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Sodium Phenylbutyrate and Edaravone Abrogate Chronic Restraint Stress-Induced Behavioral Deficits: Implication of Oxido-Nitrosative, Endoplasmic Reticulum Stress Cascade, and Neuroinflammation

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Abstract Chronic stress exposure can produce deleterious effects on the hippocampus (HC) which eventually leads to cognitive impairment and depression. Endoplasmic reticulum (ER) stress has been reported as one of the major culprits in the development of stress-induced cognitive impairment and depression. We investigated the neuroprotective efficacy of sodium phenylbutyrate (SPB), an ER stress inhibitor, and edaravone, a free radical scavenger, against chronic restraint stress (CRS)-induced cognitive deficits and anxiety- and depressive-like behavior in mice. Adult male Swiss albino mice were restrained for 6 h/day for 28 days and injected (i.p.) with SPB (40 and 120 mg/kg) or edaravone (3 and 10 mg/kg) for the last seven days. After stress cessation, the anxiety- and depressive-like behavior along with spatial learning and memory were examined. Furthermore, oxido-nitrosative stress, proinflammatory cytokines, and gene expression level of ER stress-related genes were assessed in HC and prefrontal cortex (PFC). CRS-exposed mice showed anxiety- and depressive-like behavior, which was significantly improved by SPB and edaravone treatment. In addition, SPB and edaravone treatment significantly alleviated CRS-induced spatial learning and memory impairment. Furthermore, CRS-evoked oxido-nitrosative stress, neuroinflammation, and depletion of Brain-derived neurotrophic factor were significantly ameliorated by SPB and edaravone treatment. We found significant up-regulation of ER stress-related genes in both HC and PFC regions, which were suppressed by SPB and edaravone treatment in CRS mice. Our study provides evidence that SPB and edaravone exerted neuroprotective effects on CRS-induced cognitive deficits and anxiety- and depressive-like behavior, which is possibly coupled with inhibition of oxido-nitrosative stress, neuroinflammation, and ER stress cascade.

Keywords Anxiety · Cognitive impairment ·
Depression · Endoplasmic reticulum stress ·
Neuroinflammation · Oxido-nitrosative stress

Introduction

Substantial experimental evidences suggest the involvement of oxido-nitrosative stress and neuroinflammatory cascade in the pathogenesis of cognitive dysfunction, anxiety, and depression (Maes 2008; Jangra et al. 2014a; Kasbe et al. 2015). Chronic restraint stress (CRS) has been shown to induce cognitive impairment and anxiety- and depressive-like behavior in rodents (Chiba et al. 2012; Huang et al. 2015a, b). CRS-induced behavioral anomalies are accompanied by oxidative stress and neuroinflammation in different brain regions such as hippocampus (HC) and prefrontal cortex (PFC) (McEwen and Magarinos 1997; Chiba et al. 2012). The hippocampus plays a key role in learning and memory function and is much more susceptible to CRS-evoked damage than any other brain areas

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(Tse et al. 2014). In addition, several studies suggested that oxidative and neuroinflammatory damage particularly in the hippocampal region has been associated with the pathogenesis of anxiety and depression (Bannerman et al. 2014; Jangra et al. 2014a; Sulakhiya et al. 2014). CRS-evoked reactive oxygen species (ROS) activates nuclear factor kappa B (NF-κB) signaling pathway, which in turn triggers the activation of proinflammatory cytokines (such as IL-1, IL-6, and TNF-α), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression. The resulted neuroinflammation can trigger apoptotic cell death and depletion of neurotrophic factors, thereby causing neurobehavioral alterations (Zhang et al. 2014; Radecki et al. 2005).

Endoplasmic reticulum (ER) is a site for synthesis, maturation, and folding of proteins. Several stimuli such as stress, ROS, oxidative stress, hypoxia, inflammation, obesity, hypoglycemia, and calcium depletion can disrupt protein folding process, and consequently leads to accumulation of unfolded and misfolded proteins in the ER, a condition termed "ER stress." To manage the unfolded and misfolded proteins, ER stress triggers a signaling pathway called Unfolded Protein Response (UPR). UPR activation results in translation attenuation, promotes degradation of misfolded proteins, and raises the levels of ER chaperones, glucose-regulated protein 78 (GRP78), which in turn enhance the protein folding capacity of ER. However, under prolonged ER stress condition, cells may undergo apoptosis through up-regulation of the C/EBP homologous protein (CHOP) which plays a key role in the regulation of cellular redox condition and cell death (Oyadomari et al. Oyadomari and Mori 2004; Zhao et al. 2015). Emerging experimental evidences showed that ER stress is activated in the HC region which plays a major role in the pathogenesis of memory dysfunction and depression (Huang et al. 2013; Zhang et al. 2014).

Sodium phenylbutyrate (SPB) and edaravone are well-known ER stress inhibitors which exert neuroprotective activity in several animal models (Gardian et al. 2005; Yuan et al. 2008; Itoh et al. 2010; Srinivasan and Sharma 2011, 2012). In addition to ER stress inhibitory property of edaravone, its neuroprotective action also attributed to the potent free radical scavenging activity (Kikuchi et al. 2011). Previous studies have shown the potential application of SPB and edaravone against neuronal cell death and cognitive dysfunction (Ricobaraza et al. 2009; Jiao et al. 2011). Therefore, based on the role of oxido-nitrosative stress and ER stress in RS-induced behavioral anomalies, we have chosen edaravone (ROS scavenger) and SPB (ER stress inhibitor) as pharmacological interventions in our experiment. In the present study, we investigated the effects and underlying mechanisms of SPB and edaravone against CRS-induced memory deficits and anxiety- and depressive-like behavior in mice. We have examined the oxido-nitrosative stress, brain-derived neurotrophic factor (BDNF), proinflammatory cytokine level, and expression of GRP78 and CHOP in HC and PFC regions as well as corticosterone level in the serum. We found that CRS-exposed animals exhibited learning and memory dysfunction as well as anxiety- and depressive-like behavior after 28 days. Treatment with edaravone and SPB alone alleviated the behavioral alterations via inhibition of CRS-induced neurochemical anomalies such as oxidative stress, neuroinflammation, and ER stress in the HC region.

Materials and Methods

Animals

Adult male Swiss albino mice, weighing 22–25 g, were included in the present study and provided with rodent pellet diet (Pranav Agro Industries Ltd., Pune, India) and water ad libitum. The mice were kept at a constant temperature of $24 \pm 1\ °C$ and humidity of $65 \pm 5\ \%$ under a 12:12-h light/dark cycle. The mice were allowed to acclimatize to the laboratory conditions for 7 days prior to the initiation of experiments. The study was approved by the Institutional Animal Ethics Committee (IAEC), Gauhati Medical College & Hospital (CPCSEA Registration No. 351, 3/1/2001). All the experimental protocols were strictly carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Chemicals

SPB and edaravone were purchased from Santa Cruz, CA, USA. Total RNA extraction kit (HiMedia, India), cDNA synthesis kit (Thermo Fisher Scientific, India), SYBR Green PCR kit (QIAGEN India Pvt. Ltd., India), Primers (Imperial Life Sciences (P) Limited, India), and IL-1β and TNF-α ELISA kits (Thermoscientific, India, and Invitrogen co., Carlsbad, CA, USA, respectively) were used. BDNF kit was purchased from Promega, India. All other chemicals used were of analytical grade.

