0.095 nM/mg) in comparison to the reserpine-treated group. Whereas, a higher dose of teriflunomide (20 mg/Kg) significantly reverted the TBARS uplifting effect of reserpine. A significant reduction in the TBARS level was observed with the 20 mg/Kg dose of teriflunomide (0.238 ± 0.082 nM/mg) as compared to the reserpine group. The outcome of teriflunomide (20 mg/Kg) + LY294002 group showed that LY294002 treatment significantly abolished the TBARS lowering effect of teriflunomide (20 mg/Kg). A significant increase in the brain TBARS level was observed with the LY294002 treatment (0.419 ± 0.084 nM/mg) (Fig. 6B).

3.8. Effect on the brain TNF-α level

The reserpine-treated group significantly increased the level of serum TNF-α (458.000 ± 104.370 pg/mL) as compared to the control group (155.667 ± 49.166 pg/mL) (Fig. 7). The standard drug imipramine significantly decreased the level of serum TNF-α (192.667 ± 16.073 pg/mL) in comparison to the reserpine group. Moreover, the outcomes of teriflunomide (20 mg/Kg) showed a significant reduction in the serum TNF-α level (267.000 ± 49.122 pg/mL) as compared to the reserpine group. However, no effect was observed on serum TNF-α level with a 10 mg/kg dose of teriflunomide (324.000 ± 50.090 pg/mL) in comparison to the reserpine group. Furthermore, the LY294002 treatment (429.333 ± 92.635 pg/mL) partly reverted the serum TNF-α uplifting effect of teriflunomide (Fig. 7).

4. Discussion

Depression is a pervasive and enduring medical condition that can impact cognition, emotions, and physical well-being. It manifests through persistent feelings of low mood, diminished energy, sadness, sleep disturbances, and an inability to experience pleasure in life (Cui, 2015). Pain is recognized to have both a sensory dimension (intensity) and an affective dimension (unpleasantness) (Talbot et al., 2019). The coexistence of pain and depression can exert deleterious effects on patient health, as well as impair personal and social functioning (Han and Pae, 2015). Evidence states that Approximately 19 % of adults in Europe and 20.4 % in the United States experience chronic pain, with depression being the most commonly associated comorbidity. The prevalence of depression in European and American societies stands at 7 % and 7.5 %, respectively (Tenti et al., 2022). The use of rodent models has facilitated preclinical research into the mechanisms and manifestations

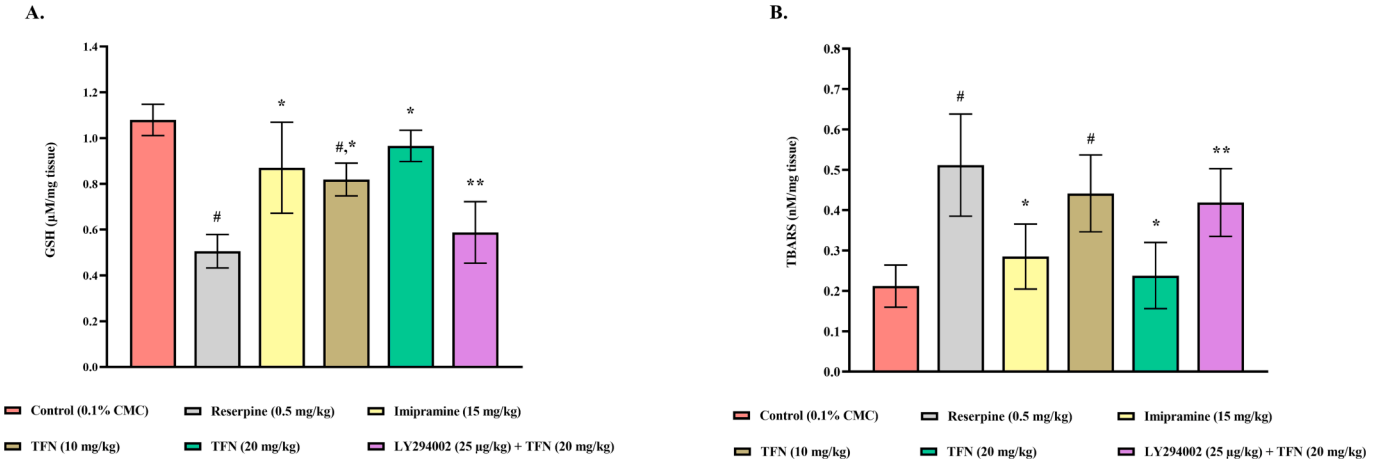

Fig. 6. Effect of various pharmacological interventions on (A) the brain GSH and (B) TBARS level. One-way analysis of variance (ANOVA) followed by Tukey's test was used to establish the statistical significance. a = $p < 0.05$ vs control, b = $p < 0.05$ vs reserpine, c = $p < 0.05$ vs imipramine, #: $p < 0.05$ vs control, *: $p < 0.05$ vs reserpine and **: $p < 0.05$ vs TFN (20 mg/Kg). [TFN, teriflunomide].

