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Research article

Pharmacological investigation of taxifolin for its therapeutic potential in depression

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ABSTRACT

The current study aimed to investigate the influence of taxifolin on depression symptoms alleviation in Male Sprague-Dawley rats by targeting underlying pathways of depression. Molecular docking analyses were conducted to validate taxifolin's binding affinities against various targets. *In silico* analysis of taxifolin revealed various aspects of post docking interactions with different protein targets. Depression was induced in rats via intraperitoneal injection of Lipopolysaccharide (LPS; 500 μ g/Kg) for 14 alternative days. Rats (n = 6/group) were randomly assigned to four groups: (i) Saline/Control, (ii) Disease (LPS 500 μg/kg), (iii) Standard (fluoxetine 20 mg/kg), and (iv) Treatment (taxifolin 20 mg/kg). At the end of the in vivo study, brain samples were used for biochemical and morphological analysis. Taxifolin exhibited neuroprotective effects, as evidenced by behavioral studies, antioxidant analysis, histopathological examination, immunohistochemistry, ELISA and RT PCR, indicating an increase number of surviving neurons, normalization of cell size and shape, and reduction in vacuolization. Taxifolin also decreased inflammatory markers such as TNF-α, NF-κb, IL-6 and COX-2, while significantly upregulating and activating the protective PPAR-γ pathway, through which it reduces the oxidative stress, neuroinflammation, neurodegeneration, thereby ameliorating depression symptoms in experimental rat model of depression. Our finding suggests that taxifolin act as neuroprotective agent partially mediated through PPAR-γ pathway.

1. Introduction

The most common mental illness among the general public is depression, which is characterized by feelings of sorrow, guilt, low self-worth, a lack of interest or enjoyment, irregular eating or sleeping patterns, fatigue, and difficulty concentrating. Depression typically has a chronic course and significantly impairs individuals' capacity to work and their overall quality of life [1]. According to the World Health Organization (WHO), depression is anticipated to be the second most common cause of disease and disability by 2030, making it a major public health concern [2]. Over 300 million individuals worldwide suffer from depression, it is considered as one of the most frequent, costly and debilitating mental disorders [3].

Recent data suggests that depression is connected with increased oxidative stress and pro-inflammatory cytokine levels. Depression is a complex and multifaceted condition that involves both psychological and physiological factors, Patients with depression have

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higher levels of cytokines like TNF-α, IL-6, and NF-κB, indicating the chronic inflammation in depression [4]. However, the exact underlying mechanisms of depression are still not fully understood [5]. This is why ongoing research is essential to improve our understanding and treatment of depression. Neuroinflammation has indeed gained significant attention as a potential participant in progress and development of depression. The innate defense mechanism as well the inflammatory responses within the brain play a crucial role in maintaining brain health. However, when these processes become dysregulated they can lead to neuroinflammation which involves the triggering of immune cells and discharge of inflammatory chemicals within the brain. There has been increasing evidence that suggests connection among chronic neuroinflammation and the development of depression [6].

The most commonly prescribed medications for depression was selective serotonin reuptake inhibitors, researchers now recognize the underlying causes of depression are more complex and involve multiple factors beyond just monoamine neurotransmitter levels. The monoamine hypothesis suggests that depression is caused by a deficiency of certain neurotransmitters, such as serotonin and norepinephrine (noradrenaline) in the brain. However, it's important to note that this hypothesis is a simplification of the actual biological and psychological processes that contribute to depression. Additionally, factors like neuroinflammation, neural plasticity, and stress responses contribute to the development and persistence of depression. There is growing evidence linking neuro-inflammation to depression. Researchers are investigating anti-inflammatory drugs as potential adjuncts to standard antidepressant treatments. It is imperative to do research on novel alternative pharmaceutical targets. This can further enhance the therapeutic approach in addition to traditional therapy [7]. It is hypothesized that there is a potential link between inflammation and depression [3]. Inflammation might contribute to depression for instance, chronic stress associated with depression can activate the immune system, promoting inflammation.

PPAR-γ may be a viable therapeutic target for depression since it inhibits the expression of inflammatory genes and controls oxidative stress-sensitive pathways both of which are implicated in the etiology of depression [8]. PPAR-γ has anti-inflammatory effects by regulating immune responses and inflammation-related genes. This aspect of PPAR-γ has implications for conditions like atherosclerosis, where inflammation contributes an important role in pathophysiology of extensive sort of neurodegenerative disorders [9].

Taxifolin as dihydroquercetin or 3,5,7,3,4-pentahydroxy flavanone, is a flavonoid that is a member of the flavanonol subclass of flavonoids. Like other flavonoids, taxifolin possesses antioxidant properties, which means it has the ability to protect cells from oxidative stress and counteract toxic free radicals for its antioxidant properties [10,11]. This antioxidant capacity can have potential benefits for human health, as oxidative stress is connected to a number of long-term illnesses, such as cancer, neurological issues, and cardiovascular problems [12]. Taxifolin can be extracted from herbs, grapes, citrus fruits, onions, green tea, olive oil, and wine and has the potential to function as a novel disease-modifying medication for patients suffering from neurodegenerative illnesses through multiple possible pathways [13]. Its absolute bioavailability in rats was barely 0.17 percent. According to a study by Yang et al. rats' absolute bioavailability of taxifolin was 0.49 %. Taxifolin glucuronide and methylated taxifolin glucuronide had substantially greater plasma concentrations than taxifolin. In vivo, the predominant metabolic route for taxifolin was glutaronidation. The distribution patterns varied according to metabolites and organs. The heart and brain generally contained very little amounts of taxifolin and its metabolites. All organs had larger concentrations of taxifolin than its metabolites, with the exception of the liver. The Cmax of taxifolin was 1.12, 1.3, and 1.14 μg/g in the kidney, lung, and spleen at 1.5 h, respectively. These values were considerably higher than the Cmax of taxifolin in plasma, suggesting that taxifolin had accumulated in these tissues. The heart and brain showed the highest concentration of taxifolin at 1.5 and 6 h, with values of 0.33 and 0.28 μg/g, respectively which indicates that taxifolin can cross the blood-brain barrier [14,15].

Fluoxetine belongs to the class of antidepressants known as selective serotonin reuptake inhibitors (SSRIs), which are frequently prescribed for the management of depressive disorders [16]. Several studies have demonstrated that fluoxetine affects the inflammatory system in experimental animals has demonstrated that fluoxetine acts on the inflammatory process to lower levels of proinflammatory cytokines [17] and is used as standard in experimental studies for evaluating the effect of novel compound for depression [18].

An important component toll-like receptors (TLRs), which are pattern recognition receptors (PRRs), are part of the innate immune response. Many different types of immune cells express TLRs, comprising dendritic cells, some types of T cells and macrophages. Their role is the recognition of particular molecular patterns which are linked to infections such as pathogens associated molecular patterns (PAMPs). When TLRs detect these PAMPs, they initiate a signaling chain which induce the initiation of various immune responses. This involves the generation of cytokines that promote inflammation, such as chemokines, and interferons. There are several types of Toll-like receptors, each recognizing different PAMPs for example lipopolysaccharides (LPS) is recognized by TLR4 which was found in the outer membrane of gram-negative bacteria's, providing rapid and general defense mechanisms against a wide range of pathogens [19]. The immunological, inflammatory and oxidative stress reactions could result from LPS or bacterial translocation. Repeated exposure to LPS can induced neuro-inflammatory reactions and depressive-like behavior in animal models [20]. Therefore, the current research was aims to investigate the potential role of taxifolin in mitigating LPS induced depressive like behavior, inflammation signals and a neurodegenerative model in rats via the PPAR-γ pathway.

2. Materials and methods

2.1. Chemical

Taxifolin (CAT# C13293876) and Lipopolysaccharide (LPS) (CAT. No. C15166619) acquired from Shanghai Macklin Biochemical Co, Ltd China (CAT# C13293876). The local pharmaceutical company provided the dimethyl sulfoxide (DMSO) and fluoxetine. It is

commonly prescribed to treat various mental health conditions, primarily depression Proteinase K and Phosphate buffer saline (PBS) was supplied by (MP Bio USA). Santa Cruz Biotechnology USA delivered mouse monoclonal anti-TNF Alpha (SC–52B83), mouse monoclonal anti-p-NFKB (SC-271908), 3,3′-diaminoben-Zidine peroxidase (DAB). Hydrogen peroxide ($H_2O_{2)}$ (from BDH Germany), Abcam UK supplied the xylene and mountain media. Formaldehyde, Glutathione-S-transferase (GST) (CAT # CS0410), catalase, and reduced glutathione (GSH), tricholoroacetic acid (TCA), as well as other substances CDNB (1-chlor-2,4-di-nitobenzene) (Pub-chem CID:6), was obtained from (Sigma-Aldrich, United States). The Abcam (Cambridge, UK) provided us with a horseradish peroxidase-conjugated secondary antibody (ab-6789). The ELISA kit COX-2 (CAT# PRS-30205Ra) and the ELISA kit PPAR gamma (CAT# E-EL-R0724) were supplied by Elabscience.

2.2. Animals

Adult male Sprague-Dawley rats weighing (180–200 g) were accommodated 6 per group issued from the animal house of Riphah Institute of Pharmaceutical Sciences (RIPS), Islamabad. The animals will be kept under standard environmental conditions temperature:25 °C ± 1 °C, humidity 50 % ± 10 % and 12-h cycle light and dark cycle with water and food available ad-libitum. The approval was given by Research Ethical Committee of the Riphah Institute of Pharmaceutical Sciences, Islamabad, Reference No. REC/RIPS/2023/32, dated 15th April 2023) to all experimental techniques in compliance with the National Research Council's (1996) recommendation from the Institute of Laboratory Animal Resources, Commission on Life Sciences University.

2.3. Experimental design

Rats were divided randomly into four groups each containing n = 6 animals in each group.

(i) Saline/Control group (administered 10 mL/kg normal saline with 5 % DMSO)
(ii) Disease group (LPS 500 μg/kg).
(iii) Standard group (fluoxetine 20 mg/kg).
(iv) Treatment group (taxifolin 20 mg/kg)

Normal saline (with 5 % DMSO) was used to dissolve LPS and fluoxetine the doses chosen as previously stated. The dosing protocol was 14 days. Saline group received normal saline for 14 alternative days however LPS (500 μg/kg) were administrated for 14 alternative days to induce depression model [21]. Fluoxetine and taxifolin administrated once daily through intraperitoneal route 1 h after the LPS injection for fourteen days. After that these behavior studies elevated plus maze test (EPM), forced swim test (FST), open field test (OFT), sucrose splash test (SST), and the light-dark box (LDB), experiments were performed using rats as experimental subjects. One cohort was processed for morphological analysis and samples were stored in 4 % paraformaldehyde and sample from another cohort were instantly frozen and kept at −80 °C for biochemical analysis such as antioxidant assay such as ELISA, PCR.