Drug Administration and Experimental Plan

Male Swiss albino mice were randomly divided into six experimental groups, each containing 24 mice (total 144 animals). The experimental groups were as follows:

1. Group 1 was treated with normal saline for seven days. This group was assigned as normal control group.

2. Group 2 animals were restrained (6 h/day for 28 days) and given normal saline for the last seven days. This group was assigned as restraint control.
3. Group 3 animals were restrained (6 h/day for 28 days) and treated with edaravone (3 mg/kg, i.p.) for the last 7 days of restraint stress.
4. Group 4 animals were restrained (6 h/day for 28 days) and treated with edaravone (10 mg/kg, i.p.) for the last 7 days of restraint stress.
5. Group 5 animals were restrained (6 h/day for 28 days) and treated with SPB (40 mg/kg, i.p.) for the last 7 days of restraint stress.
6. Group 6 animals were restrained (6 h/day for 28 days) and treated with SPB (120 mg/kg, i.p.) for the last 7 days of restraint stress.

Adult male mice were subjected to CRS for 6 h (09:00–15:00) daily up to 28 consecutive days in well-ventilated 50-ml polystyrene tubes. SPB and edaravone were dissolved in normal saline and administered intraperitoneally (i.p.) in a volume of 10 ml/kg body weight 30 min prior to the onset of restraint stress. The study plan is diagrammatically described in Fig. 1. Different doses of SPB and edaravone were selected on the basis of previous experimental studies (Qi et al. 2004; Srinivasan and Sharma 2011; Sriram et al. 2016). Morris water maze test was performed from the 24th to the 28th day to assess the memory function. Anxiety-like behavior

was assessed by elevated plus maze test and light–dark box test at 24 h after cessation of restraint stress protocol. Depressive-like behavior was evaluated by forced swimming test, tail suspension test, and sucrose preference test. Behavioral and biochemical tests were carried out on different animals to avoid the possible influence of behavioral tests on biochemical parameters. Animals were sacrificed by cervical dislocation 24 h after cessation of restraint stress protocol. HC and PFC regions were dissected out from the brain, homogenized (10 % w/v) in chilled 0.1 M phosphate buffer (pH 7.4), and centrifuged, and the supernatants were collected and stored at −80 °C which were further used for biochemical analysis.

Behavioral Tests

Morris Water Maze (MWM) Test

The spatial learning and memory performance was determined using the MWM test (Vorhees and Williams 2006). The MWM consisted of a circular tank (120 cm diameter, 50 cm height) and a platform (10 cm diameter). The tank was filled with colored water (25 ± 1 °C). During the training session, the platform was placed approximately 2 cm below the water level. The animal was kept in the pool, facing toward the tank wall, and allowed to locate the platform for 120 s during the training phase. Mice were
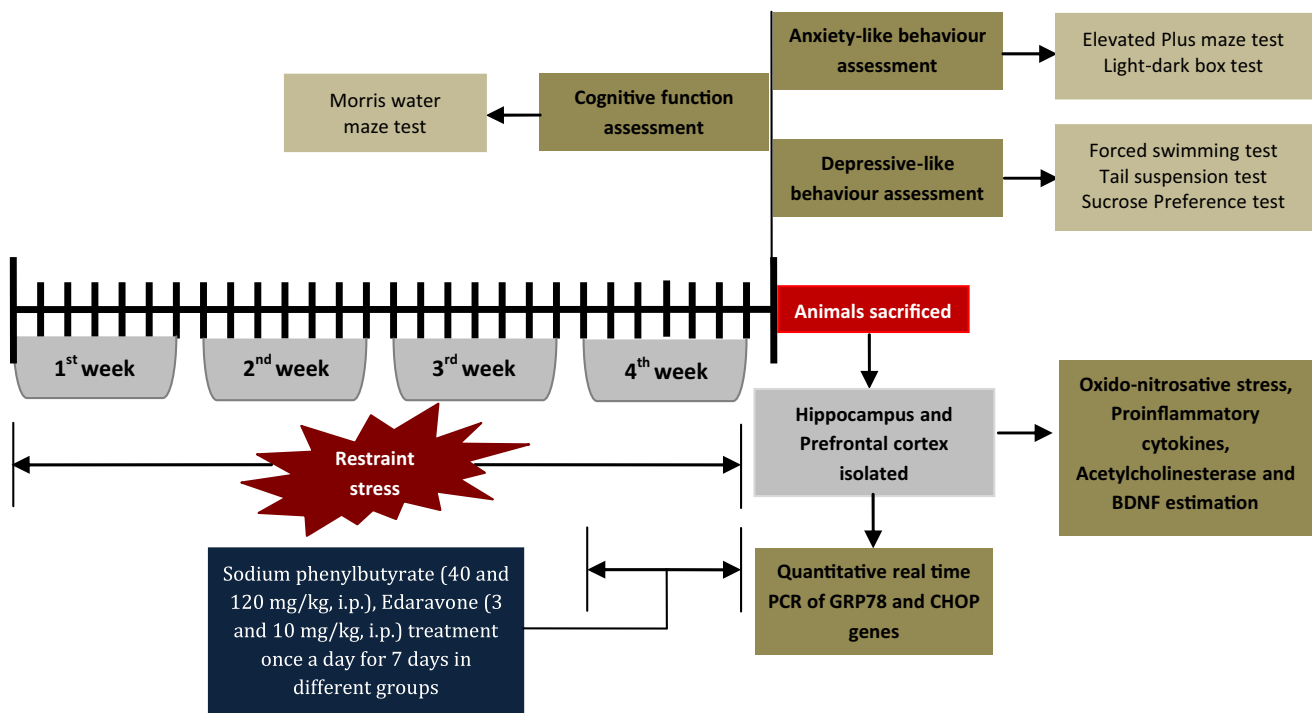
Fig. 1 Schematic representation of experimental study plan

given four trials per day for four consecutive days. The time to reach the platform was recorded in each trial. If the animal was failed to locate the platform in 120 s, then the animal was placed on the platform for 30 s. On the fifth day, spatial probe trial was conducted by removing the platform. The time spent in the target quadrant by the animal was calculated.

Elevated Plus Maze (EPM) Test

EPM test was carried out to evaluate the influence of CRS on the anxiety behavior in mice. EPM was consisted of two open arms (35×5 cm^2) perpendicular to two closed arms of the same size with a small central square (5×5 cm^2) between the arms. The maze was elevated 50 cm from the floor in a dimly illuminated room. Each animal was placed at the center of maze with head facing toward the open arm. The total number of entries into the open arm and the time spent in the open arm during the 5-min period were calculated and presented (Jangra et al. 2014a).

Light–Dark Box Test

The light–dark box consisted of two different compartments: a light side ($42 \times 30 \times 20$ cm^3; white walls brightly illuminated with 40 W bulb) and a dark side ($42 \times 30 \times 20$ cm^3; opaque black walls and dark), with an opening (6×6 cm^2) between the two compartments. The animal was placed on a dark side with head facing toward the light side and allowed to explore in the box for 10 min. Time spent in the light compartment and the number of light–dark transitions were calculated and presented (Bassi et al. 2012).

Forced Swimming Test (FST)

On the 29th day, FST was performed as previously described with minor modifications (Porsolt et al. 1977). The animal was placed in a cylinder of 25 cm height and 10 cm diameter, filled with water (25 ± 1 °C) up to 20 cm for a total time period of 6 min. Initial 1 min was considered as acclimatization period and the last 5-min period was taken into account to evaluate the depressive-like behavior (Jangra et al. 2015a). Animal was considered as being immobile if it either remained static or made only little movements required to keep their head above the water level.

