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Research Article

EFFECTS AND UNDERLYING MECHANISM OF 5-LIPOXYGENASE INHIBITOR (ZILEUTON) ON MICE DEPRESSIVE-LIKE BEHAVIOR

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ABSTRACT

Objective: Treatment experiment was conducted to investigate the effectiveness and mechanism of the action of zileuton in corticosteroid-induced depressive mice model through neuroinflammation.

Methods: The mice were randomly separated into four groups: (Veh+Veh), (Corticosteroid+Veh), (Corticosteroid+ZIL50), and (Corticosteroid+ZIL100). Intraperitoneal injection of corticosterone (CORT) (20 mg/kg for 6 weeks) was used in the mice to induce depression and neuroinflammation diverse from the Veh+Veh group, which was injected only physiological saline. The drug-treated groups (Corticosteroid+ZIL50 and Corticosteroid+ZIL100) were orally administered with the mentioned doses of zileuton. After confirming the effectiveness of zileuton through the behavioral tests, the mechanism of the action of the drug was explored through a set of biochemical assays.

Results: Zileuton (50/100 mg/kg) administration improved the performance of the mice in the behavioral experiments ($p<0.05$ or 0.01). Immunohistochemistry detection of Iba1+ revealed over activation of microglial cells in the corticosteroid-treated mice which was suppressed by the zileuton (50 or 100 mg/kg [$p<0.05$ or 0.01]). Through Western blotting tests, it had been found that CORT (i.p.) administration led to the increment of the protein 5-Lipoxygenase in the mouse hippocampus associated with neuroinflammation, which was decreased significantly by zileuton ($p<0.05$ or 0.01). Level of tumor necrosis factor-alpha, interleukin-1 beta, nuclear factor kappa B p65 protein (for neuroinflammation), Bax, and cleaved caspase-3 and TUNEL assay increased, and Bcl-2 expression decreased in the CORT-induced depressive mice. These were significantly reversed by zileuton (50 or 100 mg/kg [$p<0.05$ or 0.01]).

Conclusion: It can be concluded that selective 5-lipoxygenase inhibitor zileuton can efficiently inhibit the depressive-like behavior/activity in CORT-induced depressive mouse model. Moreover, the underlying mechanism may be the inhibition of hippocampal neuroinflammation and apoptosis.

Keywords: Depression, Zileuton, 5-Lipoxygenase, Neuroinflammation, Apoptosis.

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INTRODUCTION

Major depression and mania are two extremes of emotional disorders, which refer to the pathophysiological changes in mood state [1]. Depression disorder is a severe psychiatric abnormality characterized by a diffusive and chronic low mood that is accompanied by low self-esteem, psychomotor retardation or agitation, change in appetite, and an absence of interest or usually enjoyable pleasant attack [2]. Still now, the pathological mechanism of depression has not been well defined. Decreases of monoamine deficiency, the hypothalamic-pituitary-adrenal (HPA) axis hyperactivity, and neurotrophin are involved in the pathophysiology of depression [3].

Chronic stress leads to the regulation of HPA axis within the neuroendocrine system, and it was observed that the level of corticosterone (CORT) in the cycle disrupted the circadian regulation of CORT secretion and the glucocorticoid (GC) receptor negative recompose circuit [4]. Proved that, chronic stress could cause an imbalance in the HPA axis in neuroendocrine system. In patients with depression, hippocampal feedback was impaired, leading to excessive activity of HPA axis and elevated levels of humeral corticosteroids [5,6]. As a result, Cushing's disease with patients or those receiving long-term pharmacotherapy with GC shows an exceptionally high rate of depression [7]. Stimulation and continued actions of the HPA axis are attenuated through the adverse feedback circulating GC following exogenous CORT administration, and this is generally related to the improvement of psychosomatic disorders, which lead to severe changes in indicative or consistent emotional behavior accompanied by depressive-like symptoms [8,9]. HPA axis is activating by high dose of CORT administration, will increment depressive behavior in rodents, as held by a significantly diminish in sucrose consumption [8].

Arachidonic acid (AA) metabolized into inflammatory molecules called as leukotrienes (LTs) that have been metabolized by 5-lipoxygenase (5-LOX) inhibitor, which are capable mediators of several inflammatory and vascular diseases.

Zileuton attenuates brain inflammation in cerebral ischemia by inhibiting the nuclear factor-kappa B (NF-κB) p65 and downstream inflammatory mediators. However, it is important to further discover the potential mechanism of zileuton's neuroprotection against central nervous system (CNS) disorders.