2.4. Docking analysis

In *silico* analysis of taxifolin and fluoxetine was performed for exploratory research against a number of targets related to the pathophysiology of major depression disorder. The three-dimensional structures of both test and reference drugs were obtained in sdf format from the PubChem database and afterwards transformed into protein data bank (PDB) format through the utilization of Open Babel Gui. Target proteins, peroxisome proliferations-activated receptor γ (PPAR-γ), brain-derived neurotrophic factor (BDNF), cyclooxygenase-2 (COX-2), c-Jun N-terminal kinase (JNK), toll like receptor-4 (TLR4), monoamine oxidase A (MAO-A), heme oxygenase-1 (HO-1), phosphoinositide 3-kinase (PI3k), cyclooxygenase-1 (COX-1), sodium channels (NA^+), glutamate receptor (GRM2), tumor necrosis factor-alpha (TNF-α), prostaglandins (PGE2), mitogen activated protein kinases (MAPK), beta- 2 adrenergic receptor (ADRB2), neurokinin receptor (NK-1), procaspase activating compound (PAC-1), nuclear factor kappa B (NF-κB), nitric oxidase synthesis (iNOS), interleukin-4 (IL-4), high mobility group box 1 (HMGB1), C-Fos Proto-Oncogene Protein (c –Fos), beta catenin (β-Catenin), vasoactive intestinal peptide (VIP), serotonin receptors (SERT), nuclear factor erythroid 2-related factor 2 (Nrf2), gamma-aminobutyric acid (GABA A), peptidoglycan (PG), interleukin-2 (IL-2), dopamine receptor (D2). The one optimal position with the lowest binding energy value using Biovia Discovery Studio Visualizer Client 2016 (DSV v16.1.0.15350) for post-docking analysis. Furthermore, ligands and amino acid residues interactions, was determine for that 2D and 3D pictures were assessed, including valine, methionine, serine, arginine, aspartic acid, cysteine, glutamine, threonine, alanine, arginine, histidine, isoleucine, phenylalanine, proline, and serine.

2.5. Behavioral testing

The behavioral studies were performed on rats (n = 6/group) using the elevated plus maze test (EPM), light-dark box (LDB), forced swim test (FST), open field test (OFT), and sucrose splash test (SST).

2.5.1. Sucrose splash test (SST)

Grooming behavior is a measure of depressed symptoms that are measured in seconds. A shorter period of grooming suggests a high level of depression.

Spraying 10 % sucrose solution to the rat's dorsal surface. This test assesses grooming behavior, which is characterized as licking fur to clean it, biting, scratching the fur to remove the solution. The total time spend in grooming is 5 min. A five-minute video was recorded [22].

2.5.2. Forced swim test (FST)

Plexiglas cylinder with a diameter and height of 70 cm at a temperature of 23 $^{\circ}C \pm 1^{\circ}C$ was used to performed force swim test on rat. The antidepressant-like activity was measured 24 h prior to the test using a pre-swim exposure test. Using a pre-swim ensures that on test day, the rats take up an immobile position right away, making it possible to clearly the effects of the tested drug. Seven minutes of the test were videotaped, with the final 4 min being randomly evaluated every 5 s. Immobility period (i-e time during which animal kept it immobile with very little/negligible movement to keep its head above the water). Struggling period (i-e period during which rat continuously move to escape) was also observed from video trapped [23].

2.5.3. Open field test (OFT)

A square, white Plexiglas open field measuring 1.22 m on each side and 45 cm in height was used for testing [24]. Rats was placed in the apparatus to freely move. The video was trapped for 10 min and the parameters observed was duration of time spent in the inner and outer zones and numbers of rearing. The field apparatus was cleaned with alcohol between testing, olfactory cues were reduced.

2.5.4. Elevated plus maze test (EPM)

To measured anxiety-provoking behaviors in LPS induced depression model. That apparatus had two oppositely open and closed arm that were 10 cm wide and 50 cm long. A 50 cm elevation above the floor [25]. The behavior test was performed in sound proof and dim light. Individually rat was positioned in the middle, its face turned to any open arms. The duration of time in open arms as well as the frequency of enteritis in to open arm, were noted and video trapped for 5 min. The apparatus was cleaned with alcohol between testing, to reduce the olfactory cues.

2.5.5. Light -dark box test (LDB)

The Assessment conducted initially established on the model introduced by Crawley and Goodwin (1980), although numerous authors have utilized it with a number of structural changes [26]. The light/dark box was divided into two halves, a gap of 15 cm high by 610 cm wide connects one bright (50 cm length, 680 cm width, and 660 cm height) and one dark (30 cm length, 680 cm width, and 660 cm height). The upper light zone was not covered and got light in the room. The animals may easily enter any of the two chambers due to the small opening between them [27]. Rat was placed inside the dark chamber of the light-dark box for 5 min, where it was free to roam about the light-dark- compartments. Alcohol was used to clean te apparatus reduce the odor cues.

2.6. Antioxidant assays

After completion of the experimental study duration, rats from each group were anesthetized with chloroform [28] and sacrificed through cervical dislocation [29]. Brain tissues of experimental rats were homogenized in 20 mM Tris-HCl with a homogenizer. The homogenates were then centrifuged at 6000 rpm for 10 min at 4 °C. The supernatant of the homogenate from each rat was taken for analysis of following biochemical/oxidative stress markers.

2.6.1. Determination of lipid peroxidation (LPO)

LPO is a major oxidative stress marker [30] and Malondialdehyde (MDA), the end product of LPO, was used to assess its level (MDA) in tissue (brain) homogenate of all the experimental groups, by previously published methods with slight modification [31–33]. In short, 200 μL of supernatant layer solution, 200 μL of 100 mM ascorbic acid, 580 μL of 0.1 MPBS (pH 7.4), and 20 μL of ferric chloride were combined to create the mixture. This mixture was then incubated in a water bath at 37 °C for 1 h. To halt the reaction, 1000 μL of 10 % trichloroacetic acid (TCA) and 1000 μL of 0.66 % thiobarbituric acid (TBA) were added to this solution. These sample tubes were then centrifuged at 3000 g for 10 min, after which they were incubated once again for 20 min using a water bath. Using an appropriate blank, the absorbance of this resulting combination was measured at 535 nm to determine the quantity of TBARS, by comparing with the standard curve. The concentration of TBARS was expressed as (nM/min)/mg protein.

2.6.2. ***Reduced glutathione (GSH)*** *level*

A slightly modified version of a previously used method was used [33,34]. A 0.2 M sodium phosphate solution was used to dissolve 0.6 mM DTNB, and 2 mL of this combination was combined with 0.2 mL of previously obtained supernatants of the tissue homogenates. The final volume was made up with 0.2 M PBS to yield a 3 mL solution. After 10 min, the absorbance of the test liquid was measured at 412 nm. Phosphate buffer and DTNB solution served as negative and positive controls, respectively, and were used to adjust the measured absorbance. The results were represented as μmol/mg of protein. The level of GST markers was also evaluated following a previously reported protocol with slight modification [35].

2.6.3. Glutathione-S-transferase (GST) level

A previously published method was used to determination of GST levels, with slight modifications [36]. 1-chloro-2,4-dinitrobenzol (CDNB) was utilized as a substrate to measure GST activity in homogenized tissue samples. A freshly produced solution containing 20 μl of 1 mM CDNB, 100 μl of Phosphate buffer solution, and 15 μl of 5 mM reduced glutathione was added to each well of the microplate

reader along with 20 μl of the collected supernatant. The absorbance of reaction mixture was measured at room temperature using micro plate reader with a wavelength of 412 nm. Similarly, obtained values were expressed in μmol/mg of proteins.

2.6.4. Catalase activity

Catalase enzyme activity was determined according to method of Aebi, 1984 [37] by measuring decomposition of hydrogen peroxide (H_2O_2) into water and oxygen. The reaction mixture was prepared by adding 1.95 ml of phosphate buffer (50 nM, pH 7.4), 0.05 ml of tissue homogenate and 1 ml of H_2O_2 (30 mM). Then, absorbance of all assay mixtures was determined at 340 nm for 1 min with the interval of 15 s. The unit of measurement for catalase activity is μmoles H_2O_2 decomposed per mg of protein per minute [38].

2.7. Hematoxylin and eosin (H and E) staining

H&E staining was carried out using the procedures provided in previous investigations with slight modifications [39,40]. Initially, tissue sections were applied on the glass slides, their wax coating was being removed, by using xylene (100 %) for deparaffinization forwarded by rehydration with graded ethanol series (100 %, 90 %, 80 %, 70 %). After rinse with distilled water and then hematoxylin and eosin nuclei was used for staining. Keep the dye at least for 10–15 min. The stained section was washed with running tap water, after drying these slides has been dehydrated with graded ethanol series (70 %, 95 %, and 100 %) and xylene was used to clean it after that mounting media was mounted. Images were taken with a light microscope, which were then stored in TIFF format for later quantification using Image J software [41].

2.8. Cresyl violet staining

The slides were rinsed in distilled water then immersed in 0.1 M PBS for 10 min. The solution containing 0.5 % (w/v) Cresyl violet acetate (Sigma) was mixed with a few drops of glacial acetic acid before used. Cresyl violet solution was administered to stain the brain sections for 20 min subsequently distilled water was used to rinse the slides, and differentiated in ethyl alcohol (70, 80, 90, and 100 %). The xylene was used to wash the slides after that apply mounting media and covered through glass cover slip. The light microscope was used to take images and saved in TIFF format and Image J software was used for further quantification [42].

2.9. Immunohistochemical analysis (IHC)

We used an immunohistochemistry analysis method that has been previously described with slight modification [34]. Slides were deparaffinized, treated with an enzyme technique to retrieve antigens, and then rinsed three times in a succession in PBS for 5 min to prevent the activity of endogenous peroxidase. As a blocking, normal goat serum was added, for 2 h' then slides were incubated. The primary antibodies TNF-α, IL-6, and NF-κB was applied on slides for an entire night and the next morning, PBS was used to clean the slides and then, secondary antibody was put for 90 min, and then incubated for 60 min in a humidified box using an ABC reagent from Santa Cruz. After that, slides were dehydrated with ethanol (70 %, 80 %, 90 % and 100 %) PBS was used to cleaned it, DAB was used for staining. Slides were dehydrated, xylene was used for fixing, mounting medium was used to covered. Light microscopes was used to capture the images, which were then recorded in TIFF format so that Image J software [41] could further quantify them [57].

2.10. Enzyme linked immunosorbent assay (ELISA)

Peroxisomes proliferation-activated receptor-γ (PPAR-γ), Cyclooxygenase-2 (COX-2) concentration in rat's cortex tissues was determine through ELISA [43] by following the manufacturers' instructions. The samples were homogenized in PBS at 4000 RPM, then the supernatant was collected. Total protein concentration was assessed using the BCA method (Elabscience), whereas Peroxisomes proliferation-activated receptor-γ (PPAR-γ), Cyclooxygenase-2 (COX-2) concentrations were measured using the ELISA microplate reader (Bio-Tek Instruments, Winooski, VT, USA). The concentrations (pg/mL) were then adjusted to total protein content (pg/mg).

2.11. RT-PCR analysis (real time polymerase chain reaction)

Subsequently the cortical tissues (n = 6/group) were homogenized, the trizol method was used to extract total ribonucleic acid (RNA), adhering to the manufacturer's instructions. A thermocycler was utilized to amplify the cDNA produced by reverse transcriptase from 1 to 2 μg of total RNA by real-time PCR. The levels of GAPDH expression were used to normalize the mRNA expression. Relative gene expression was determined by real-time quantitative PCR using the 2ˆΔΔ-CT technique. The following are the GAPDH and PPAR-γ primer sequences [44].