Tail Suspension Test (TST)

TST was performed in both acoustically and visually isolated place on the 29th day. Mice were hanged at 40 cm above the floor by adhesive tape on tip of the tail in a dark

room. The immobility time of each mouse was observed for 6 min, and the immobility time during the last 5 min was also observed, analyzed, and presented (Sriram et al. 2015).

Sucrose Preference Test (SPT)

Before the initiation of CRS, all mice were given 2 % sucrose solution and drinking water to find out the baseline of sucrose preference. After 28 days of CRS, mice were given free choice between two bottles of either 2 % sucrose solution and drinking water for 8 h. At the beginning and end of the experiment, bottles were weighed and sucrose preference was calculated using the following formula: Sucrose preference (%) = sucrose intake/(sucrose intake + water intake) $\times$ 100. No mice showed <65 % sucrose preference before the initiation of the CRS. Therefore, mice which have sucrose preference <65 % were considered as anhedonic (Cline et al. 2015).

Biochemical Analysis

Malondialdehyde (MDA) Level

MDA content was determined quantitatively, according to the method of Ohkawa et al. (1979). In brief, 100 µl of sample was added to 100 µl of 8.1 % sodium dodecyl sulfate, 0.75 ml of 20 % acetic acid solution (pH 3.4), and 750 µl of 0.8 % thiobarbituric acid. The mixture was made up to 3 ml with distilled water. The resultant mixture was then heated on a water bath at 95 °C for 60 min, cooled, and then centrifuged at 10,000 rpm for 10 min. Supernatant was collected and the absorbance was taken at 532 nm. Results were expressed as µmole MDA/g of tissue weight (Jangra et al. 2015a).

Reduced Glutathione (GSH) Level

The reduced glutathione content was estimated using the method of Beutler et al. (1963). The supernatant of the homogenate was mixed with trichloroacetic acid (10 % w/v) in 1:1 ratio and centrifuged at $1000 \times g$ for 10 min at 4 °C. The supernatant obtained (500 µl) was mixed with 2 ml of 0.3 M disodium hydrogen phosphate. Thereafter, 250 µl of 0.001 M freshly prepared DTNB [5, 5′-dithiobis (2-nitro benzoic acid) dissolved in 1 % w/v sodium citrate] was added. The absorbance was taken at 412 nm and the results were calculated as µg/g of tissue weight.

Nitrite Level

Nitrite content was determined using Griess reagent commercially obtained from Sigma-Aldrich Corp., St Louis, USA. 100 µl of the supernatant was mixed with 100 µl of

the Griess reagent and kept at room temperature for 15 min. Thereafter, absorbance was taken at 560 nm on a microplate reader and the results were expressed as μmole/mg of protein. Protein level was estimated by the method of Lowry et al. (1951).

Acetylcholinesterase (AChE) Level

Acetylcholinesterase (AChE) assay was performed using the method of Ellman et al. (1961). 400 μl of sample was mixed with 2.6 ml phosphate buffer (0.1 M, pH 8) and 100 μl of DTNB. The contents were mixed in the cuvette thoroughly by bubbling. Then, 20 μl of acetylthiocholine was added and change in absorbance was recorded for a period of 5 min at the intervals of 1 min at 412 nm. The AChE enzyme activity was calculated using the formula: $R = A \times 10^6/13600$, where $R =$ micro moles of substrate hydrolyzed/min and $A =$ change in absorbance/min. The AChE activity was expressed as μmole/min/mg of protein.

Proinflammatory Cytokines, Brain-Derived Neurotrophic Factor (BDNF) and Corticosterone Estimation

IL-1β and TNF-α levels were determined according to manufacturer's protocol available with the respective kits. Concentration of IL-1β and TNF-α was expressed as picogram per milliliter (Pg/ml). Quantitative determination of BDNF level was performed using ELISA kit purchased from Promega, Madison, WI, USA (BDNF assay kit). Concentration of BDNF was expressed as nanogram per milligram of protein (ng/mg of protein). Corticosterone (CORT) was determined in the serum by corticosterone assay ELISA kit (Abnova Corporation, Taiwan). Corticosterone concentration was measured according to the manufacturer's protocol and expressed as nanogram per milliliter (ng/ml).

Determination of GRP78 and CHOP mRNA Level by Real-Time PCR

Total RNA was extracted from freshly isolated HC and PFC using HiPuraA™ Total RNA Miniprep purification kit (HiMedia, India). 1 μg of RNA was diluted in 20 μl of retrotranscription reagent and used to synthesize cDNA with the RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific, India). To quantitatively determine GRP78 and CHOP gene expression, real-time PCR analysis was performed using QuantiTect SYBR Green PCR 9 Master Mix and the ABI 7300 Real-Time PCR System (Applied Biosystems, USA) according to the manufacturer's instructions. ER stress-related transcripts were quantified using real-time PCR. Primers used for amplification were GRP78: forward 5′-GTTTGCTGAGGA AGACAAAAAGCTC-3′ and reverse 5′-CACTTCCATAG AGTTTGCTGATAATTG-3′; CHOP: forward 5′GGAGC TGGAAGCCTGGTATGAGG-3′ and reverse 5′-TCCCTG GTCAGGCGCTCGATTTCC-3′; and β-actin: forward 5′-T CCTCCCTGGAGAAGAGCTAC-3′ and reverse 5′-TCC TGCTTGCTGATCCACAT-3′ (Kudo et al. 2008; Sriram et al. 2015). The relative gene expressions of GRP78 and CHOP were determined by the $2^{-\Delta\Delta C_T}$ method for real-time quantitative PCR.

Statistics

Data were analyzed with Jandel Sigma Stat Version 3.5 software. The data were analyzed using one-way analysis of variance (ANOVA) for multiple comparisons. If the ANOVA showed statistically significant difference, post hoc analysis was performed with Tukey's test. A p value < 0.05 was considered statistically significant.
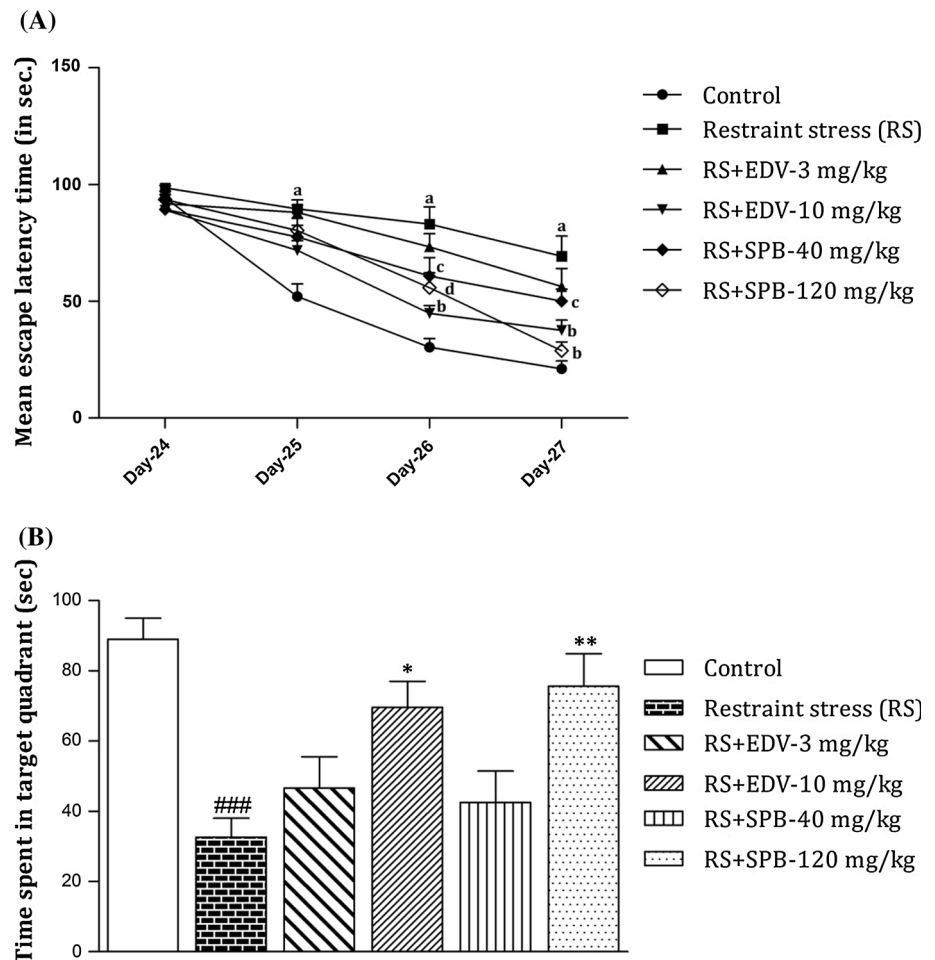
Results