Given this background, in this research work, we focused to give a glimpse the effects of 5-LOX drug zileuton (50 mg/kg and 100 mg/kg) treatment on the tail suspension test (TST), forced swimming test (FST), novelty suppression feeding test (NSFT), and open-field test (OFT) in mice that show repetition of exposure to CORT. Nowadays, many evidence suggested that neuroinflammation is responsible for the pathophysiology of depression [9,10]. We can show remarkable influence in both patients suffering depression and animal model of depression in the presence of high levels of pro-inflammatory cytokines, for example, interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) [10,11]. These pro-inflammatory cytokines show

an impact on the plasticity and neurotransmission, therefore, suppress the neurogenesis in the brain, and appliance of antidepressants suppresses these cytokines activation. The conversion of AA to 5-hydroxyperoxyeicosatetraenoic acid is carried out in the presence of a pro-inflammatory enzyme named 5-LOX and afterward the catalysis, it also produces hydroxyeicosatetraenoic acid (5-HETE). Therefore, it metabolized in several LTs [12]. 5-LOX and the LTs in the CNS perform both neuromodulatory and neuroendocrine effects [13] and lead to inflammatory response characterized by the increments of IL-1β, TNF-α, and NF-κB. Nowadays, a mass of researchers focused on 5-LOX. 5-LOX is responsible for the various type of CNS damage such as cerebral ischemia, cognitive deficit, traumatic brain injury, and anxiety by implicating its novel pathophysiological role [14-16]. Therefore, the relationship and the mechanism between 5-LOX and depressive-like behaviors need to be clarified. During this study, we first observed whether 5-LOX inhibitor zileuton prevents depressive behaviors and neuroinflammatory responses in CORT (corticosteroid)-induced mice model [17].

Furthermore, we tend to additionally evaluate whether the behavioral antidepressant-like effects of zileuton associated with alterations in hippocampal cell proliferation and neuronal commitment, as well as astrocytic hyperactivation.

MATERIALS, EQUIPMENT, AND METHODS

Materials

The pure drug zileuton was generously obtained from Dalian Meilun Biological Co., Ltd., China, CORT (corticosteroid) was purchased from Dalian Meilun Biological Co., Ltd., China, streptavidin-biotin complex (SABC) immunohistochemistry (IHC) kit was purchased from Wuhan Boster Biological Technology, Ltd., China. Trizol reagents and bovine serum albumin were purchased from Nanjing SunShine Biotechnology Co., Ltd., China; Western blot markers were obtained from Thermo Scientific, USA. Chemiluminescence detection reagents were purchased from Tanon Science and Technology Co., Ltd., China. Caspase-3 rabbit monoclonal antibody, Bcl-2 rabbit monoclonal antibody, and Bax rabbit monoclonal antibody were purchased from Cell Signaling Technology Ltd., USA.

Equipment

Tanon 4200 chemiluminescent protein (Tanon Science and Technology Co., Ltd., China). XW-80A type vortex mixer (Shanghai Huxi Analysis Instrument Factory Co., Ltd., China), CM1950 cryostat (LEICA, Germany), SR-5 stereotaxic instruments (Narishige, Japan).

Animals

Institute of Cancer Research mice were used for this experiment, took 12 mice in each group. All experiments were conveyed according to NIH guide for the care, and animal care committee in China Pharmaceutical University approved the use of laboratory animal (NIH publications No. 80-23, received, 1996) and the procedures. All animals were placed on a 12-h dark/light cycle with free access to water and standard chow and adapted for 1 week before the start of the experiment.

The experimental design is illustrated in Fig. 1. Animals were divided into four groups: Vehicle+Vehicle normal physiological saline (Veh+Veh), corticosteroid 20 mg/kg+Vehicle (CORT+Veh), corticosteroid 20 mg/kg+zileuton 50 mg/kg (CORT+ZIL50), and corticosteroid 20 mg/kg+zileuton 100 mg/kg (CORT+ZIL100). CORT administers by intraperitoneal (i.p) route and Zileuton administer by intragastric (i.g.). Every day CORT [{20 mg/kg body weight}, 5 ml/kg 0.1 ml/20 g of body weight (dissolve in dimethyl sulfoxide 0.1%, Tween-80 [0.1%] and require volume makeup by physiological saline)] was intraperitonially administration in mice. After 3 weeks of CORT administration, zileuton (50 mg/kg or 100 mg/kg), 0.2 ml/10 g of body weight (dissolved in methanol [0.5% methanol] and physiological saline), was orally given in mice daily by intragastric route (i.g.). After 21 days for behavioural tests were performed. After the behavioral tests, the mice were sacrificed by cervical dislocation and the full brain was extracted for IHC and Western blotting (WB).

Behavior tests

FST

The FST was performed [18]. In short, separately placed the mice in a glass cylinder (diameter 19 cm, height 25 cm) containing water (25±1°C) to a depth of 13 cm and allowed to swim for 6 min. Record the behavior of the mice and explored by TSE behavioral software. After a 6 min test, removed the mouse from the tank and thoroughly dried with dry cloth then returned to the colony room in their home cage. Change the tank's water after each swim session. Judged the immobility time as the absence of active, unwanted behavior, such as jumping, swimming, rearing or diving. Measured the total immobility time during the final 4 min of a 6 min test season by an observer blind to the treatment conditions.

TST

TST was performed [19]. After FST, mice were conducted 24 h. Shortly, moved the mice from housing room to the testing room in their home cages, at least 1 h before testing to allow for adapt to the new environment. During the dark period of the circadian cycle, mice were tested. Each mouse was hanged by his or her tail with adhesive tape to the hook in the soundproof box. The total immobility period during 6 min test was explored by ANY-MAZE software. The immobility period during initial 2 min of the 6 min task was discounted while the last 4 min task was explored statistically.