GAPDH forward: CATCACTGCCACCCAGAAGACTG.
GAPDH reverse: ATGCCAGTGAGCTTCCCGTTCAG.
PPAR-γ forward: CCCTTTACCACGGTTGATTTCTC.
PPAR-γ reverse: GCAGGCTCTACTTTGATCGCACT.

2.12. Statistical analysis

Hematoxylin and eosin staining, cresyl violet staining and IHC data was analyzed via Image J software. Data was expressed as mean

Table 1
The best conformational pose, binding energy (kcal/mol), number of hydrogen bonds, bonding residues forming other hydrophobic interactions, of taxifolin and fluoxetine with target proteins such as peroxisome proliferator-activated receptor gamma (PPAR-γ), cyclooxygenase-2 (COX-2), Toll like receptor-4 (TLR4), c-Jun N-terminal kinase (JNK), brain-derived neurotrophic factor (BDNF), monoamine oxidase A (MAO-A), heme oxygenase-1 (HO-1), cyclooxygenase-1 (COX-1), sodium channels (NA+),Glutamate receptor (GRM2), phosphoinositide 3-kinase (PI3k), tumor necrosis factor-alpha (TNF-α), prostaglandins (PGE2), mitogen activated protein kinases (MAPK), Beta- 2 adrenergic receptor (ADRB2), Neurokinin receptor (NK-1), Procaspase activating compound (PAC-1), nuclear factor kappa B (NF-κB), nitric oxidase synthesis (iNOS), interleukin-4 (IL-4), high mobility group box 1 (HMGB1), Protein -c –fos, Beta catenin (β-Catenin), serotonin receptors (SERT), nuclear factor erythroid 2-related factor 2 (Nrf2), vasoactive intestinal peptide (VIP), Gamma-aminobutyric acid (GABA A), peptidoglycan (PG), interleukin-2 (IL-2), Dopamine receptor (D2).

Taxifolin						Fluoxetine			
Target Proteins	PDB ID	E Value (Kcal/mol)	H-Bonds	H-Bond Residues	H-Bond Residues Forming other hydrophobic interactions	E Value (Kcal/mol)	H-Bonds	H-Bond Residues	H-Bond Residues Forming other hydrophobic interactions
PPAR-γ	4JAZ	−7.3	4	ARG 397 ARG 443 TYP 320 GLU 448	TYP 320 ARG 397 ARG 443 THR 447 GLN 444 GLU 448 VAL 446	−5.7	2	SER 482 SER 482	VAL 450 VAL 446 TYP 320 THR 447 ARG 443
COX-2	1CX2	−7.3	3	GLU 319 HIS 320 GLY 551	LYS 56 ASP 58 PRO 547 GLU 553 SER 548	−6.3	3	LYS 253 SER 566 THR 561	LYS 252 SER 566 LYS 293 HIS 242 ILE 558
TLR-4	4G8A	−7.7	5	MET 437 GLN 436 HIS 458 HIS 432 LYS 435	HIS 458 HIS 431 LYS 436 GLN 361 MET 437 GLU 439	−6.7	2	SER 482 SER 482	ARG 460 THR 459 HIS 458 SER 82 GLN 502
JNK	5AWN	−8.1	3	GLU 107 SER 153 MET 109	VAL 38 ALA 51 GLU 107	−7.2	–	–	VAL 156 ALA 51 ARG 443
BDNF	1B8M	−10.2	2	TYP 55 ARG 98	ARG 98 TYR 55 ARG 88	−6.3	1	SER 21	TYR 86 SER 21 ALA 118
MAO-A	2ZSX	−9.9	2	TYR 444 GLY 443	TYP 444 GLY 443 TYR 407	−8.6	1	TYP 444	GLY 67 TYP 69 GLN 215 VAL 303
HO-1	IUBB	−8.7	1	ARG 136	ARG 136 LEU 54 MET 51	−8.6	1	ARG 136	PHE 167 ARG 136 VAL 50 PHE 37
COX-1	6Y3C	−8.4	2	TYP 385 ASN 382	ALA 202 LYS 193 ALA 232	−7.4	1	GLN 203	ALA 199 LEU 390 VAL 447
NA^+ Channel	6AGF	−8.2	4	GLY 1245 PHE 1243 ASP 406 GLN 405	ASP 406 GLN 405 TYP 407 PHE 1243	−7.6	1	TYP 1593	ILE 431 TYR 1593 VAL 1589
GPM2	5KZN	−7.7	1	GLY 451	GLU 227 LYS 193 ALA 223	−7.7	1	ASP 444	GLU 227 LYS 193 ALA 202
PI3K	5NGB	−7.7	2	LEU 735 PRO 812	LEU 735 GLN 795 MET 788 GLN 792	−7.3	4	LEU 612 GLN 792 LEU 613 GLN 795	CYS 815 GLN 795 MET 788 GLN 610
TNF	5WUX	−7.6	3	GLU 116 GLN 102	GLN 102 CYS 69	−7.3	1	GLU 116	CYS 101 TRP 114
PGE2	6AK3	−7.2	2	THR 61 TRP 344	−7.8	−8.6	–	–	LEU 341 TRP 344
MAPK	5UOJ	–7.3	2	THR 68 LYS 53	ARG 67 TYR 35 THR 68	−6.7	2	THR 68 PHE 169	PHE 169 TYP 35 THR 68
ADR2	3NYA	−7.2	2	SER 329 TYP 141	SER 329 ALA 271 THR 68 ASP 331	−7.3	2	LEU 275 TYR 141	ALA 271 LEU 275 GLU 268 TYR 141

(*continued on next page*)

Table 1 (*continued*)

NKR	6HLL	−7.2	2	GLN 239 SER 226	SER 226 ALA 243 GLN 239	−7.1	–	–	ILE 135 ILE 134 VAL 240
PAC-1	6LPB	−7.1	4	ASP 240 ARG 42 ARG 263 THR 258	ARG 263 THR 258 PRO 261 THR 242	−6.2	–	–	PRO 261 MET 376 ILE 373 ILE 372
NFKB	4Q3J	−7.0	3	ASN 240 ARG 263 ARG 232	HIS 183 GLU 184 ARG 232 CYS 149	−6.8	3	ARG 232 THY 227 GLU 233	TYP 227 HIS 183 GLU 184 GLY 190
INOS	3E7G	−6.8	2	SER 276 GLY 279	ARG 301 GLY 279	−5.4	1	GLU 320	VAL 326 PRO 323
IL-4	2B84	−6.5	3	TYP 56 SO 41001 SO 41003	ARG 88 LYS 84 TYR 56	−6.1	3	ARG 85 SO 41001 TYR 56	TYR 56 ARG 81 ARG 87
HMGB1	2RTU	−5.4	4	ALA 69 ASP 70 GLN 24 PHE 21	ASP 70 ALA 69 MET 66 PHE 21 GLN 24	−5.3	–	–	SER 18 TYR 19 GLU 64
C-FOS	1FOS	−6.3	4	SER 177 GLN 299 ASP 174 THR 295	GLN 299 THR 295 GLU 173 SER 177 ASP 174	−5.8	1	ARG 288	LYS 292 GLU 291 ARG 288 ALA 287 GLU 284
B-Catenin	3OUW	−6.4	2	GLU 462 THR 418	GLN 238 ILE 251 TRP 253	−6.2	–	–	ASP 459 PRO 505 GLU 462 THR 418 GLY 422 PHE 2
Serotonin Receptor	516X	−6.6	1	ILE 251	VAL 37 GLY 72 HIS 20 ASN 74	−5.7	–	–	LEU 577 LEU 245 VAL 479
NRF2	2LZ1	−6.4	1	GLY 721	THR 7 TYR 10 ARG 14 LEU 13 MET 17	−5.9	1	PRO 34	PRO 34 ILE 33 LYS 29 ALA 28 GLU 25 LEU 89
VIP	2RRH	−5.2	2	ARG 14 THR 7	ASN 11 VAL 108 HIS 110 ARG 136 SER 104 LYS 103 ASP 101	−5.0	–	–	TYR 10 LEU 13 ARG 68 MET 17
GABA-A	6D6T	−6.2	3	ARG 136 ASP 101 SER 104	ILE 190 TYR 191 LEU 206 VAL 203	−5.9	1	THR 133	LYS 103 THR 133 ASP 56 ASP 48 LYS 106 LYS 105
Peptidoglycan	3PBI	−6.6	–	–	THR 123 ARG 120 MET 46 ALA 112	−6.8	–	–	TRP 119 MET 153
IL-2	1M47	−5.7	1	THR 123	ALA 379 ALA 376 TYR 209 LEU 206	−5.3	1	GLU 67	LEU 63 ASN 90 LYS 64 SER 87
Dopamine Receptors	3 CM4	−6.5	–	–	ALA 379 ALA 376 TYP 209 LEU 206	−6.9	–	–	TYR 29 ALA 379 ALA 376

Amino acids are: alanine (ALA), arginine (ARG), asparagine (ASN), aspartic acid (ASP), cysteine (CYS), glutamine (GLN), glutamic acid (GLU), glycine (GLY), histidine (HIS), isoleucine (ILE), lysine (LYS), methionine (MET), phenylalanine (PHE), proline (PRO), serine (SER), threonine (THR), tryptophan (TRP), tyrosine (TYR) and valine (VAL).

± standard error of mean (SEM) and statistically evaluated by applying *t*-test for comparison between Saline and LPS groups and one-way ANOVA followed by Tukey's Test for comparison among LPS, LPS + Taxifolin and LPS + Fluoxetine groups. Statistical significance was shown as # symbol (relative to saline group), * symbol (relative to disease group) represents $^{###}P < 0.001$, $^{##}P < 0.01$, $^{*}P < 0.05$ $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. In-silico analysis

Taxifolin and fluoxetine exhibited different binding affinities for distinct protein targets. Taxifolin and fluoxetine showed different atomic energy against different protein targets. The best-docked positions of the drug-target complex with corresponding scoring values, the number of hydrogen bonds, hydrogen bonding residues, and other hydrophobic interactions are summarized in Table 1. The 2D structure of test and standard compound taxifolin and fluoxetine was showed in Supplementary Figs. S1–S30.

3.2. Effects on sucrose splash test (SST)

Saline group showed significant increase grooming time (35.6 ± 1.5), while LPS induced group showed significant decrease ($^{###}P < 0.001$ vs. saline group) grooming time (22.5 ± 0.97). LPS + taxifolin group reverse the effects of LPS treated group and shows significantly increase.

($^{*}P < 0.05$ vs. LPS group) grooming time (32.9 ± 1.24). LPS + fluoxetine shows significantly increase ($^{**}P < 0.01$ vs. LPS group) grooming time (37.9 ± 0.83) (Fig. 1).