Effect of SPB and Edaravone Treatment on CRS-Induced Learning and Memory Impairment

In order to investigate the learning–memory ability of CRS-exposed mice, MWM test performed. A repeated-measures two-way ANOVA revealed that CRS exposure caused significant ($P < 0.001$) learning–memory impairment during the MWM test (Fig. 2a, b). The escape latency of CRS group was significantly ($P < 0.001$) higher as compared to normal control group during training phase. There was no significant difference for the escape latency between CRS group and normal control group mice on the first day of MWM test. However, escape latencies of CRS group mice were significantly longer than that of the normal control group from the second day to the fourth day of the test. MWM test results indicated that CRS exposure leads to learning deficits in mice. Edaravone-10 mg/kg treatment group showed significantly shorter escape latencies ($P < 0.001$) as compared to CRS group on the third and fourth days. On the other hand, edaravone-3 mg/kg did not show any significant improvement in escape latencies as compared to CRS group mice. However, low dose of SPB (40 mg/kg) significantly ($P < 0.05$) shortened escape latencies as compared to CRS group. Similarly, SPB-120 mg/kg treatment also significantly shortened the escape latencies on the third ($P < 0.01$) and fourth ($P < 0.001$) days as compared to CRS group mice (Fig. 2a). Therefore, it can be concluded that edaravone-10 mg/kg and SPB-120 mg/kg elicit statistically significant improvement in CRS-induced learning deficits.

To evaluate the retention memory in different experimental groups, platform was removed on the 28th day. We

found that CRS group significantly failed to recall the position of the platform, thus spending significantly less time on the former location of the platform as compared to normal control group (Fig. 2b). However, edaravone-10 mg/kg ($P < 0.05$)- and SPB-120 mg/kg ($P < 0.01$)-treated groups focused their search on the former location of the platform as compared to CRS group mice, which clearly indicates improvement in cognitive performance. Edaravone-3 mg/kg and SPB-40 mg/kg did not show any significant improvement in memory retention (Fig. 2b).

Effect of SPB and Edaravone Treatment on CRS-Induced Anxiety- and Depressive-Like Behavior

We investigated the effect of CRS exposure and drug treatments on anxiety- and depressive-like behavior in mice. EPM and light–dark box tests were performed for anxiety-like behavior assessment, and FST and TST were carried out to assess the depressive-like behavior. As shown in Fig. 3a, the number of entries and time spent in open arms of CRS group mice were significantly ($P < 0.001$ and $P < 0.01$, respectively) reduced as

compared to normal control group mice that indicates anxiety behavior in CRS-exposed mice. Treatment with edaravone-10 mg/kg and SPB-120 mg/kg caused alleviation of CRS-induced anxiety behavior as indicated by more number of entries ($P < 0.01$ and $P < 0.01$, respectively) and time spent in open arms ($P < 0.05$ and $P < 0.01$, respectively) (Fig. 3b).

To verify the CRS-induced anxiety behavior, we examined mice using the light–dark box test. CRS-exposed mice showed anxiety behavior as indicated by significant reduction in the number of light–dark transitions ($P < 0.001$) and time spent in the light compartment ($P < 0.001$) as compared to the normal control group (Fig. 4a, b). However, the number of light–dark transitions was increased after treatment with edaravone-10 mg/kg ($P < 0.01$) and SPB-120 mg/kg ($P < 0.05$). Consistently, the time spent in light compartment was also markedly increased in edaravone-10 mg/kg ($P < 0.05$) and SPB-120 mg/kg ($P < 0.01$) treatment groups compared to that of CRS group. Therefore, it can be concluded by the results of EPM and light–dark box tests that edaravone and SPB prevent the anxiety behavior in CRS-exposed mice.

Fig. 3 Effects of edaravone (EDV) and sodium phenylbutyrate (SPB) on the restraint stress (RS)-induced anxiety-like behavior in elevated plus maze (EPM) test. **a** Number of entries in open arm. **b** Time spent in open arm. Data are represented as mean ± SEM ($n = 8$). $^{##}P < 0.01$, $^{###}P < 0.001$ compared with control group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with RS group
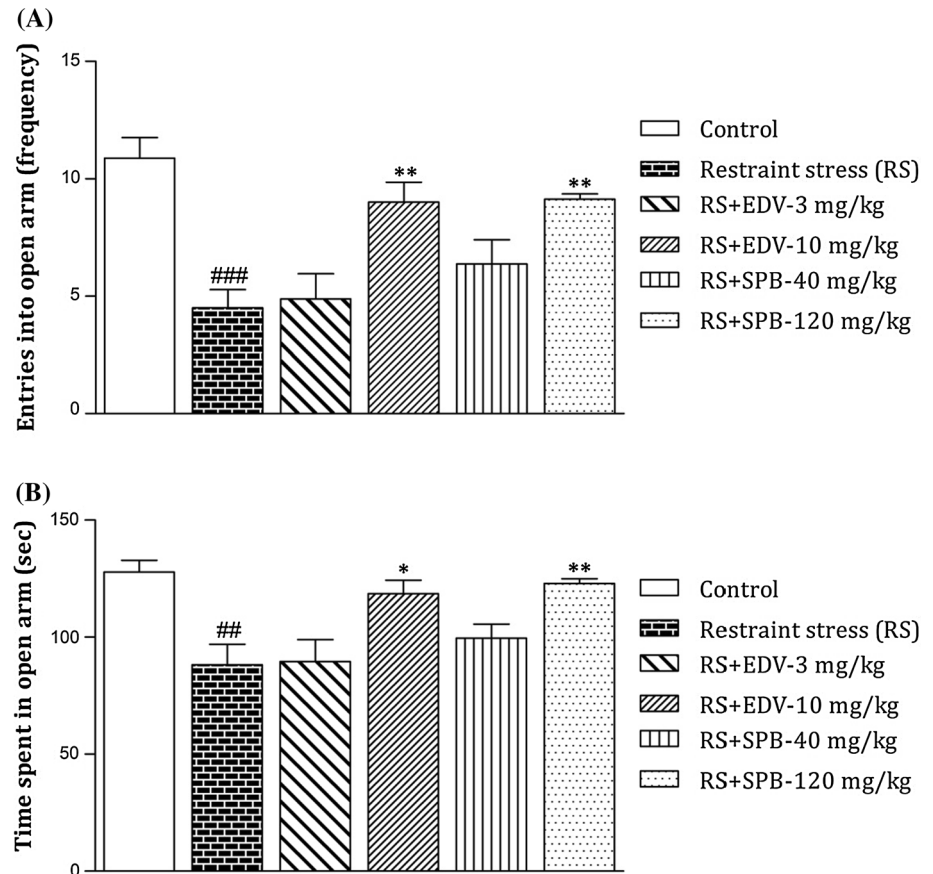
To assess the depressive-like behavior in CRS group and drug treatment groups, we performed FST and TST. Figure 5a, b depicts that immobility time in FST and TST was significantly ($P < 0.001$) increased in CRS group, indicating depressive-like behavior caused by CRS. Edaravone-10 mg/kg treatment group mice showed marked reduction in the immobility time in FST ($P < 0.01$) and TST ($P < 0.01$) as compared to CRS group mice. Moreover, we found that SPB-120 mg/kg treatment significantly reduced the immobility time in FST ($P < 0.001$) and TST ($P < 0.001$) compared to CRS group. So, FST and TST results revealed the anti-depressant action of edaravone and SPB against CRS exposure.