OFT

The OFT was performed [20]. The mice were conducted 24 h after TST. The open field contained (40×40×38 cm) square arena with clean Plexiglas wall and floor. At first, mouse was placed in one corner of the open space and during a 5 min test, session allowed to move arena freely. Measured peripheral and central activities using a computer-assisted activity system with software to easily the data collection and analysis.

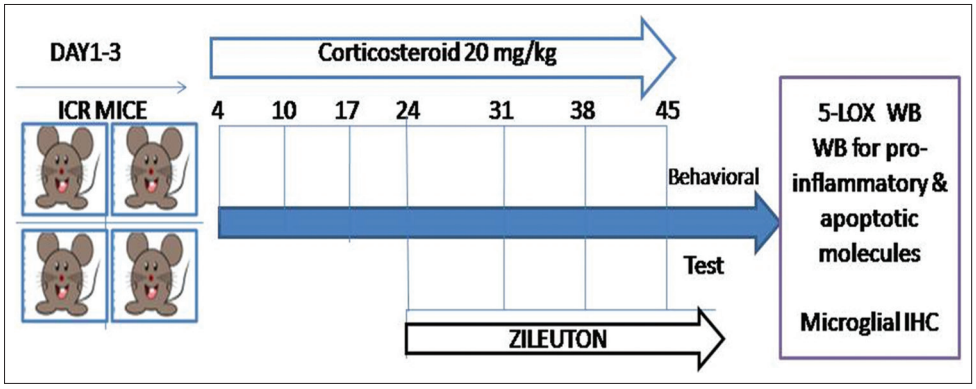

Fig. 1: Study design and animal treatments

NSFT

After habituation in the testing room, mice were subjected to the NSFT test [21]. The testing instrument consists of a plastic box (50×50×20 cm), covered by wooden bed 2 cm. Twenty-four hours before this behavioral test, all food was removed from their cage. At testing time, a single pellet of food was placed on a white paper platform in the center of the testing box. The mouse has placed in the center of the box. The mouse was placed in the corner of the box and instantly started the stopwatch. The latency to eat (it means the mouse sitting on its haunches and biting the pellet with the use of forepaws) was recorded.

Tissue preparation and immunohistochemical analyses

Tissue preparation, at first anesthetized the mice and perfused transcardially with 0.1 M phosphate-buffered saline (PBS) (pH-7.4), contains 4% paraformaldehyde and 5 U/ml heparin. Then, the brain was collected and fixed in 4% paraformaldehyde for 18–20 h followed by 30% sucrose solution for 24 h. After then, the brain was dissected and embedded into optimal cutting temperature compound on dry ice and cryosectioned at 30 μm. Following the manufacturer's instructions, immunohistochemical staining was performed using SABC IHC kit. After washing, the section with 0.1 M PBS solution heated the sections on water bath for 4 h in 0.3% Triton X-100 at 60°C. Then again washed the section and treated with 3% H_2O_2 at normal room temperature for 10 min and washed with PBS for 3×5 min followed by blocking 5% BSA for 30 min. After then, the sections were incubated overnight at 4°C with primary antibody for Iba 1 (rabbit immunoglobulin G [IgG], 1:1000) diluted in 5% BSA. Next day, washed the sections in PBS for 3×5 min at room temperature and incubated with biotinylated mouse anti-rabbit IgG for 20 min. After then, again rewashed in PBS (3×5 min, 37°C) and incubated with SABC at 37°C for 20 min. Again, sections were washed in 0.1 M PBS for 4×5 min and mounted on the glass slides. Then added the diaminobenzidine followed by gradient dehydration ([1] 70% ethanol for 5 min, [2] 95% ethanol for 5 min, [3] 100% ethanol for 2×5 min, and [4] xylene for 2×5 min). Next, sections were covered with DPX mounting solution and cover glass. At last, photomicrographs were obtained using a Nikon DS-Fi2 camera connected to a Nikon Eclipse Ti microscope and analyzed by the Image-Pro Plus software.

Total protein and nuclear protein extraction

Homogenized the mouse hippocampus in an ice-cold radioimmunoprecipitation assay buffer, it contains 0.1% phenylmethylsulfonyl fluoride (PMSF). The homogenate was centrifuged at 12,000 g for 15 min and collected the supernatant. Total protein concentration was determined. The supernatant was used to determine using the bicinchoninic acid protein kit and stored at −20°C.

Nucleoprotein extraction kit was used for nuclear extraction. Shortly, chopped the mouse hippocampus into small pieces, then homogenized in the ice-cold hypotonic buffer, contains 1% PMSF, 0.5% phosphate inhibitor, 0.1 % DL-Dithiothreitol (DDT) and then centrifuged at 4°C 3000 g for 5 min. Then washed the precipitate with hypotonic buffer solution and centrifuged at 4°C, 5000 g for 5 min. Finally, 0.2% lysis buffer containing 1% PMSF, 0.1% DDT, and 0.5% phosphatase buffer was added into the precipitate, cooled for 20 min, and centrifuged at 4°C, 15,000 g for 10 min. Subjected the supernatant nuclear extract was detected by WB assay for NF-κB p65 and histone H3 using as the control.