3.3. Effects on force swim test (FST)

Saline group demonstrated increase struggling time (Fig. 2A) and decreases immobility time (Fig. 2B) (142.5 ± 2.5 and 102.5 ± 2.5, respectively). Similarly, LPS treated rats showed significant decrease ($^{###}P < 0.001$ vs. saline group) in struggling time (67.5 ± 2.5) and significant increase ($^{###}P < 0.001$ vs. saline group) in immobility time (168.5 ± 1.5). The treatment group LPS + taxifolin showed significantly increase ($^{**}P < 0.01$ vs. LPS group) struggling time 102.5 ± 2.5 but significantly decrease ($^{**}P < 0.01$ vs. LPS group) immobility time 138 ± 3. The LPS + fluoxetine showed significantly increase ($^{***}P < 0.001$ vs. LPS group) struggling time

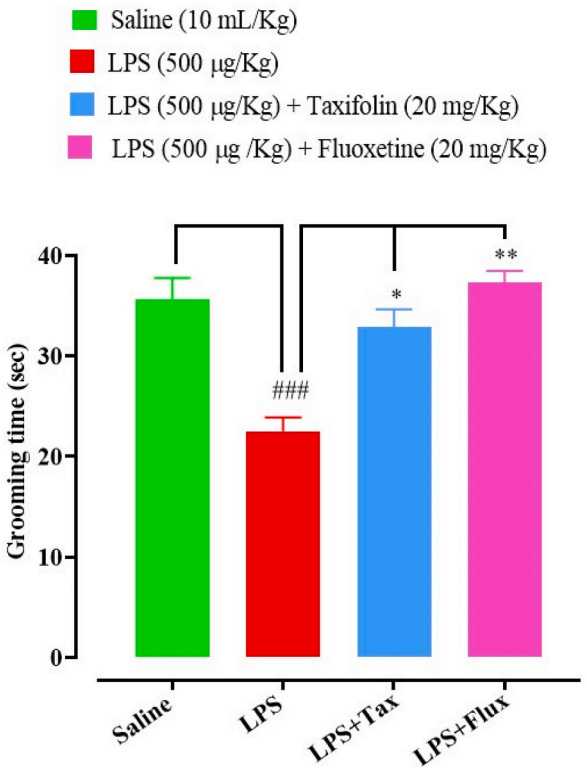

Fig. 1. Effect of Taxifolin and Fluoxetine against grooming time in rat's sucrose splash test. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{###}P < 0.001$ vs. saline group, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. LPS group.

140.5 ± 1.5 but significantly decrease (***$P < 0.001$ vs. LPS group) immobility time 102 ± 2.5 (Fig. 2).

3.4. Effect on open field test (OFT)

Saline group showed increase time (254.33 ± 3.67) spend in inner zone (Fig. 3A) but decrease time spend (335.16 ± 5.16) in outer zone (Fig. 3B) and rears (9.16 ± 1.5) (Fig. 3C). The LPS induced group showed significant increase ($^{###}P < 0.001$ vs. saline group) time spend in outer zone and rears 468 ± 5, 41.73 ± 1.48 but significant decrease ($^{###}P < 0.001$ vs. saline group) time spend in inner zone 74.5 ± 5.5. The LPS + taxifolin reversed this effects and shows significantly decrease (*$P < 0.05$, **$P < 0.01$ vs. LPS group) time spend in outer zone 370.16 ± 5 and rears 23.93 ± 1.27 but significant increase (**$P < 0.01$ vs. LPS group) time spend in inner zone 174.5 ± 5. LPS + fluoxetine shows significantly decrease (*$P < 0.05$, **$P < 0.01$ vs. LPS group) time spend in outer zone 343 ± 5 and rears 13.66 ± 2 but significantly increase (***$P < 0.001$ vs. LPS group) time spend in inner zone 235 ± 5 (Fig. 3).

3.5. Effects on elevated plus maze test (EPM)

Saline group, reveals a longer time (97.5 ± 2.5 Sec.) spend in the open arm (Fig. 4A) as well as higher number of entries (7.7 ± 0.2) in the open arm (Fig. 4B). LPS induced group showed significant decrease ($^{###}P < 0.001$ vs. saline group) time spend and number of entries (2.3 ± 0.2) in open arm. LPS + taxifolin and LPS + fluoxetine showed significantly increased (**$P < 0.01$, ***$P < 0.001$ vs. LPS group) time spent (64.5 ± 3.5, 5.415 ± 0.085, respectively) and number of entries (90.5 ± 0.5, 5.75 ± 0.095 respectively) in the open arm (Fig. 4).

3.6. Effects on light dark box test (LDB)

Saline group spend longer (159.5 ± 3.5 Sec.) time in light (Fig. 5A) and shorter time (134 ± 3 Sec.) in dark area (Fig. 5B). LPS induced group showed significant decrease and increase ($^{###}P < 0.001$ vs. saline group) time spend in light region 63 ± 3 and time spend in dark region 230.5 ± 3. LPS + taxifolin and LPS + fluoxetine showed significantly increase (*$P < 0.05$, **$P < 0.01$ vs. LPS group) time spend in light area 97 ± 4, 194.5 ± 4.5 but showed significantly decrease (**$P < 0.01$ vs. LPS group) time spend in dark area 136 ± 4, 155.5 ± 4.5 (Fig. 5).

3.7. Effects on oxidative stress markers

The enzymatic and non-enzymatic oxidants GSH, GST, catalase and LPO levels were assessed in the prefrontal cortex (Table 2a) and

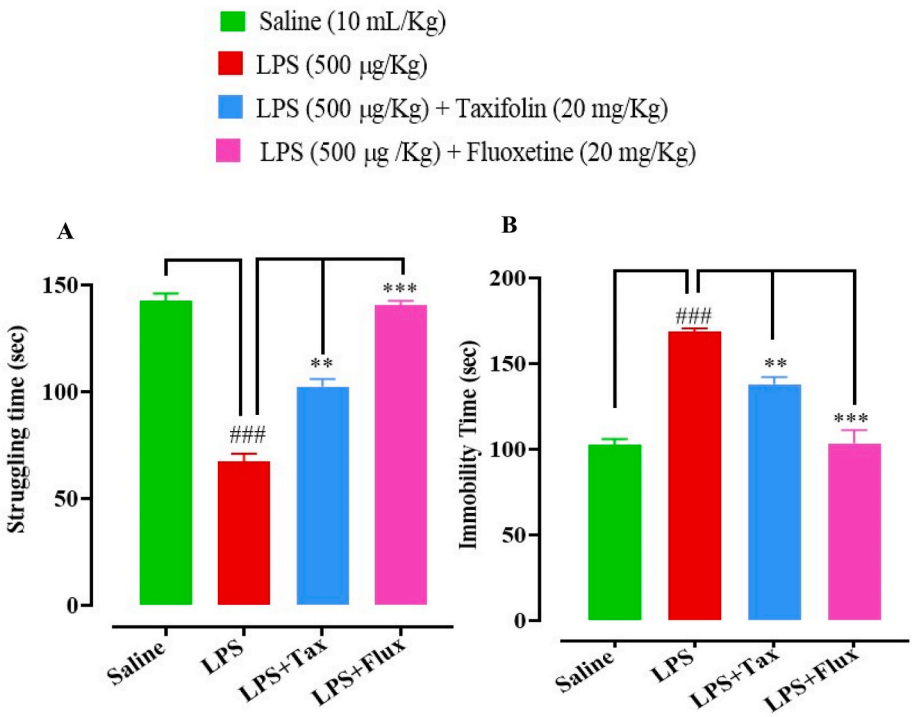

Fig. 2. Effect of Taxifolin and Fluoxetine against struggling time **(A)** and immobility time **(B)** in rat's Force swim test. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{###}P < 0.001$ vs. saline group, **$P < 0.01$, ***$P < 0.001$ vs. LPS group.

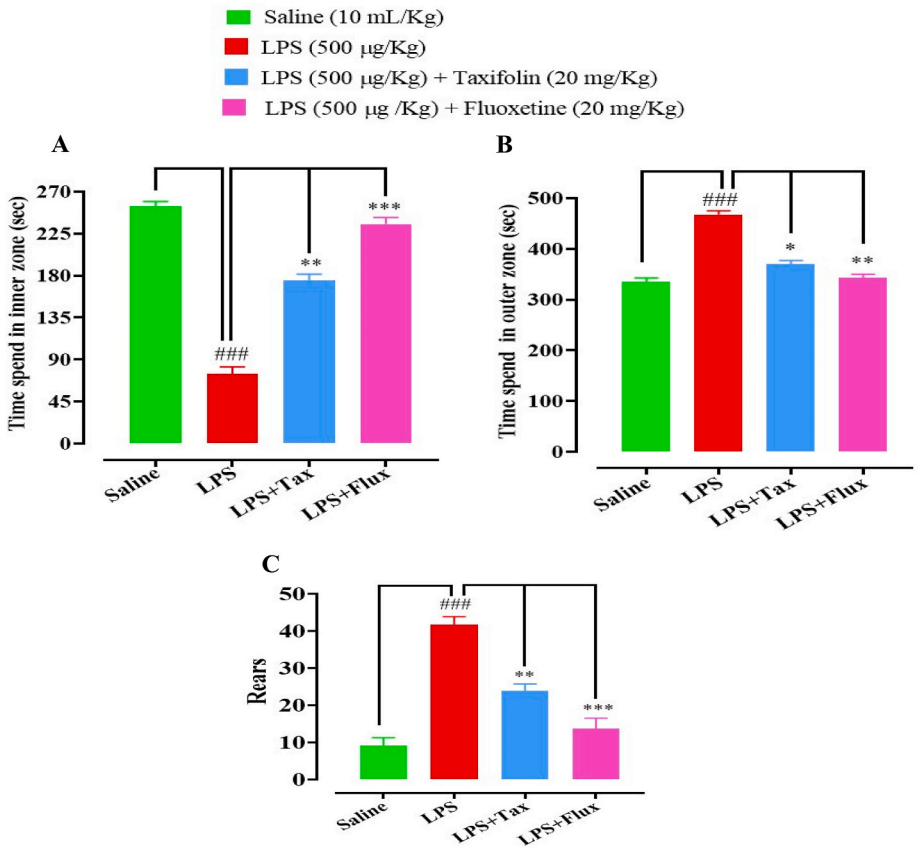

Fig. 3. Effect of Taxifolin and Fluoxetine against time spend in inner zone **(A)** and outer zone **(B)** and number of rearing **(C)** in rat's Open filed test. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{###}P < 0.001$ vs. saline group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. LPS group.

also determined GSH, GST, catalase and LPO level in hippocampal regions (Table 2b) in order to evaluate the neuroprotective potential of taxifolin. The LPS induced group shows notable decrease in GSH, GST, catalase level as compared to saline group but, the levels of LPO was increase in both prefrontrol cortex and hippocampus of LPS treated group as compared to saline group. LPS + taxifolin treated group restored antioxidant expression level in the prefrontal cortex and the hippocampal regions significantly increase in level of GSH, GST, catalase and remarkable decrease in LPO level as compare to LPS treated group. LPS + fluoxetine shows increased level of GSH, GST, catalase and marked decrease in LPO level as compared to LPS treated group in cortex and hippocampus region of rat brain.