Furthermore, we performed sucrose preference test to assess the anhedonic response after CRS exposure. Prior to CRS exposure, we found that no animal showed sucrose preference <65 %. Thereafter, animals were randomly divided into the different experimental groups. On the 28th day, no normal control animal exhibited sucrose preference below 65 %. However, CRS-exposed group showed a significant reduction ($P < 0.01$) in the sucrose preference as compared to normal control group, indicating anhedonic behavior caused by CRS exposure (Fig. 5c). Both edaravone-10 mg/kg and SPB-120 mg/kg treatment

significantly alleviated CRS-induced anhedonic behavior as indicated by higher sucrose preference by edaravone-10 mg/kg ($P < 0.05$) and SPB-120 mg/kg ($P < 0.05$) treatment groups as compared to CRS-exposed group.

Effect of SPB and Edaravone Treatment on CRS-Induced Oxido-Nitrosative Stress in Hippocampal (HC) and PFC Regions

Different biochemical tests were performed to assess CRS-induced neuronal damage. CRS-exposed animals showed a significant increase in oxidative damage as evident by increased MDA (HC: $P < 0.001$, PFC: $P < 0.01$), nitrite level (HC: $P < 0.001$, PFC: $P < 0.01$), and depleted reduced glutathione level (HC: $P < 0.001$, PFC: $P < 0.05$) in HC and PFC regions as compared to normal control group (Table 1). We found that edaravone and SPB treatment caused profound protection against CRS-evoked oxidative stress. Edaravone-10 mg/kg treatment significantly reduced the MDA level in HC ($P < 0.01$) and PFC ($P < 0.05$) regions, whereas edaravone-3 mg/kg treatment group showed a marked reduction in the MDA level only in the HC region ($P < 0.05$). Moreover, edaravone-10 mg/kg treatment caused a significant increase in the level of

Fig. 4 Effects of edaravone (EDV) and sodium phenylbutyrate (SPB) on the restraint stress (RS)-induced anxiety-like behavior in light–dark box test. **a** Number of light–dark transitions. **b** Time spent in light compartment. Data are represented as mean ± SEM ($n = 8$). $^{###}P < 0.001$ compared with control group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with RS group
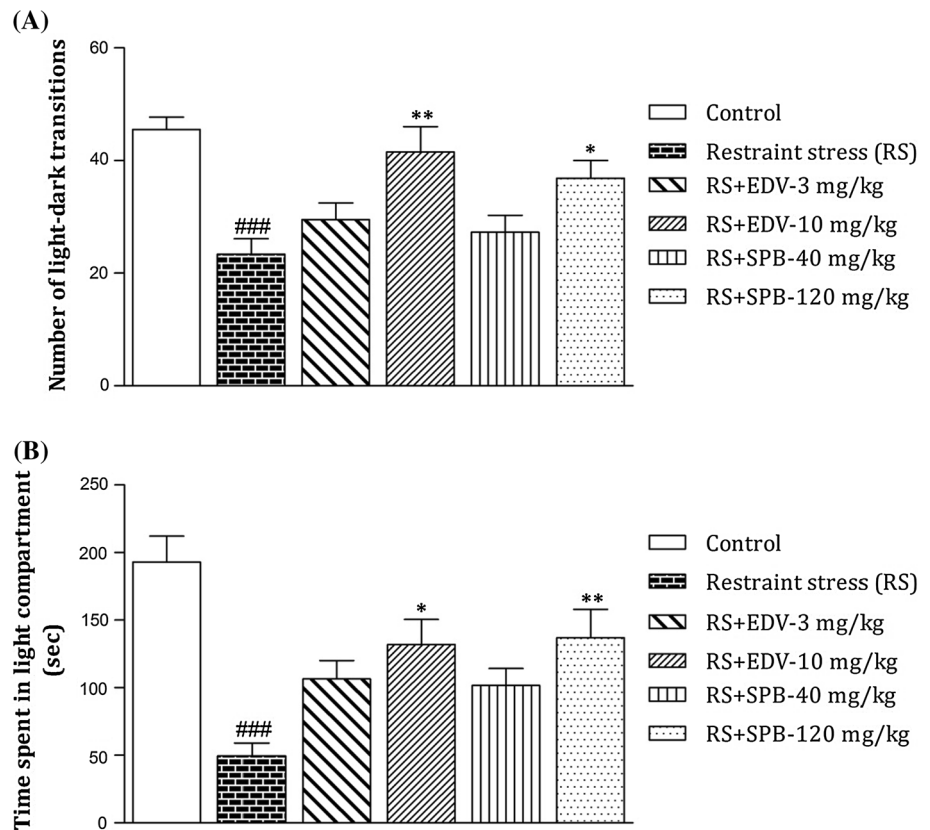
reduced glutathione level in HC ($P < 0.01$) and PFC ($P < 0.05$) regions compared to CRS group. Nitrite content was significantly reduced by the treatment of edaravone-10 mg/kg in HC ($P < 0.01$) and PFC ($P < 0.05$) regions. SPB-120 mg/kg significantly reduced the MDA level ($P < 0.001$) and increased the reduced glutathione level ($P < 0.001$) only in the HC region. SPB treatment failed to affect the nitrite level both in HC and PFC regions as compared to CRS group.

Effect of SPB and Edaravone Treatment on CRS-Induced Changes in Acetylcholinesterase (AChE) Level in HC and PFC Regions

Cholinergic dysfunction was assessed by AChE activity in the HC and PFC regions. A variable effect of CRS on brain AChE activity was observed in different brain regions. CRS exposure for 28 days caused a marked elevation of AChE enzyme activity only in the HC region ($P < 0.001$) of CRS group mice as compared to normal control group mice (Table 1). In the PFC region, AChE level was found to be unaltered in the CRS group animals when compared to the normal control group. However, edaravone-10 mg/kg treatment ($P < 0.001$) prevented the elevation of AChE level in the HC region as compared to CRS group. Similarly, AChE level in the HC region was found to be

significantly ($P < 0.05$) lower by the treatment of SPB-120 mg/kg compared to CRS group. Edaravone-3 mg/kg and SPB-40 mg/kg failed to alter the AChE level both in HC and PFC regions as compared to CRS group.