Western blot analysis

The sample was run to evaluate the protein and isolate the protein bands by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Then transferred the bands onto polyvinylidene difluoride membrane and blocked with 5% skim milk prepared in Tris-buffer saline solution. Membranes were incubated at 4°C overnight with the respective primary antibody for 5-LOX (1:1000), IL-1β (1:500), TNF-α (1:500), Caspase-3 (1:1000), Bcl-2 (1:200), and Bax (1:1000); β-actin (1:3000) was used as control.

For NF-κB p65 (1:1000), histone H3 (1:1000) loading control. After the overnight incubation, membranes were washed with Tris buffer saline – Tween 20 and incubated for 2 h at room temperature, with a horseradish peroxidase-conjugated secondary antibody (1:5000–1:10000). Finally, visualized the antibody reactive bands using enhanced chemiluminescence detector reagents (1:1) by gel imaging system.

Statistical analysis

Data shown are expressed as mean±standard error of the mean using analysis of variance (ANOVA) software to analyze the behavioral tests (TST, OFT, FST, and NSFT). All other data were analyzed by one-way ANOVA for various comparisons followed by Dennett's *post hoc* analyzer. All analyses were carried out using the SPSS 22.0. Considered that, $p<0.05$ was statistically significantly difference between the groups.

RESULTS

CORT-induced depressive-related behaviors are ameliorated by zileuton

To evaluate that, whether 5-LOX inhibition prevents CORT-induced depression-related behaviors, we treated the mice using a specific 5-LOX inhibitor zileuton and performed different behavior tests. Hope that the mice reveal to CORT showed an increase of immobility time in the TST (Fig. 2a, $F(3,32)=8.183$, $p<0.01$). Treatment with zileuton (50 mg/kg or 100 mg/kg) significantly reduced the immobility time in the TST (Fig. 2a, CORT+Zil50 and CORT+Zil100: $p<0.01$) compare to the CORT-treated group. Similarly, same result was obtained in the FST. In contrast with the Veh+Veh group, mice treated with CORT expend longer immobility time (Fig. 2b, $F(3,32)=4.888$, $p<0.01$), and this was minimized by zileuton treatment (50 mg/kg or 100 mg/kg) (Fig. 2b, CORT+Zil50: $p<0.05$ and CORT+Zil100: $p<0.01$). In addition, the anxiety-related behaviors were assessed employed NSFT, in which mice displayed an increase in the latency to feed in the novel environment (Fig. 2c, $F(3,32)=7.218$, $p<0.01$). While no differences observed in home cage consumption index (Fig. 2d, $F(3,32)=0.327$, $p>0.05$) after CORT injection, zileuton treatment (50 mg/kg or 100 mg/kg) suppressed the latency to feed in CORT-induced mice (Fig. 2c, CORT+Zil50 and CORT+Zil100: $p<0.05$). The locomotor activities were observed in the OFT, and there was no difference between all groups (Fig. 2e, CORT+Zil50 and CORT+Zil100: $p>0.05$). Furthermore, our studies also expressed that zileuton (50 mg/kg and 100 mg/kg) administration diminished depressive behavior in compared to CORT-induced depressive mice model. Taken together, hippocampal 5-LOX inhibitor zileuton may significantly prevent CORT-induced depressive-like behaviors.

Zileuton blocks CORT-induced microglial activation

It informed that CORT activates the mediated inflammatory response through microglia, whose changes are characterized by improved cell numbers and conformational changes, as well as size enlargement and thicker processes [22]. To study the effects of hippocampal 5-LOX enzyme on microglia, we detected the activation levels of microglia by immunohistochemical method. As shown in figure, the number of IBA1 positive cells in the hippocampus of CORT-induced mice was significantly prolonged (Fig. 3a, $F(3,12)=13.115$, $p<0.01$) compared to the Veh+Veh group, while zileuton (50 mg/kg or 100 mg/kg) significantly decreased IBA1 staining cells (Fig. 3b CORT+Zil50: $p<0.05$ and CORT+Zil100: $p<0.01$).

Zileuton prevents CORT-activated NF-κB signaling and production of pro-inflammatory cytokines

Evidence exhibits that 5-LOX regulates inflammatory response by controlling NF-κB pathway [23], and CORT activated the NF-κB pathway by triggering the nuclear translocation of the p65 subunit. Herein, we are curious about whether 5-LOX involved in the CORT-activated NF-κB pathway. Actively, after the 5-LOX inhibitor, zileuton (50 mg/kg or 100 mg/kg), treatment in the hippocampus was significantly inhibited by NF-κB signaling (Fig. 4a, $F(3,32)=11.858$, $p<0.01$), which can block the expression of hippocampal 5-LOX (Fig. 4b, CORT+Zil50: $p<0.05$ and CORT+Zil100: $p<0.01$).