3.8. Effects on histopathological examination

H&E staining revealed morphological alterations in the prefrontal cortex and hippocampal regions (Fig. 6A). The saline group displayed round, well demarcated, well-defined, undamaged cells without nuclear condensation or distoration and basophilic cytoplasm. Significant histopathological changes, such as changed neuronal size and shape and other characteristic features including inflated, neurons that are kryolitic, atrophied, and flattened with pyknotic nuclei and decreases in number of neurons that survived, were seen in the LPS-treated group. LPS + taxifolin significantly decreased these morphological damages, as demonstrated by an increase in the number of intact neurons and increase number of survival neurons in the examined hippocampal and cortical areas. The effects of LPS treatment on rats were likewise reversed by LPS + fluoxetine, which resulted in notable increases in the number of surviving neurons and confined cells free of nuclear condensation or distoration with basophilic cytoplasm (Fig. 6B).

3.9. Effects on cresyl violet staining

The extent of neuronal cell death induced by LPS was detected through cresyl violet staining which examined the neuroprotection produced through taxifolin treatment in cortex and hippocampus of LPS-induced rat (Fig. 7A). The number of survival neurons in the cortex and hippocampus regions were significantly reduced in LPS treated rat as compare to saline group. After treatment with taxifolin and fluoxetine the number of survival neurons were significantly upregulated in the cortex and hippocampus (Fig. 7B).

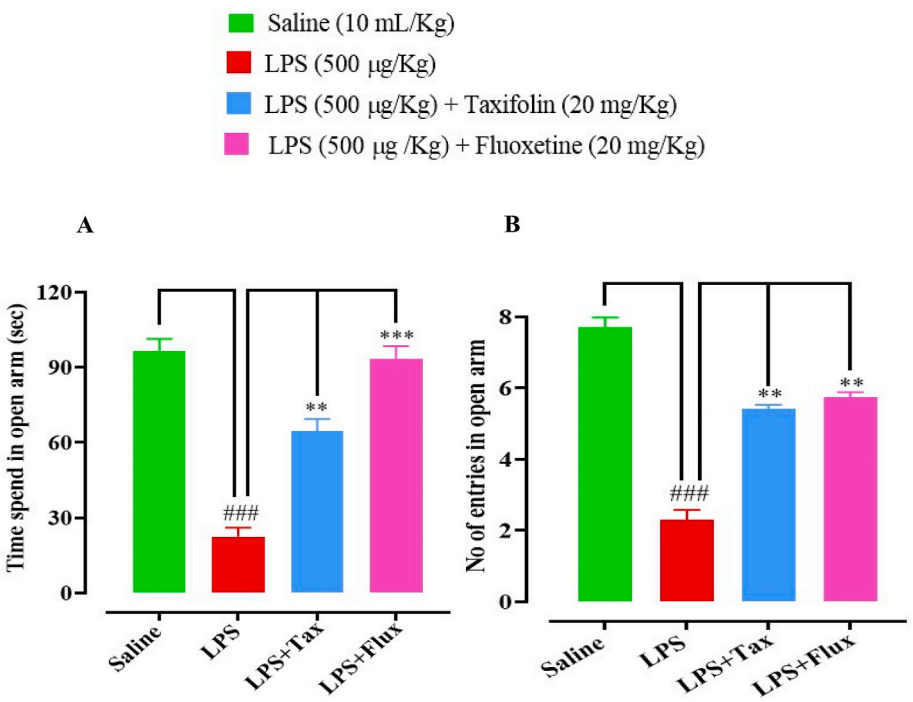

Fig. 4. Effect of Taxifolin and Fluoxetine against time spend in open arm **(A)** and number of entries **(B)** in rat's Elevated plus maze test. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{###}P < 0.001$ vs. saline group, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. LPS group.

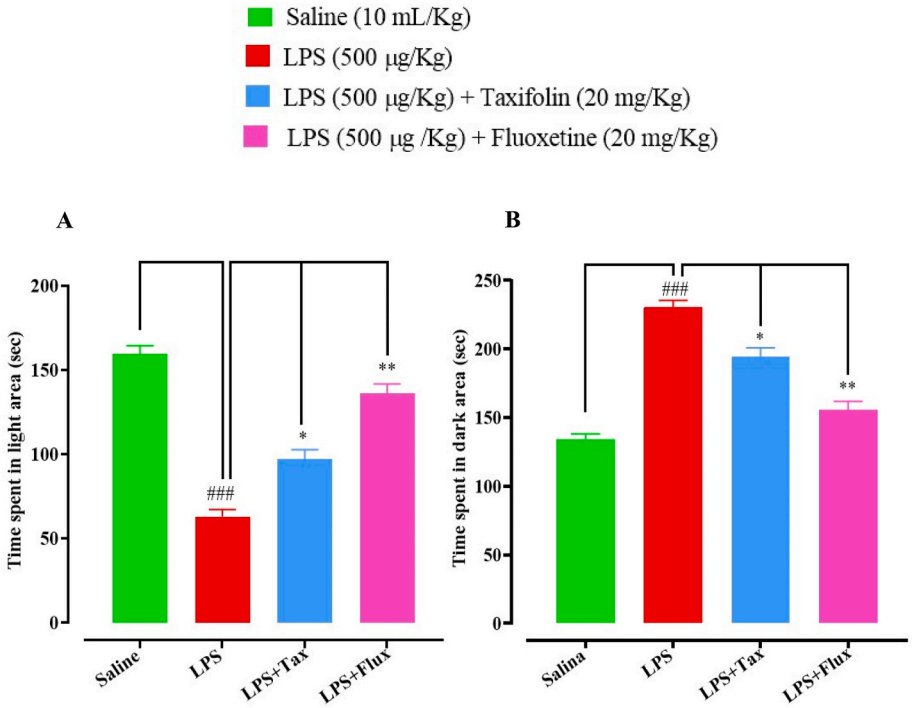

Fig. 5. Effect of Taxifolin and Fluoxetine against time spend in light area **(A)** and dark area **(B)** in rat's Light dark box test. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{###}P < 0.001$ vs. saline group, $^{*}P < 0.05$, $^{**}P < 0.01$, vs. LPS group.

Table 2a
Effects of taxifolin and fluoxetine against reduced glutathione (GSH), glutathione sulfotransferase (GST), catalase and lipid peroxidase (LPO) in rats cortex tissues.

Group	GSH (μmoles/mg protein)	GST (μmoles CDNB conjugate/min/mg protein)	Catalase (μmoles H_2O_2/min/mg protein)	LPO (TBARS nmoles/min/mg protein)
Saline (10 mL/Kg)	13.19 ± 0.013	35.39 ± 0.013	8.143 ± 0.05	13.61 ± 2.836
LPS (500 μg/Kg)	4.34 ± 0.02$^{\#\#\#}$	13.67 ± 0.015$^{\#\#\#}$	5.98 ± 0.005$^{\#\#\#}$	42.55 ± 1.78$^{\#\#\#}$
LPS (500 μg/Kg) + Taxifolin (20 mg/Kg)	8.04 ± 0.02**	28.66 ± 0.035**	7.36 ± 0.012**	23.77 ± 1.85***
LPS (500 μg/Kg) + fluoxetine (20 mg/Kg)	11.45 ± 0.03**	11.45 ± 0.03*	7.83 ± 0.035***	20.88 ± 1.02***

Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $\#\#P < 0.01$, $^{\#\#\#}P < 0.001$ vs. saline group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. LPS group.

Table 2b
Effects of taxifolin and fluoxetine against reduced glutathione (GSH), glutathione sulfotransferase (GST), catalase and lipid peroxidase (LPO) in rats hippocampus tissue.

Group	GSH (μmoles/mg protein)	GST (μmoles CDNB conjugate/min/mg protein)	Catalase (μmoles H_2O_2/min/mg protein)	LPO (TBARS nmoles/min/mg protein)
Saline (10 mL/Kg)	16.04 ± 0.015	41.26 ± 0.50	12.85 ± 0.039	19.09 ± 8.31
LPS (500 μg/Kg)	3.75 ± 0.03$^{\#\#\#}$	18.24 ± 0.06$^{\#\#\#}$	8.82 ± 0.025$^{\#\#\#}$	48.41 ± 7.6$^{\#\#\#}$
LPS (500 μg/Kg) + Taxifolin (20 mg/Kg)	9.86 ± 0.06**	27.75 ± 0.011**	10.82 ± 0.272***	32.71 ± 0.014***
LPS (500 μg/Kg) + fluoxetine (20 mg/Kg)	13.48 ± 0.017**	35.42 ± 0.01**	11.95 ± 0.178***	25.72 ± 6.8***

Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{\#\#\#}P < 0.001$ vs. saline group, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. LPS group.

3.10. Effects on immunohistochemistry staining

IHC was performed to determine the important role that inflammatory mediators had in the neurological inflammation brought on by LPS. The cortical and hippocampal tissues of the LPS-treated group were shown to have higher levels of NF-κB, TNF-α, and IL-6 markers than those of the saline group where as treatment of taxifolin and fluoxetine reverse the effects and decreases the over-expression of inflammatory marker such as TNF-α (Fig. 8A and B), NF-κB (Fig. 9A and B) and IL-6 (Fig. 10A and B).

3.11. Effects on inflammatory markers

PPAR-γ levels was increase in saline group 173 ± 2.62 (Fig. 11A). LPS induced group showed significant decrease ($^{\#\#\#}P < 0.001$ vs. saline group) level of PPAR-γ 61.27 ± 1.7.36. LPS + taxifolin and LPS + fluoxetine group showed significant increase ($^{*}P < 0.05$, $^{**}P < 0.01$ vs. LPS group) level of the PPAR-γ 124.40 ± 2.39, 182.15 ± 2.03. In saline group C0X-2 level in the cortex tissue was 41 ± 2.12. The prefrontal cortex regions of the rat brain exhibit significant increase ($^{\#\#\#}P < 0.001$ vs. saline group) COX-2 levels 120.5 ± 2.82 in the LPS-treated group ((Fig. 11B). LPS + taxifolin and LPS + fluoxetine group have significant decrease ($^{*}P < 0.05$, $^{**}P < 0.01$ vs. LPS group) COX-2 level 105 ± 2.82 and 69 ± 2.12. (Fig. 11).

3.12. Effect on mRNA expression of PPAR-γ

PPAR-γ fold expression in LPS-treated group was evaluated using RT-PCR. The expression PPAR-γ mRNA is downregulated in the LPS-treated group as compare to saline group. The effects were reversed in the LPS + taxifolin group, and the level of PPAR- γ increased noticeably. The PPAR-γ level was also increased in the LPS + fluoxetine group (Fig. 12).

4. Discussion

The current study confirmed the anti-oxidant and anti-inflammatory activity of taxifolin against LPS induced depression model in rats.