Effect of SPB and Edaravone Treatment on CRS-Induced Changes in Proinflammatory Cytokine and BDNF Levels in HC and PFC Regions

IL-1β and TNF-α are important inflammatory mediators which play a major role in neuroinflammation. We found a clear elevation of IL-1β level in HC ($P < 0.001$) and PFC ($P < 0.001$) regions that was significantly reduced by edaravone-10 mg/kg (HC: $P < 0.001$, PFC: $P < 0.05$) and SPB-120 mg/kg (HC: $P < 0.01$, PFC: $P < 0.01$) treatment (Table 2). However, edaravone-3 mg/kg and SPB-40 mg/kg failed to show any significant effect on IL-1β both in HC and PFC regions as compared to CRS group. Similarly, the negative impact of CRS exposure on TNF-α level was observed in the HC region. A significantly higher level of TNF-α level was found only in the HC region ($P < 0.001$) of CRS-exposed mice as compared to normal control group. Treatment with edaravone-10 mg/kg and SPB-120 mg/kg significantly reduced the TNF-α level ($P < 0.01$) in the HC region which indicates the anti-inflammatory action of edaravone and SPB. To determine

Fig. 5 Effects of edaravone (EDV) and sodium phenylbutyrate (SPB) on the restraint stress (RS)-induced depressive-like behavior in **a** forced swimming test (FST), **b** tail suspension test (TST), and **c** sucrose preference test. Data are represented as mean $\pm$ SEM ($n = 8$). $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with control group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to RS group
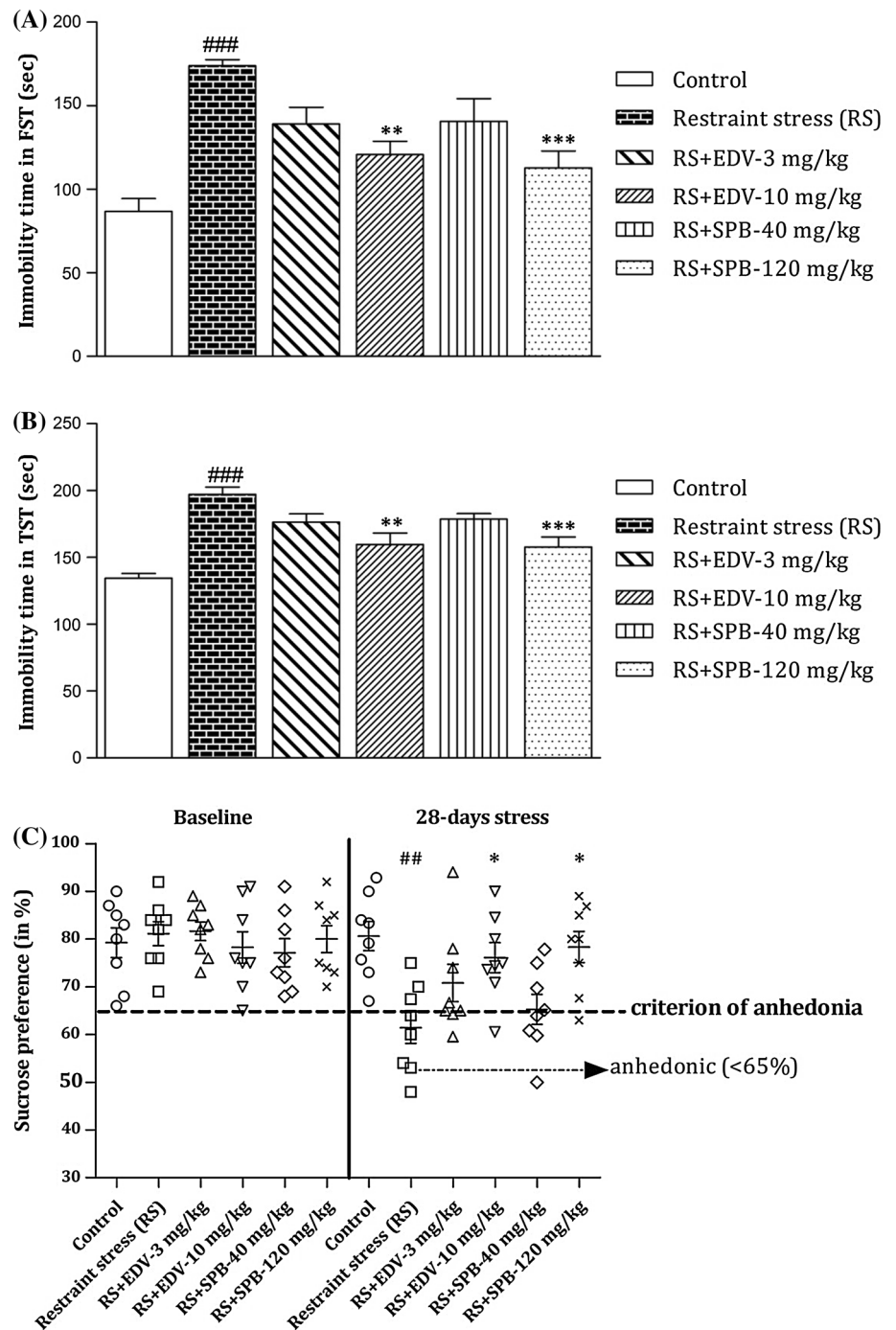
whether BDNF level could correlate with the neuroprotection offered by edaravone and SPB treatment, we examined BDNF level in HC and PFC regions. We found a marked reduction of BDNF level both in HC ($P < 0.001$) and PFC ($P < 0.05$) regions after CRS exposure as compared to normal control group. However, edaravone-10 mg/kg ($P < 0.01$) and SPB-120 mg/kg ($P < 0.05$) treatment significantly increased the BDNF in the HC region as compared to CRS group. SPB-120 mg/kg treatment caused a significant increase in the BDNF level in the PFC region ($P < 0.05$), whereas both the doses of edaravone failed to increase the CRS-induced BDNF depletion in the PFC region. Therefore, it can be concluded that protection against CRS-induced behavioral anomalies offered by edaravone and SPB may be partly due to amelioration of neuroinflammation and BDNF depletion.

Table 1 Effects of restraint stress (RS) and treatment with edaravone (EDV) and sodium phenylbutyrate (SPB) on oxido-nitrosative stress and acetylcholinesterase (AChE) level in HC and PFC regions