Activated microglia and NF-κB pathway may regulate various pro-inflammatory cytokines involved in the onset of depression [24] to

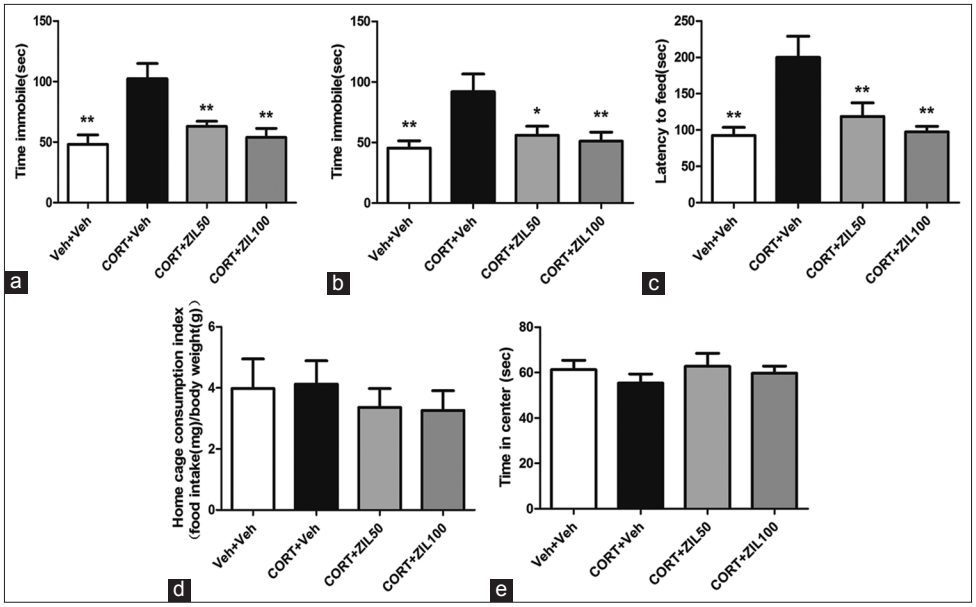

Fig. 2: Zileuton prevents corticosterone (CORT)-induced depression-related behaviors. (a) (Tail suspension test) immobility time, (b) (forced swimming test) immobility time, (c) latency to feed, (d) (novelty suppression feeding test) home cage consumption index and (e) (open-field test) line crossings were shown. Mice were injected with CORT (20 mg/kg, i.p.), for 6 weeks period; 2 h later, they were treated drugs, respectively, once daily for the past 3 weeks and then subjected all mice for behavior tests. The data are indicated as mean±standard error of the mean (n=9). *p<0.05, **p<0.01 versus CORT+Veh group

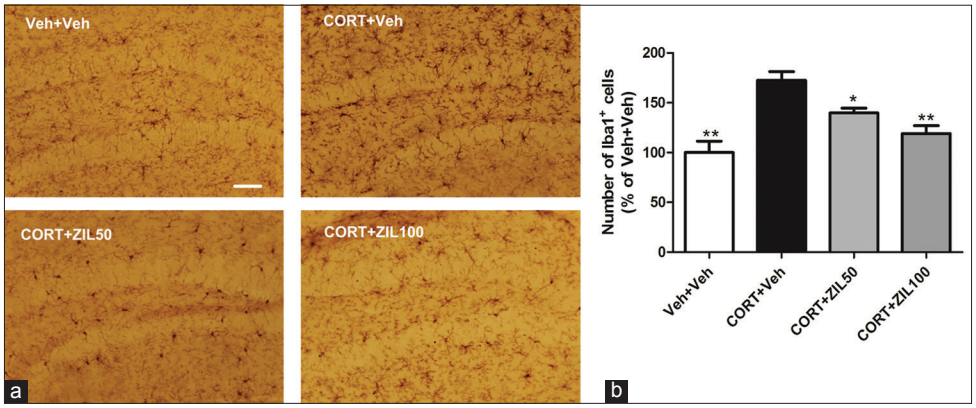

Fig. 3: Zileuton blocks corticosterone (CORT)-induced microglial activation. Sacrificed the mice and sections the mice brains for immunohistochemical staining for the microglial marker Iba1. (a) Representative microphotographs show Iba1 immunostained microglia of the mouse hippocampus. (b) The number of Iba1 antibody-stained microglia was normalized in the same corresponding area, as the ratio (in %) of the Veh+Veh group is shown. Administration of CORT and drugs was the same as that in Fig. 2. Data shown are revealed as mean±standard error of the mean (n=4). *p<0.05, **p<0.01 versus CORT+Veh group. These data suggest that 5-Lipoxygenase inhibitor zileuton blocks CORT-induced microglial activation

investigate the effect of zileuton on the neuroinflammatory process induced by CORT.