Docking is the fundamental technique for structured virtual screening, and research in this field is still going strong [45]. The utilization of structure-based drug design has proven to be pivotal in drug discovery, it involves the use of detailed knowledge about the three-dimensional structure of a target protein to design new drugs. Several processes are involved in the process, including

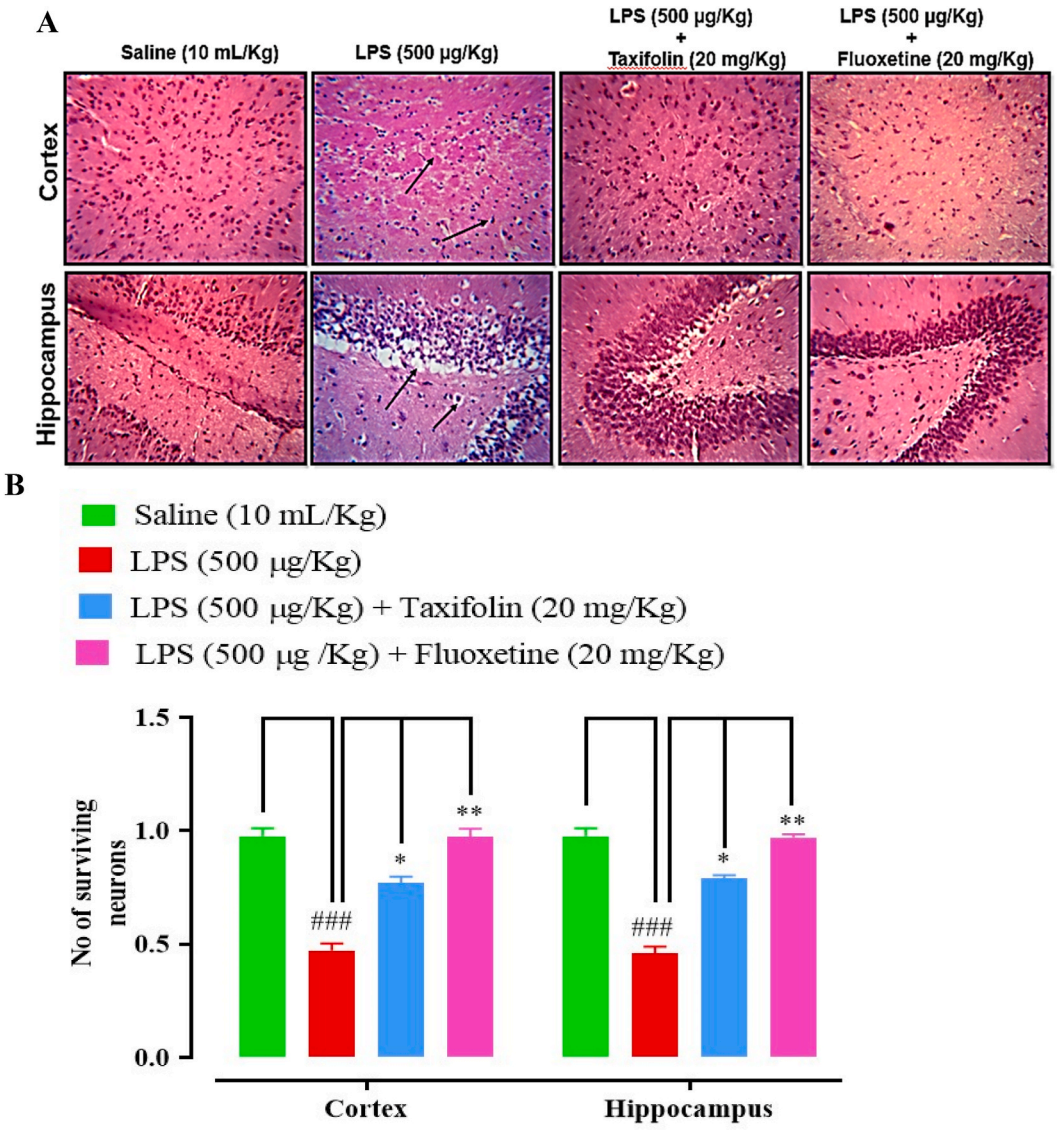

Fig. 6. **A** and **B** represent the effect of Taxifolin and Fluoxetine against surviving neuron expression in rat's cortex and hippocampus tissues, using the H and E staining histopathological technique. Bar 50 µm, magnification 40x. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{###}P < 0.001$ vs. saline group, $^{*}P < 0.05$, $^{**}P < 0.01$, vs. LPS group.

retrieving and preparing the protein structure, creating the ligand archives, and manually designing new, unique compounds [46]. The ligands (taxifolin and fluoxetine) were docked with the active binding sites of PPAR-γ, COX-2, TLR-4, JNK, BDNF, MAO-A, HO-1, COX-1, NA^{+} channels, GPM2, PI3K, TNF-α, MAPK, DR2, NK1R, PAC-1, NFKB, INOS, IL-4, HMGB1, C-FOS, β-Catenin, Seretonin, NRF2, VIP, GABA-A, Peptidoglycan, IL-2, Dopamine receptor. To evaluate ligand affinity to their specific target protein, in-silico experiments are utilized as a preliminary approach. The order of ligand affinity against taxifolin and fluoxetine was determined using ACE-values against various selected target proteins PPAR-γ > BDNF, MAO-A > HO-1 > JNK > COX-1 > NA^{+} channels > JNK > TLR-4 > GPM2 > PI3K > TNF-α > COX-2 > MAPK > PGE2 > PAC-1> NFKB > INOS > COX-2 > IL-4 >C-FOS > β-Catenin > Seretonin receptor, Peptidoglycan > NRF2 > GABA-A > IL-2, Dopamine receptor > HMGB1 >VIP. Docking studies with taxifolin and fluoxetine revealed differences in affinities for numerous target proteins involved in depression pathophysiology. Taxifolin, a natural flavonoid, has been demonstrated to exhibit antidepressant properties via interactions with several target proteins involved in depression pathophysiology [47]. whereas, fluoxetine, a regularly prescribed antidepressant, has been shown to increase the expression of brain-derived neurotrophic factor (BDNF) in dopaminergic areas, potentially contributing to its therapeutic effects [48]. These findings emphasize the distinct pharmacological profiles of taxifolin and fluoxetine, emphasizing their complimentary roles in depression therapy.

Current antidepressant medications generally target the monoaminergic system to induce therapeutic responses by boosting neurotransmitters including serotonin, noradrenaline, and dopamine. However, the therapeutic delay (at least several weeks) and

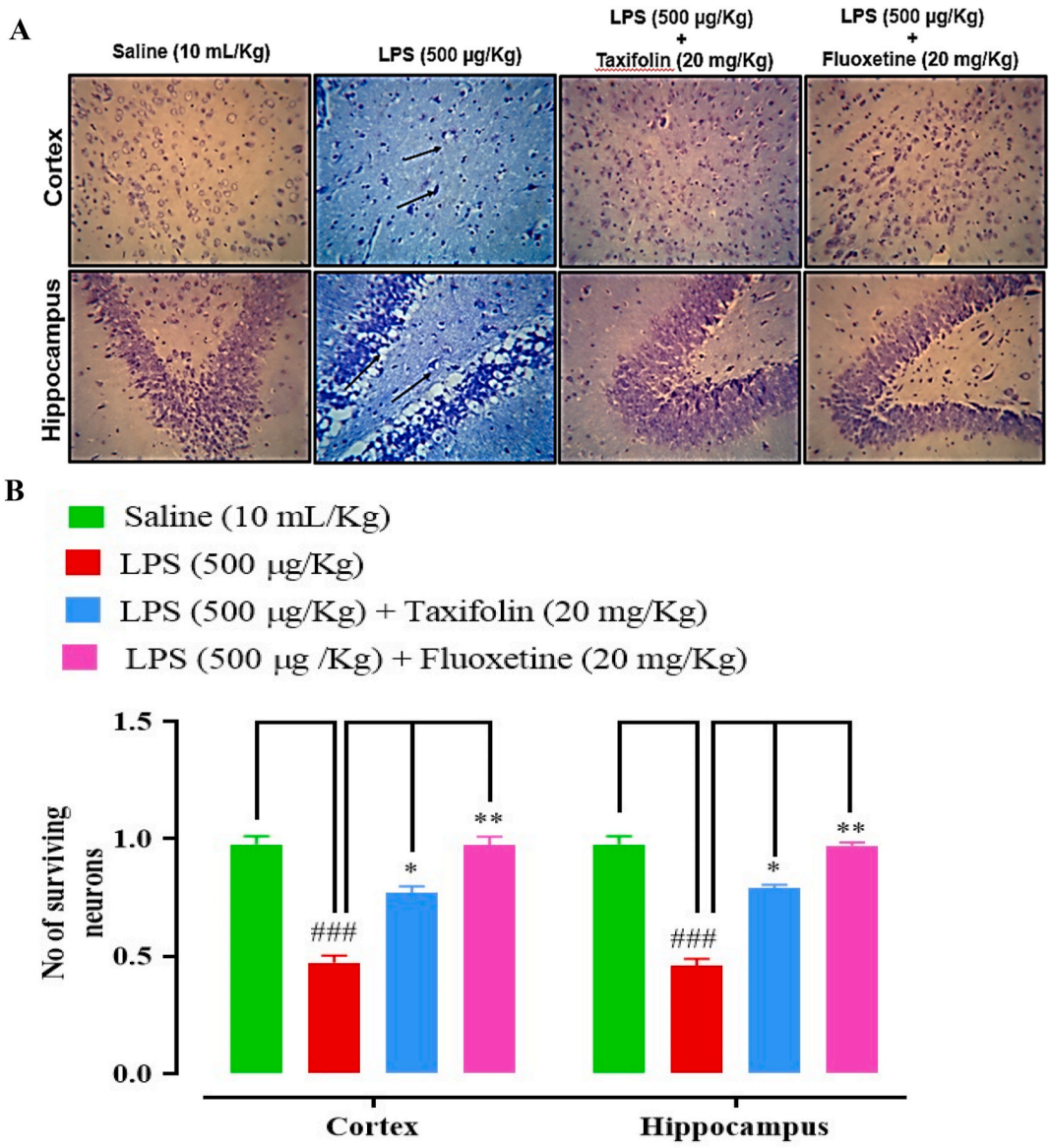

Fig. 7. **A** and **B** represent the effect of Taxifolin and Fluoxetine against surviving neuron expression in rat's cortex and hippocampus tissues, using the cresylic violet staining technique. Bar 50 µm, magnification 40x. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{###}P < 0.001$ vs. saline group, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. LPS group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

unavoidable side effects are key limitations of current depression therapy [49]. In this study, taxifolin is being investigated as a treatment for depression disorder induced by LPS. The inflammatory process is linked in a bidirectional manner ("cytokine theory of MDD") [50]. Gram negative bacteria outer cell walls contains lipopolysaccharide (LPS) which was used in research to induce an immune response and has been linked to the development of depressive-like symptoms in animal models. In this study, the depressive-like behaviour is induced by LPS [51]. Taxifolin is being tested as a potential treatment to mitigate the symptoms associated with induced depression. Overall, this research focuses on multiple aspects, including behavior, inflammatory mediator modulation and oxidative stress. The effects from such studies can provide valuable insights into potential new treatments for depression and related mood disorders.