Sl no.	Biochemical parameters		Control	Restraint stress (RS)	RS+EDV (3 mg/kg)	RS+EDV (10 mg/kg)	RS+SPB (40 mg/kg)	RS+SPB (120 mg/kg)
1.	MDA Level (μM/g of tissue wt)	HC	211.75 ± 23.28	563 ± 27.68[a]	437.50 ± 29.91[f]	328.12 ± 30.38[e]	471.32 ± 34.82	289.39 ± 25.82[d]
		PFC	161.75 ± 23.93	330.37 ± 35.05[b]	294.62 ± 41.29	198.50 ± 30.72[f]	300 ± 22.79	233.47 ± 12.39
2.	Reduced glutathione (μg/g of tissue)	HC	2694.37 ± 169.65	1271 ± 141.11[a]	1700.62 ± 163.85	2166.87 ± 122.79[e]	1911.37 ± 200.38	2319 ± 192.38[d]
		PFC	2625.59 ± 209.87	1644.87 ± 118.94[c]	1843.12 ± 197.68	2313 ± 165.37[f]	1927.59 ± 232.39	2223.38 ± 128.78
3.	Nitrite level (μM/mg of protein)	HC	2.38 ± 0.28	4.45 ± 0.20[a]	3.48 ± 0.20	2.87 ± 0.21[e]	3.9 ± 0.18	3.5 ± 0.12
		PFC	1.93 ± 0.34	3.78 ± 0.33[b]	3.26 ± 0.18	2.34 ± 0.39[f]	3.18 ± 0.47	2.5 ± 0.23
4.	Acetylcholinesterase activity (μM/min/mg of protein)	HC	0.163 ± 0.0059	0.292 ± 0.012[a]	0.230 ± 0.092	0.188 ± 0.020[d]	0.260 ± 0.017	0.209 ± 0.230[f]
		PFC	0.115 ± 0.0093	0.126 ± 0.029	0.141 ± 0.0088	0.122 ± 0.015	0.152 ± 0.011	0.135 ± 0.013

Data are represented as mean ± SEM ($n = 6$)

[a] $P < 0.001$ compared with control group

[b] $P < 0.01$ compared with control group

[c] $P < 0.05$ compared with control group

[d] $P < 0.001$ compared with restraint stress (RS) group

[e] $P < 0.01$ compared with restraint stress (RS) group

[f] $P < 0.05$ compared with restraint stress (RS) group

Table 2 Effects of restraint stress (RS) and treatment with edaravone (EDV) and sodium phenylbutyrate (SPB) on proinflammatory cytokines and BDNF level in HC and PFC regions and corticosterone level in the serum

Sl no.	Parameters		Control	Restraint stress (RS)	RS+EDV (3 mg/kg)	RS+EDV (10 mg/kg)	RS+SPB (40 mg/kg)	RS+SPB (120 mg/kg)
1.	IL-1β level (pg/ml)	HC	5.28 ± 0.84	14.28 ± 1.01[a]	11.32 ± 0.34	8.65 ± 0.83[d]	12.68 ± 0.68	10.40 ± 0.54[e]
		PFC	4.39 ± 0.43	9.27 ± 0.98[a]	7.12 ± 0.34	6.14 ± 0.93[f]	7.23 ± 0.76	5.37 ± 0.54[e]
2.	TNF-α level (pg/ml)	HC	25.28 ± 1.84	44.51 ± 1.01[a]	40.38 ± 3.13	31.29 ± 3.50[e]	35.32 ± 1.34	30.55 ± 1.59[e]
		PFC	32.32 ± 1.29	39.60 ± 2.21	37.80 ± 1.23	35.23 ± 2.92	36.87 ± 2.38	32.30 ± 1.20
3.	BDNF (ng/mg of protein)	HC	7.38 ± 0.29	4.19 ± 0.32[a]	5.39 ± 0.58	6.42 ± 0.32[e]	4.98 ± 0.12	6.03 ± 0.43[f]
		PFC	5.39 ± 0.43	3.82 ± 0.28[c]	3.71 ± 0.46	5.30 ± 0.27	3.99 ± 0.42	4.78 ± 0.57[f]
4.	Corticosterone level (ng/ml)	Serum	44.38 ± 4.75	139.63 ± 14.7[a]	116.88 ± 10.28	96 ± 3.18[f]	129.23 ± 7.54	118.30 ± 8.67

Data are represented as mean ± SEM ($n = 6$)

[a] $P < 0.001$ compared with control group

[b] $P < 0.01$ compared with control group

[c] $P < 0.05$ compared with control group

[d] $P < 0.001$ compared with restraint stress (RS) group

[e] $P < 0.01$ compared with restraint stress (RS) group

[f] $P < 0.05$ compared with restraint stress (RS) group
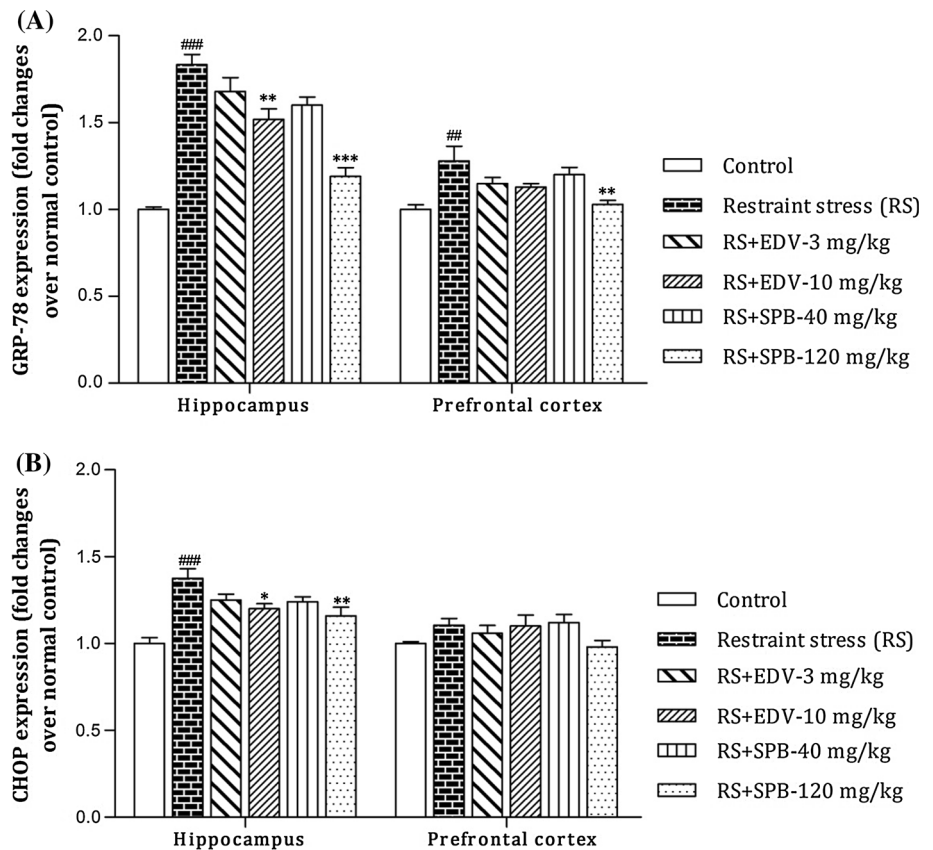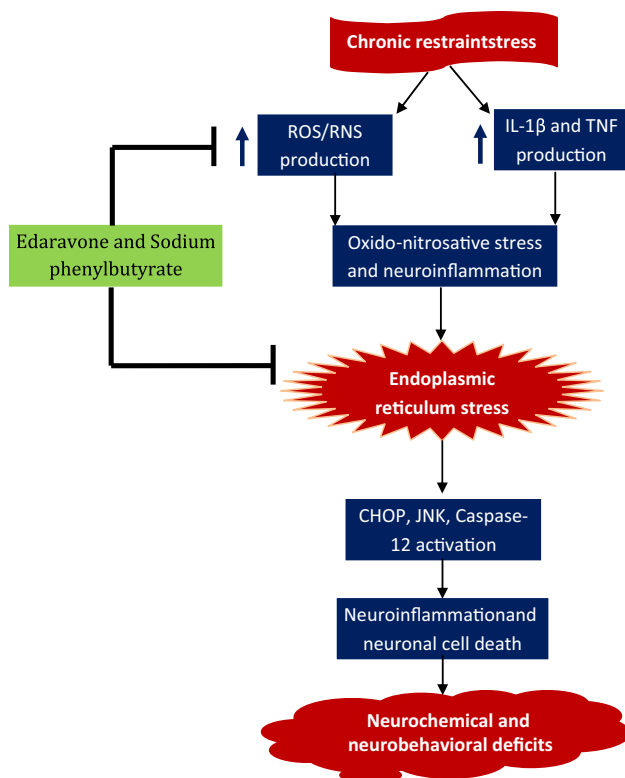