The expression of pro-inflammatory cytokines such as IL-1β and TNF-α was detected by western blot. As shown in figure, CORT expression significantly enhanced the generations of TNF-α and IL-1β in the hippocampus (Fig. 4c, $F(3,12)=13.873$, $p<0.01$; Fig. 4e, $F(3,12)=14.683$, $p<0.01$), whereas zileuton (50 mg/kg or 100 mg/kg) suppressed the production of TNF-α and IL-1β (Fig. 4d and f CORT+Zil50: $p<0.05$ and CORT+Zil100: $p<0.01$). These results suggest that 5-LOX inhibitor prevents the production of pro-inflammatory cytokines induced by CORT.

Zileuton prevents neuronal apoptosis

Evidence has shown that 5-LOX decreases pro-apoptotic molecules such as cleaved caspase-3, and Bax and TUNEL in the mouse hippocampus while the level of the anti-apoptotic protein Bcl-2 was increased. Figure (Fig. 5a, $F(3,12)=9.625$, $p<0.01$) shown that zileuton (more 100 mg/kg than 50 mg/kg dose) decreases cleaved caspase-3 in compared to CORT-induced mice model (Fig. 5b CORT+Zil50: $p<0.05$ and CORT+Zil100: $p<0.01$). Similarly, zileuton decreases Bcl-2/Bax ration in comparison to CORT-induced mice model (Fig. 5c, $F(3,12)=10.393$, $p<0.01$) (Fig. 5d CORT+Zil50: $p<0.05$ and CORT+Zil100: $p<0.01$). In immunofluorescence assay, zileuton decreases neuronal apoptosis in TUNEL-positive cells ratio in mouse hippocampus (Fig. 5e, $F(3,12)=9.806$, $p<0.01$) (Fig. 5f CORT+Zil50 and CORT+Zil100: $p<0.01$).

DISCUSSION

Through the present study provides evidence that the neuroprotective effect of zileuton has shown in CORT-induced depressive mice model through modulating loss of behavioral activities, activation of microglia, neuroinflammation, and cell apoptosis. Specifically, CORT led to the significant increase of hippocampal 5-LOX protein, produced several