In this researh, LPS-induced depression model is studied. Rats were administered LPS to develop an inflammation-related model of MDD manifested by behavioral abnormalities such as forced Swim Test in which rats spend less time in swimming and more time as immobile often considered to exhibit depressive-like behavior but treatment group improved struggling time and decrease immobility as compare to LPS treated group. In light-dark box test treatment with taxifolin and fluoxetine shows marked improvement in increase time spend in light box and decreases time spend in dark area as compare with LPS treated group. Elevated Plus Maze Test in which

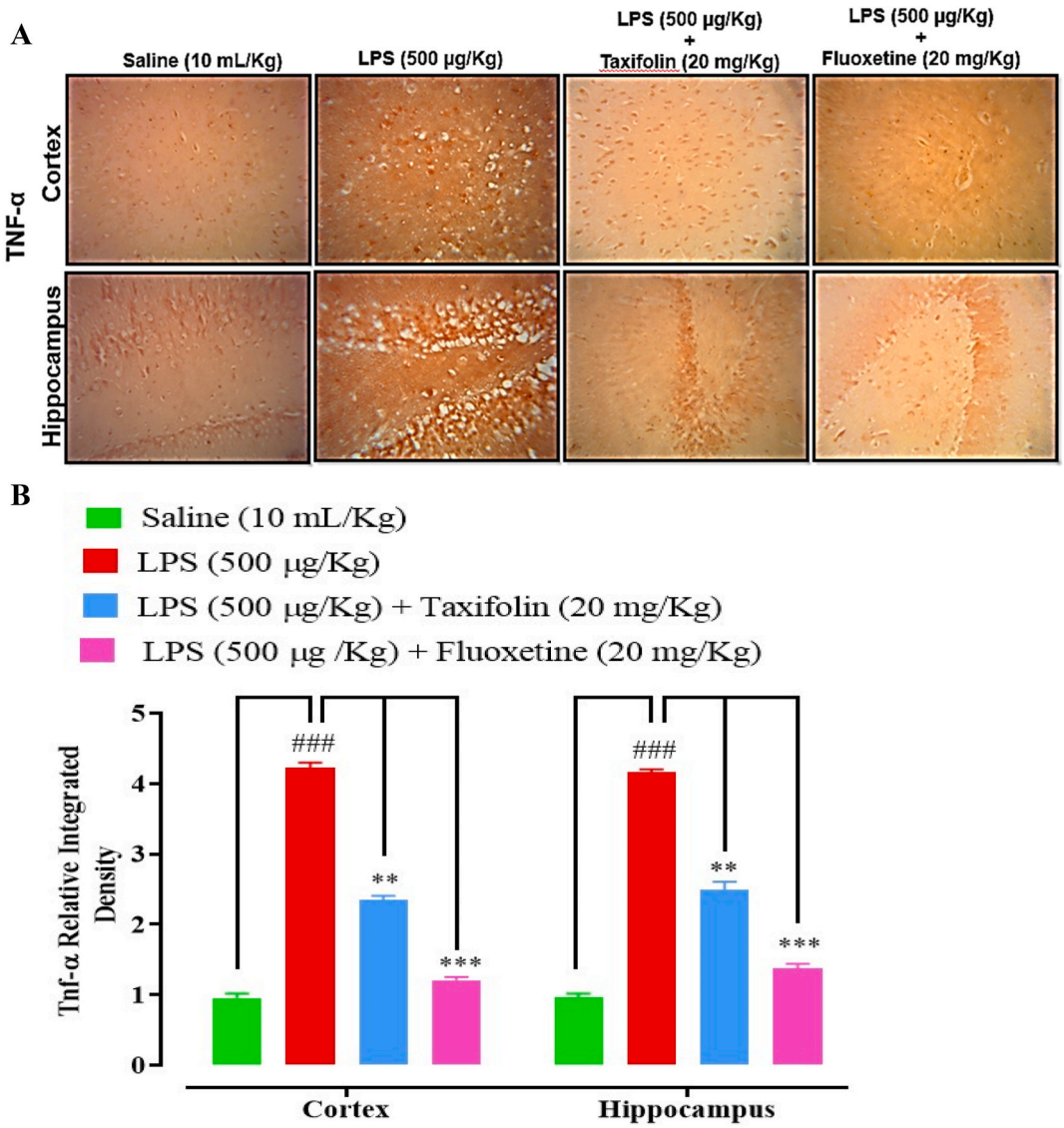

Fig. 8. **A** and **B** represent the effect of Taxifolin and Fluoxetine against tumor necrosis factor (TNF-α) expression in rat's cortex and hippocampus tissues, using immunohistochemical technique. Bar 50 µm, magnification 40x. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{###}P < 0.001$ vs. saline group, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. LPS group.

treatment with taxifolin and fluoxetine there was improvement as increase time spend in open arm and also number of entries in open arm as compare with LPS- treated group, Sucrose Splash Test in which treatment group and standard group shows increase grooming time as compared to LPS treated group. Open Field Test in which treatment and standard group shows increase duration of time spend in inner area and decrease duration of time spend in outer area and decrease in number of rearing as compared to LPS-treated group [52].

Antioxidant enzymes are substances that help protect cells from the damaging effects of ROS by neutralizing them. The enzymes mentioned are catalase, glutathione (GSH), and glutathione-S-transferase (GST), and lipid peroxidation (LPO). TBARS assess is a commonly used method for biological samples to quantify MDA levels [53]. LPS treated group elevated the level of LPO and natural oxidative response was reduced such as catalase, GSH, GST compare this to the saline group. In our treatments and standard group body's natural oxidative response upraised such as catalase,GSH, GST and reduced the level of LPO.

Histopathological studies reveals that LPS treated group showed different morphological changes as disruption of morphological cell boundries, vacuolation, well-demarcated/rounded cells, necrotic cell as compared to control group [54]. The LPS group's heightened inflammatory response and unique expression patterns may alter histological appearance, resulting in discernible differences from other groups, Inflammatory processes and disease-associated indicators can cause alterations in staining and coloring, affecting the visual appearance of histological specimens [55]. In our treatment group and standard group showed marked

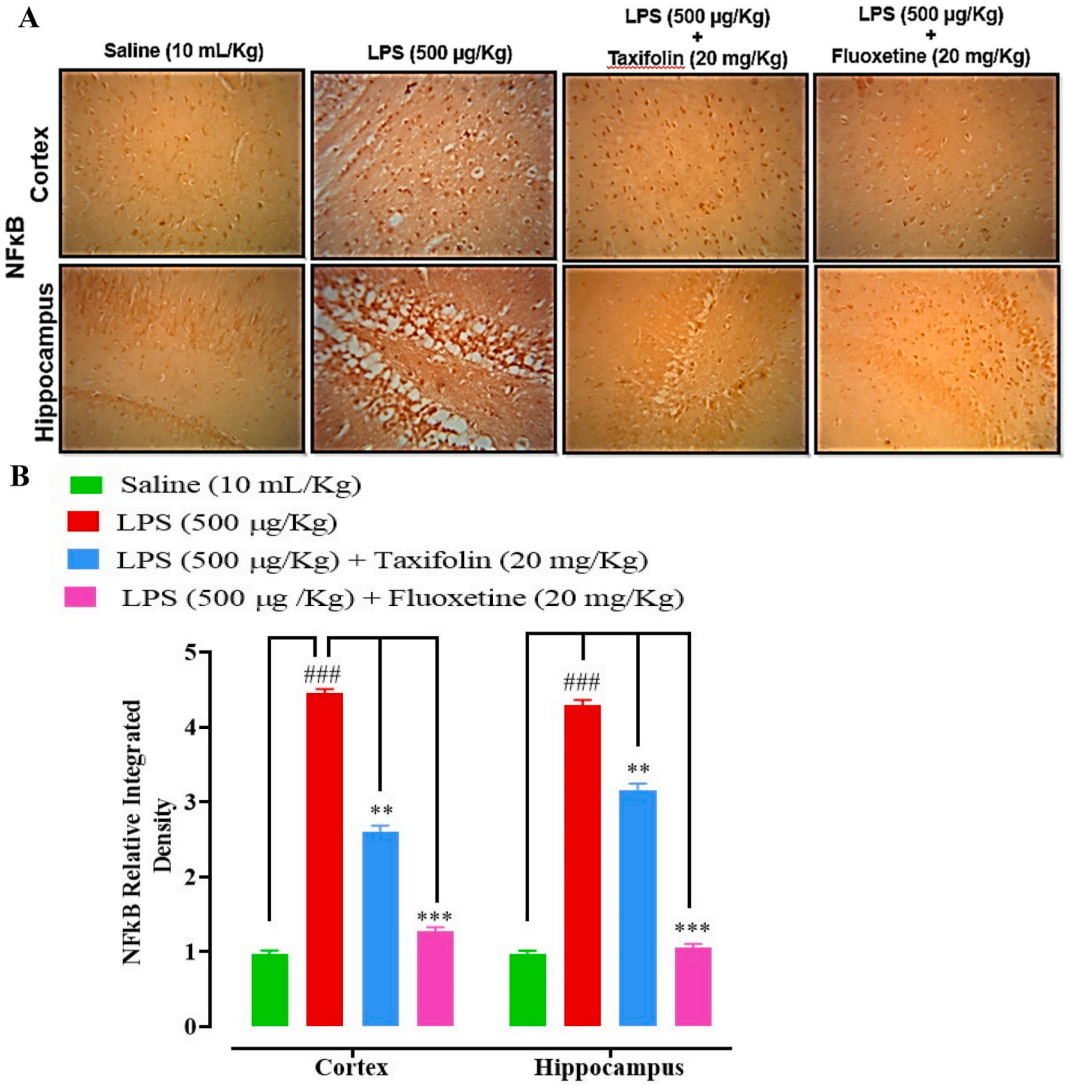

Fig. 9. **A** and **B** represent the effect of Taxifolin and Fluoxetine against nuclear factor kappa B(NF-κB) expression in rat's cortex and hippocampus tissues, using immunohistochemical technique. Bar 50 µm, magnification 40x. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{###}P < 0.001$ vs saline group, $^{**}P < 0.01$, $^{***}P < 0.001$ vs LPS group.

improvement in these morphological changes.

Some of the immune markers and inflammatory substances that have been associated with depression include IL-6, TNF-α, NF-Kb [56]. In IHC there was increased release of cytokines that promote inflammation, like TNF-α, NF-kB, and IL-6 in LPS treated group as compare to the saline group. Our treatment group and standard group showed marked reduction in these pro-inflammatory cytokines. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is involved in a number of biological processes, such as inflammation, glucose homeostasis, and lipid metabolism. In recent years, research has also highlighted its potential neuroprotective mechanisms, particularly in the context of neurodegenerative diseases and neurological disorders and target for depression disease model [57].

ELISA technique was performed to quantify COX-2, PPAR-γ in rat brain of cortex region. In LPS treated group there was remarkable increase expression of COX-2 and decrease expression of PPAR-γ in comparison to the saline group. Treatment group and standard group shows reduce expression of COX-2 and increase expression of PPAR-γ expression. Activation of PPAR-γ pathway is associated with beneficial effects in reducing inflammation and promoting neurogenic processes in stress-exposed animals. This information suggests that PPAR-γ and related pathways could be potential targets for therapeutic interventions in neuroinflammatory conditions and CNS injury [58]. In this recent study we target PPAR- γ as a pathway in depression then its involment through ELISA and RT-PCR. RT-PCR technique was carried out for further confirmation of targeted PPAR-γ. The PPAR-γ mRNA levels were determined in saline, LPS treated group and our treatment and standard group. Result showed that in LPS treated group there was decrease level of mRNA level of PPAR-γ as compare to saline group but increase level of mRNA PPAR-γ level our in treatment and standard group.