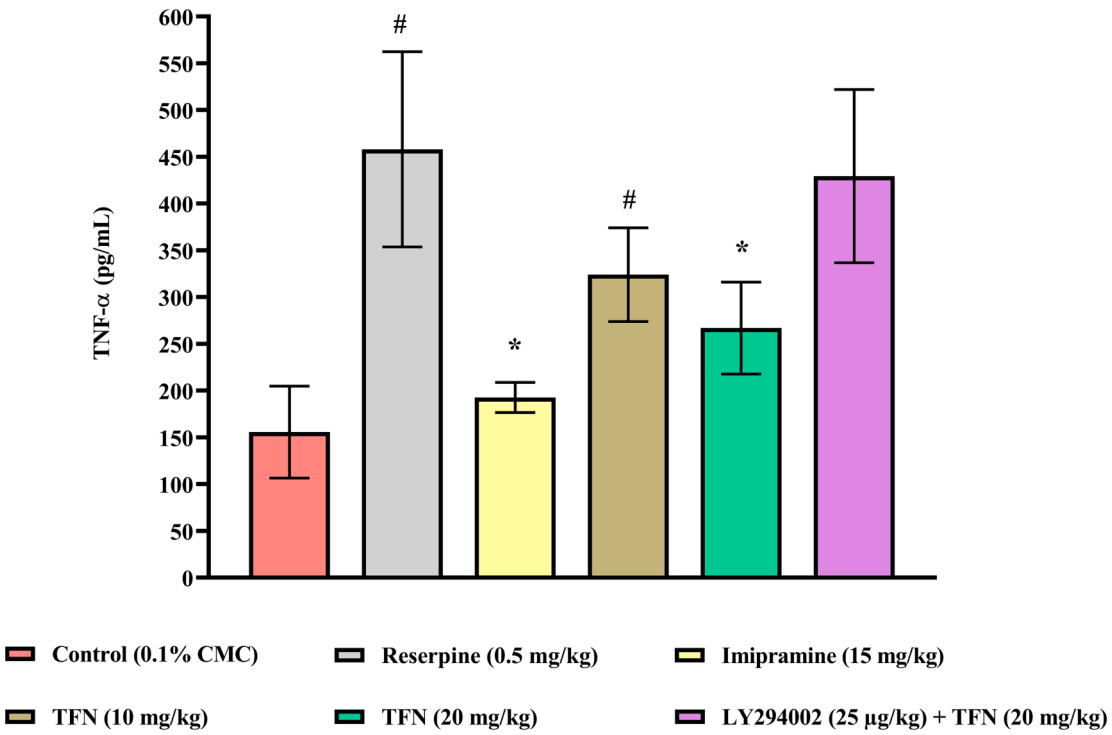

Fig. 7. Effect of various pharmacological interventions on the serum TNF-α level. One-way analysis of variance (ANOVA) followed by Tukey's test was used to establish the statistical significance. #: $p < 0.05$ vs control, *: $p < 0.05$ vs reserpine and **: $p < 0.05$ vs TFN (20 mg/Kg). [TFN, teriflunomide].

of depression triggered by pain (Singh et al., 2021c). In the present research, reserpine treatment induced the pain-depression-like state in mice. Reserpine significantly increased the immobility time in comparison to the control group of mice in FST and TST. Whereas, decreased the paw and tail withdrawal latency in Eddy's hot plate and tail flick tests, respectively. A test drug teriflunomide (10 and 20 mg/Kg) significantly reverted the depressive and pain-eliciting effect of reserpine. The results of FST and TST showed a significant decrease in immobility time in comparison to the reserpine group of mice. Whereas, increased the paw and tail withdrawal latency in Eddy's hot plate and tail flick tests, respectively. Reports delineate that depression can impact cognitive function, including memory. Individuals with depression might experience difficulties with concentration, attention, and memory. This can manifest as forgetfulness, difficulty recalling information, and impaired cognitive abilities. The cognitive symptoms associated with depression are often referred to as depressive cognitive symptoms (Levin et al., 2007). In this study, reserpine treatment uplifted the time to reach the platform in the MWM test. Teriflunomide (10 and 20 mg/Kg) treatment also reverted the memory-impairing effect of reserpine. A significant decrease in time to locate the platform was observed with both doses of teriflunomide. Overall, these results delineate that teriflunomide is an effective analgesic, anti-depressant and memory-consolidating agent at the preclinical step.