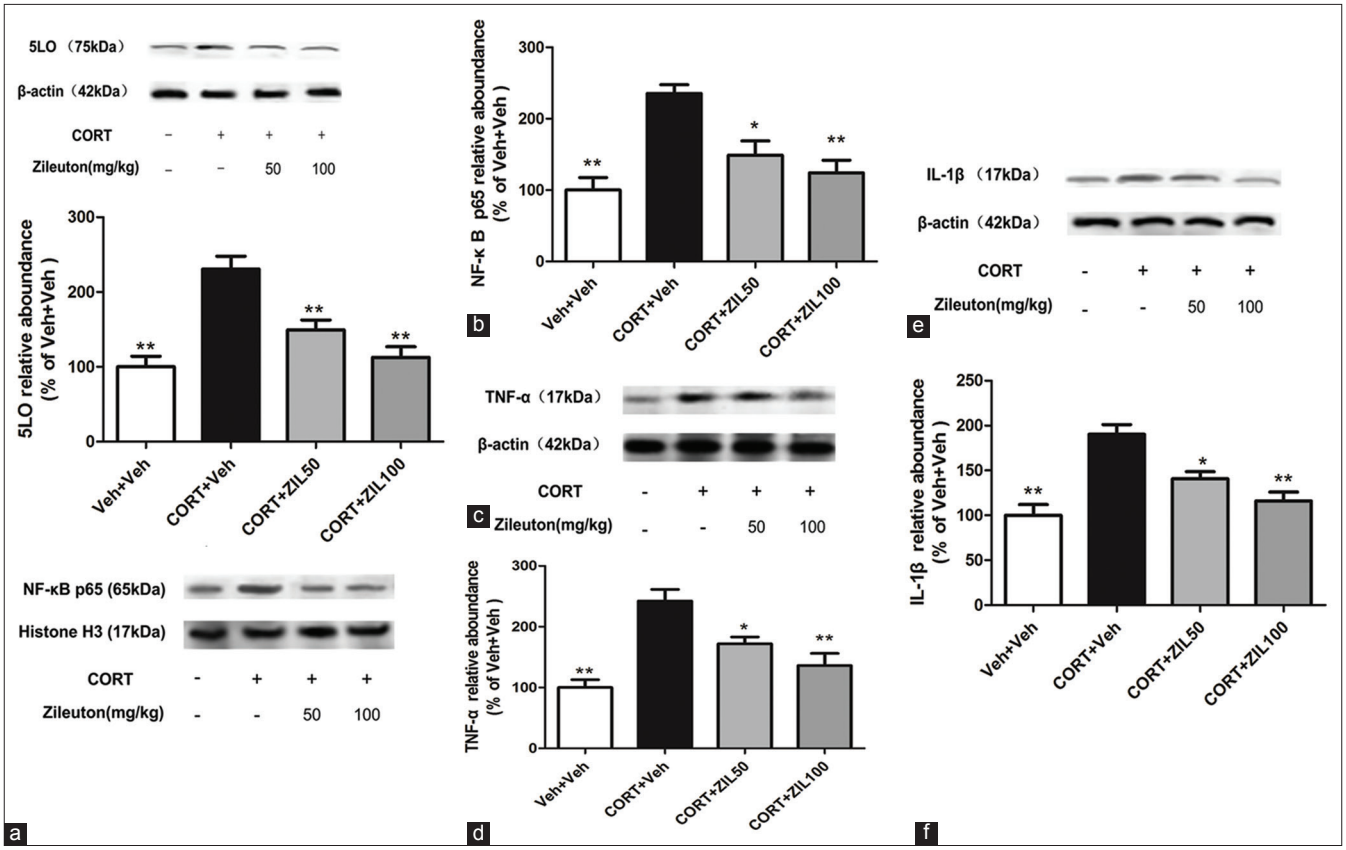

Fig. 4: Zileuton prevents corticosterone (CORT)-activated nuclear factor kappa B (NF-κB) signaling and production of pro-inflammatory cytokines. The protein expression of hippocampal nuclear (a,b) NF-κB p65, (c,d) tumor necrosis factor-alpha, (e,f) interleukin 1 beta, and β-actin or histone H3 as loading control were examined by Western blot using respective antibodies (1:1000). Quantification expressed as the ratio (in %) of Veh+Veh group is shown. Administration of CORT and drugs was the same. The data are revealed as mean± standard error of the mean (n=4). *p<0.05, **p<0.01 versus CORT+Veh group

behavioral activities including increased immobility time in the FST and TST test, and increased the latency to feed in the NSFT in mice. It was accompanied by the hippocampal inflammatory response, activated microglia, increased the expression of TNF-α, IL-1β, NF-κB p65, increased cellular apoptosis caspase-3, Bax, TUNEL, and decreased Bcl-2. 5-LOX inhibitor drug zileuton markedly attenuated these effects by inducing CORT. These results suggesting that antidepressant effect of 5-LOX inhibitor reduced neuroinflammation. In addition, increasing evidence suggested that a strong association in-between neuroinflammation and depression, which was marked by, increased levels of pro-inflammatory cytokines in the CNSs. The 5-LOX is broadly expressed in the CNS. It localizes mainly in neuronal cells [25,26]. In recent study had suggested in various regions of the brain, including the hippocampus. Where its levels was increased. We first identified the expression of hippocampal 5-LOX in depressive mice model by induced CORT, which was used to model inflammation with depressive disorders.

Therefore, we found that after the administration of zileuton produced an antidepressive effect in the behavioral tests, exhibited by inhibiting the CORT stress-induced increase in immobility time in the FST and TST and to the difference in locomotor activities by observed in OFT. In addition, depression often with anxiety in patients, so we estimated the anxiety-like behavior by the selection of NSFT. Zileuton treatment significantly opposite the anxiety-like behaviors induced by CORT, characterized by reducing the increment of latency to feed by CORT. Moreover, zileuton exhibited an antidepressant effect.

Treatment with zileuton is one of the 5-LOX inhibitors, successfully attenuated histopathological and biochemical changes by induced CORT microglial associated neuroinflammation in intraperitoneal route [24,27]. However, histological changes in microglia have considered being a pathophysiological role of depression [28]. 5-LOX effective inflammatory mediator, which plays a crucial role in the asthma patient and other inflammatory diseases [29,30].

The 5-LOX inhibitor, zileuton, is a novel class of anti-asthma drugs and well tolerated in asthma patient [31,32]. 5-LOX-mediated signaling is relative to NF-κB pathway [33-36]. NF-κB, an important transcription factor, regulates the expression of several pro-inflammatory cytokines such as TNF-α and IL-1β [37,38]. Hence, NF-κB has reported mediate microglia activation and suppressing NF-κB transcriptional activity inhibits the production of pro-inflammatory cytokines [39,40]. Administration of zileuton (50 mg/kg and 100 mg/kg) regulated these pro-inflammatory cytokines through inhibition (TNF-α and IL-1β) of NF-κB p65.

Evidence focuses that apoptosis as an essential faction contributing depression pathogenesis [41] not only inhibits the inflammatory reactions but also blocks the apoptotic processes as well [42].

In addition, microglia-derived TNF-α and IL-1β can mediate cell death [43]. Therefore, suppressing the NF-κB signaling and microglial activation is helpful against depression-related neuronal apoptosis. Earlier studies have shown that pro-apoptotic protein such as Bax, caspase-3, and antiapoptotic protein Bcl-2 is related in depressive mice model [37-38,41] and blocking NF-κB opposite changes in depressive mice [44].