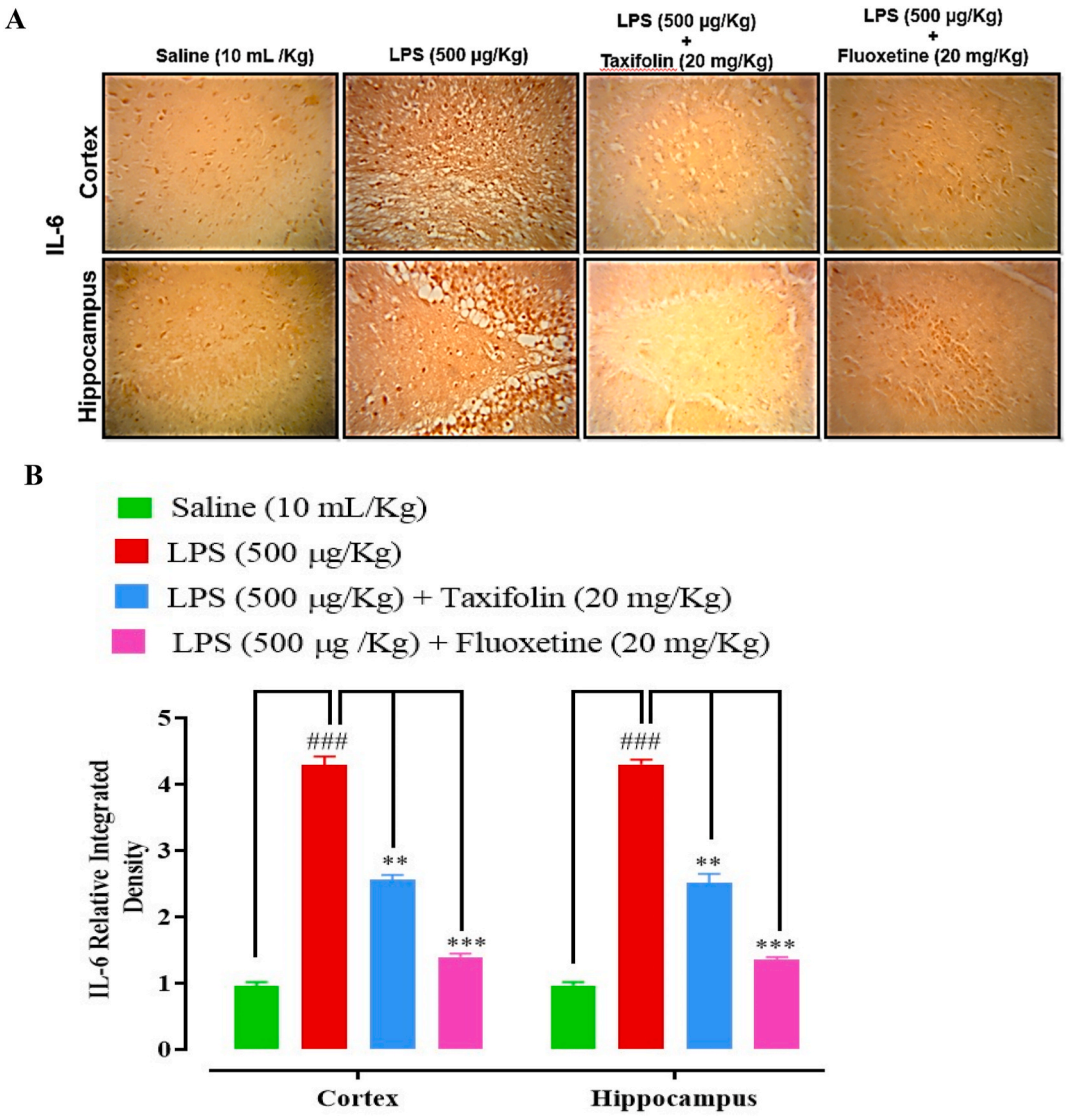

Fig. 10. **A** and **B** represent the effect of Taxifolin and Fluoxetine against IL-6 expression in rat's cortex and hippocampus tissues, using immunohistochemical technique. Bar 50 µm, magnification 40x. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey's test. $^{###}P < 0.001$ vs saline group, $^{**}P < 0.01$, $^{***}P < 0.001$ vs LPS.

5. Conclusion

The present study reveals that taxifolin possess binding energy values of −5.2 to −10.2 kcal/mol against selected targets. Taxifolin exhibits anti-depression effect, possibly mediated through activation of PPAR-γ pathway. Taxifolin also possess anti-oxidant and anti-inflammatory properties, demonstrating its therapeutic potential in depression management (Fig. 13).

Data availability statement

All data to support the conclusions have been provided in the manuscript.

CRediT authorship contribution statement

Maha Mir: Writing – original draft, Methodology, Data curation. **Arif-ullah Khan:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Conceptualization. **Aslam Khan:** Writing – review & editing, Formal analysis, Data curation.

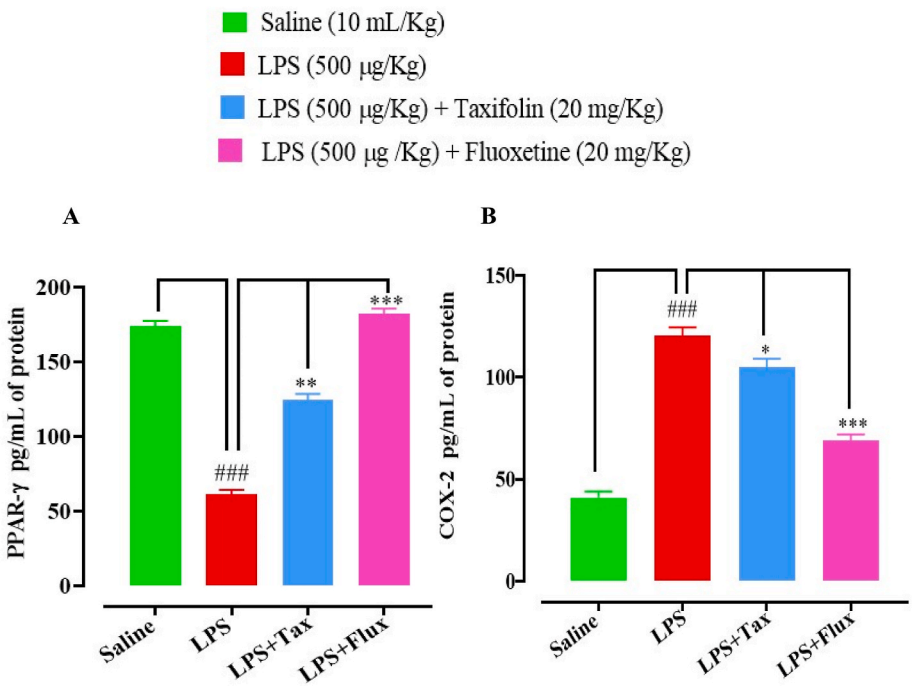

Fig. 11. Effects of Taxifolin and Fluoxetine against **(A)** Peroxisomes proliferation-activated receptor-γ (PPAR-γ) and **(B)** Cyclooxygenase-2 (COX-2) concentration in rat's cortex tissues using enzyme linked immunosorbent assay technique (ELISA). Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey' s test. $^{###}P < 0.001$ vs. saline group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. LPS group.

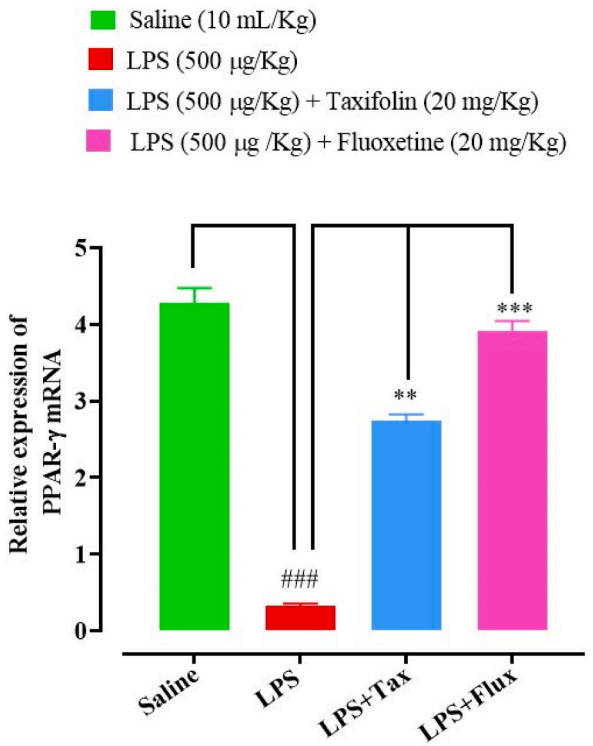

Fig. 12. Effects of Taxifolin and Fluoxetine against Peroxisomes proliferation-activated receptor-γ (PPAR-γ) by RT-PCR. Values expressed as mean ± SEM (n = 6). One-way ANOVA with post hoc Tukey' s test. $^{###}P < 0.001$ vs saline group, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. LPS group.

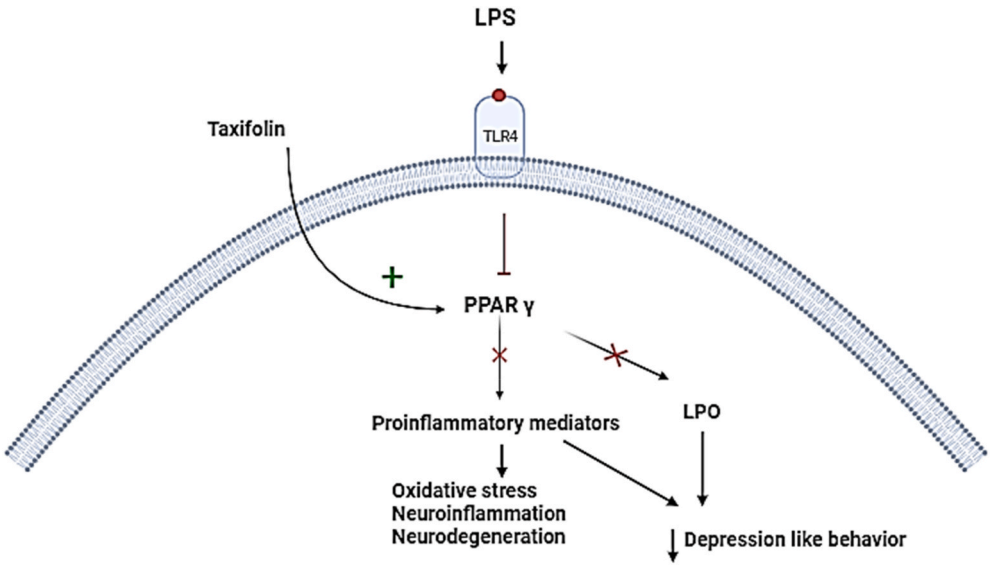

Fig. 13. A proposed pathway for the effects of Taxifolin treatment on LPS induced depression.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30467.

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