LY294002 is a chemical compound that functions as an inhibitor of the PI3K pathway (Huang et al., 2021). The PI3K pathway plays a pivotal role in regulating cell survival, growth and differentiation (Yang et al., 2019). The activation of PI3K is responsible for producing phosphatidylinositol-3,4,5-trisphosphate, which leads to Akt activation. The PI3K/Akt pathway exerts a significant influence on the regulation of multiple signaling pathways, one of which is the glycogen synthase kinase 3 beta (GSK-3β) pathway (Lindahl et al., 2005). GSK3β has been proposed as a potential contributor to the development of major depressive disorder (MDD). GSK3β is involved in the signaling pathways of various neurotransmitters such as serotonin, dopamine, and glutamate, which are implicated in mood regulation (Moreno et al., 2006). PI3K/Akt is known to phosphorylate GSK-3β, resulting in its inactivation (Lindahl et al., 2005). Thus, delineating that PI3K activation negatively regulates the GSK-3β mediated pathophysiological role in depression. Moreover, there have been reports delineating that different pharmacological agents mediate the anti-nociceptive and anti-inflammatory effects via the stimulation of the PI3K/Akt cascade (Zhang et al., 2019; Feng et al., 2020). In the present study, LY294002 treatment significantly alleviated the anti-depressant and anti-nociceptive effect of teriflunomide (20 mg/Kg). Outcomes of LY29002 treatment showed a significant increase in immobility time in FST and TST in comparison to the teriflunomide-treated (20 mg/kg) group. Whereas, decreased the paw and tail withdrawal latency in Eddy's hot plate and tail flick tests, respectively. Evidence also delineates that activation of the PI3K pathway is associated with the early stages of learning and memory processes. It plays a role in the molecular processes that support the transformation of short-term memories into more enduring long-term memories (Santini et al., 2014). In this investigation, LY294002 treatment significantly alleviated the memory-consolidating effect of teriflunomide (20 mg/Kg). LY294002 treatment significantly increased in time to locate the platform in comparison to the teriflunomide (20 mg/kg) treated group. Thus, these outcomes delineate that teriflunomide might exert its anti-depressant, anti-nociceptive and memory-improving effect by activating the PI3K pathway.

The interplay between pain and depression can arise due to neuronal tissue damage, which is associated with the occurrence of neuroinflammation as well as oxidative and nitrosative stress processes (Birmann et al., 2019). Inflammatory agents in the brain play a crucial role in sustaining the connection between depression and pain, potentially serving as underlying components of this syndrome (Campos et al., 2020). GSH has been reported to play a role in regulating oxidative stress and inflammation. These processes, in turn, may have an indirect impact on pain perception. Individuals with chronic pain conditions might have reduced glutathione levels (Borkum, 2016). Evidence states that GSH mediates protection against oxidative or nitrosative stress, either through its direct interaction with reactive oxygen or nitrogen species or as a necessary cofactor for GSH S-transferases and glutathione peroxidases (Morris et al., 2014). In the present study, reserpine

treatment significantly reduced the brain GSH and increased brain TBARS and serum TNF-α levels. Teriflunomide treatment significantly uplifted the brain GSH level (10 and 20 mg/Kg) and reduced the brain TBARS level (20 mg/Kg). Moreover, both doses of teriflunomide also downregulated the serum TNF-α level. Furthermore, the available evidence suggests that the PI3K/Akt pathway plays a pivotal role in removing reactive oxygen species, thus, maintaining the redox balance (Koundouros and Poulogiannis, 2018). The PI3K/Akt pathway has a significant role in controlling the protective response to oxidative stress through the Nrf2-ARE antioxidant pathway (de Sousa et al., 2014). In this study, LY294002 treatment significantly alleviated the GSH-increasing and TBARS-lowering effect of teriflunomide. LY294002 treatment significantly increased and decreased the brain TBARS and GSH levels. Whereas, no significant effect was observed on serum TNF-α levels. Thus, delineating that teriflunomide might alleviate oxidative stress by stimulating the PI3K pathway. Conclusively, the results of the current study delineate that teriflunomide might exert its anti-depressant, anti-nociceptive and memory-improving effect by stimulating the PI3K-mediated protection against oxidative stress. Though this study provides valuable insights into the potential of teriflunomide in alleviating the pain-depression dyad, it does have certain limitations. In this study, only TBARS and GSH estimations were performed to assess the oxidative stress levels in biological systems. Moreover, elucidating the effect of teriflunomide on the brain Nrf-2 and HO-1 could offer valuable insights for potential therapeutic strategies. Additionally, the initial assessment of the compound's impact on TNF-α levels serves as a starting point for prospective studies focused on modulating inflammatory pathways. Even though the broader objectives may not have been entirely achieved, this study has laid the foundation for future research to delve deeper insight into the oxidative and inflammatory stress pathways in the protective effect of teriflunomide against the pain-depression dyad.

Ethics approval

The experimental protocol received proper approval from the Institutional Animal Ethics Committee (IAEC) (Reg. no. CU/2022/IAEC/7/07). The experiments were conducted following the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Ministry of Environment and Forest, Government of India.

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CRediT authorship contribution statement

Kamal Nabi: Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft. **Lovedeep Singh:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Atul Kabra:** Resources, Software, Writing – review & editing. **Ashwag S. Alanazi:** Writing – review & editing, Funding acquisition. **Hany W. Darwish:** Writing – review & editing, Funding acquisition. **Bader Alsuwayt:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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