Here, this experiment increase of CORT caused increment of pro-apoptotic molecules such as caspase-3, Bax, TUNEL

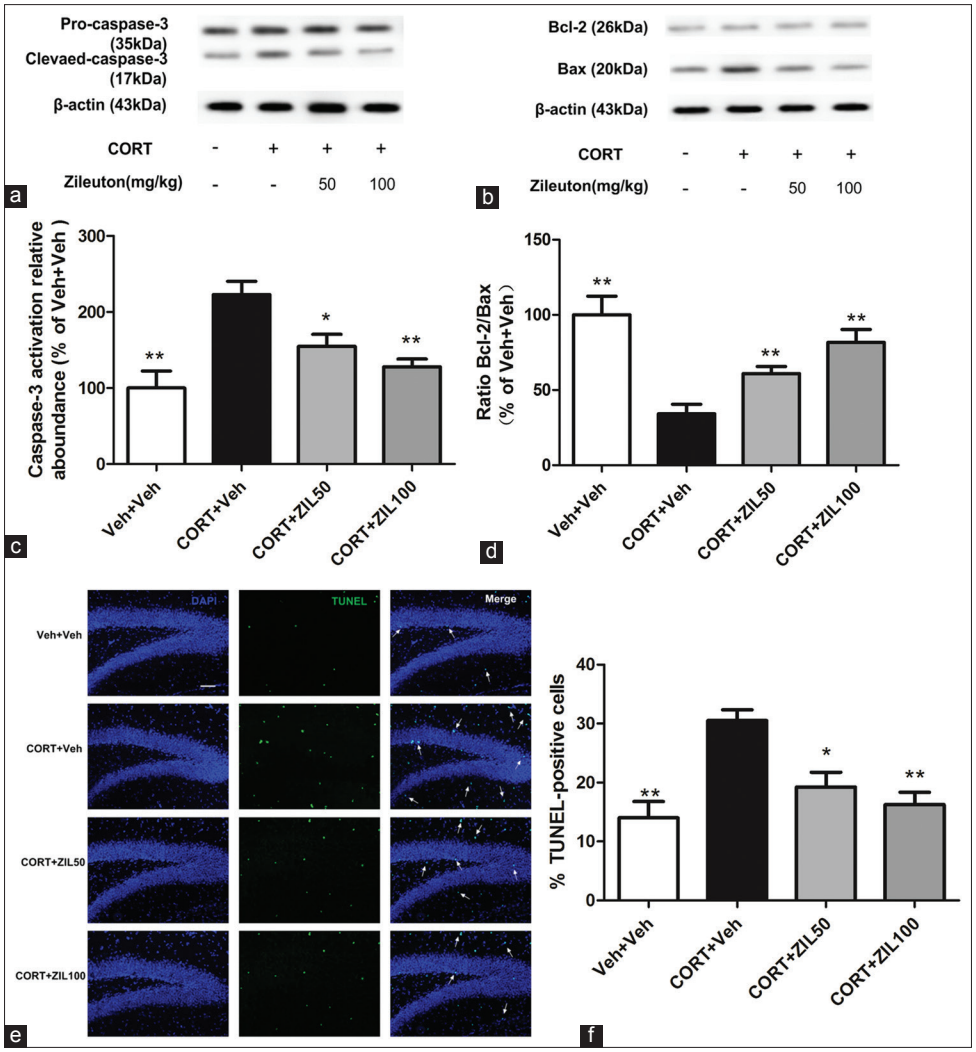

Fig. 5: Zileuton decreases the caspase 3 activation in corticosterone (CORT)-induced mice. Entire hippocampus extracts were adapted to Western blot analysis through an antibody against caspase 3, (a,b) pro-caspase 3 and cleaved caspase 3 and (c,d) Bcl-2/Bax. Quantification of the protein levels of (b) caspase 3 and (d) Bcl-2/Bax was expressed as the ratio (in %) of Veh+Veh group. (e,f) Entire hippocampus extracts were adapted to TUNEL staining by fluorescence microscopy. The results are revealed as mean±standard error of the mean (n=4). *p<0.05, **p<0.01 versus CORT+Veh group

immunofluorescence assay, and approximately Bcl-2 reduction in the mouse hippocampus. However, the treatment of zileuton (50 mg/kg and 100 mg/kg) significantly gives such response in comparison to model group. Therefore, it concluded that 5-LOX could be a promising target for the treatment of depression.

CONCLUSION

The effect of 5-lipoxygenase was founded by clinical studies targeted against neuroinflammatory cascade with the synthesis pathway in the pathogenesis of depression.

However, the study was limited to usage of the research of depressive mice model, and must be needed to discover them for depression. It was research to explore by Utilization of CORT (corticosteroid) induced mouse model of depression. My research show neuroinflammation was strongly related to behavioral activities. In this research, activation of microglia was frequent. Increases the level of proinflammatory cytokines (such as TNF-α, IL-1β) and pro-apoptotic molecules (cleaved caspase-3 and TUNEL) observed while reduces in the anti-apoptotic molecule (Bcl-2) reserved in the CORT (corticosterone in an intraperitoneal injection) induced mice, possibly through the NF-κB signaling pathway.

5-LOX inhibitor zileuton treatment, at high doses (100 mg/kg) is more than zileuton (50 mg/kg), reversed/reduced such changes and showed some outcome in the behavioral tests.

This experiment illustrates that 5-LOX enzyme as an emerging therapeutic target in depressive mouse model and motivates 5-LOX inhibitor such as Zileuton, basically used in anti-asthmatic drug, to be promoted for the treatment of depression, which can minimize the mental, clinical social and economic liability of depression.

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AUTHORS' CONTRIBUTIONS

Both authors have contributed to reviewing the preparation and editing of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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