Fig. 6 Effects of restraint stress and treatment with edaravone (EDV) and sodium phenylbutyrate (SPB) on mRNA expression levels of **a** GRP78 and **b** CHOP in HC and PFC regions. Data are represented as mean ± SEM ($n = 8$). $^{##}P < 0.01$, $^{###}P < 0.001$ compared with control group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with RS group

Fig. 7 Role of edaravone and sodium phenylbutyrate in neuroprotection against behavioral deficits induced by restraint stress (RS). RS-induced ROS/RNS generation and proinflammatory cytokines lead to endoplasmic reticulum (ER) stress. Activation of CHOP leads to neuronal apoptosis which ultimately caused neurobehavioral anomalies. Edaravone and sodium phenylbutyrate acts on oxidative stress, proinflammatory cytokines, and ER stress, thereby ameliorating RS-induced behavioral deficits

Effect of SPB and Edaravone Treatment on CRS-Induced Changes in Serum Corticosterone Level

Serum corticosterone level plays an important role in CRS-evoked detrimental outcomes. Therefore, to verify the effectiveness of edaravone and SPB, we determined the serum corticosterone level. We found a clear elevation of corticosterone level ($P < 0.001$) after 28 days of CRS exposure. Edaravone-10 mg/kg treatment significantly reduced ($P < 0.05$) the serum corticosterone level, whereas edaravone-3 mg/kg and both doses of SPB did not alter the serum corticosterone level as compared to CRS-exposed group (Table 2). So, SPB exerted its neuroprotective action without affecting the serum corticosterone level.

Effect of SPB and Edaravone Treatment on CRS-Induced Changes in GRP78 and CHOP Gene Expression Level

RT-PCR analysis revealed that CRS exposure significantly up-regulated the GRP78 mRNA expression level both in

HC ($P < 0.001$) and PFC ($P < 0.01$) regions as compared to normal control group (Fig. 6a). Nevertheless, treatment with edaravone-10 mg/kg ($P < 0.01$) and SPB-120 mg/kg ($P < 0.001$) significantly reduced the CRS-induced GRP78 mRNA expression level in the HC. In the PFC region, only SPB-120 mg/kg treatment group showed a significant reduction ($P < 0.01$) in the level of GRP78 mRNA expression as compared to CRS group. CHOP mRNA expression level was found to be up-regulated ($P < 0.001$) only in the HC region of CRS-exposed group mice (Fig. 6b). In the PFC region, CHOP mRNA expression level was not altered significantly as compared to normal control group. CHOP mRNA expression level was significantly reduced by treatment with edaravone-10 mg/kg ($P < 0.05$) and SPB-120 mg/kg ($P < 0.01$) in the HC region as compared to CRS-exposed group. Our results clearly indicated that CRS exposure elicits the ER stress particularly in the HC region which was significantly prevented by edaravone and SPB treatment.

Discussion

The present study revealed that oxido-nitrosative stress, neuroinflammation, and ER stress play a key role in CRS-induced hippocampal damage. Moreover, we demonstrate that edaravone and SPB protect against oxidative stress, neuroinflammation, and ER stress cascade-mediated behavioral deficits in CRS-exposed mice as depicted in Fig. 7. This study further supports the role of oxido-nitrosative stress, neuroinflammation, and ER stress in stress-induced behavioral deficits (Kumari et al. 2007; Ishisaka et al. 2011; Huang et al. 2013; Tan et al. 2015). We found that the neuroprotective role of edaravone and SPB involves augmentation of reduced glutathione level, BDNF content as well as a reduction of proinflammatory cytokines, AChE level, and ER stress particularly in the HC region. Thus, the current results provide a substantial support to those previously observed reports of neuroprotection by targeting oxidative–nitrosative stress, neuroinflammation, and ER stress cascade (Sokka et al. 2007; Srinivasan and Sharma 2011, 2012; Jangra et al. 2015a; Kasbe et al. 2015).

In rodents, sucrose preference test is commonly used to assess the anhedonic response. In the present study, CRS-exposed mice exhibited a reduction in sucrose preference and an increase in the immobility time in both FST and TST as compared to normal control mice. Moreover, CRS exposure induced anxiety-like behavior as evident from the results of EPM and light–dark box tests. Our results demonstrated that mice subjected to 28 days of CRS exposure induce anhedonia and anxiety- and depressive-like behavior. Chronic restraint stress paradigm induces a

number of neurobehavioral and neurochemical anomalies. Therefore, 28 days of CRS model may be an appropriate tool to explore novel mechanisms that could be involved in the pathophysiology of anxiety and depression and useful to screen the drugs for the treatment of anxiety and depression.

Endoplasmic reticulum is an organelle which plays a key role in the protein folding. Various destructive stimuli and pathological conditions such as hypoglycemia, obesity, stress, calcium depletion, inflammation, oxidative stress, and hypoxia may impair the ER function and consequently leads to the induction of a self-protecting signaling pathway known as UPR (Schönthal 2012). Activation of UPR pathway causes transcriptional induction of ER chaperons such as GRP78 and endoplasmic reticulum-associated protein degradation (ERAD) components, and attenuates the translational process. However, in case of severe damage, UPR signaling evokes the induction of CHOP and caspase-12 activation that eventually lead to apoptotic cell death (Tabas and Ron 2011). ER stress has been implicated in several inflammatory diseases such as cancer, diabetes, arthritis, atherosclerosis, neurodegenerative diseases, and irritable bowel syndrome (Chaudhari et al. 2014). Increasing evidences from the previous experimental studies suggested the role of ER stress in the pathophysiology of depression, bipolar disorder, and stress-induced cognitive dysfunction (Hayashi et al. 2009; Zhao et al. 2013; Zhang et al. 2014; Huang et al. 2015a; Tan et al. 2015). Moreover, oxidative stress and neuroinflammation also play a major role in the pathophysiology of anxiety, depression, and cognitive dysfunction (García-Bueno et al. 2008; Sulakhiya et al. 2014; Jangra et al. 2015b). Therefore, we hypothesized that oxido-nitrosative stress, neuroinflammation, and ER stress cascade could be responsible for the development of CRS-evoked cognitive deficits and anxiety- and depressive-like behavior. Furthermore, we investigated the neuroprotective role of SPB and edaravone against CRS-evoked neurobehavioral and neurochemical changes. To evaluate the ER stress after CRS exposure, gene expression level of ER stress markers such as GRP78 and CHOP was measured in HC and PFC regions. GRP78 is a heat shock protein family chaperone transcriptional factor which plays a key role in the regulation of ER functioning and UPR initiation (Wang et al. 2009). CHOP is a transcriptional factor that is activated in the ER stress condition and mediates apoptotic pathway (Nishitoh 2012). In the present study, we found that CRS exposure leads to the up-regulation of GRP78 and CHOP expressions in the HC region of mice, indicating the presence of ER stress. In the PFC region, CRS exposure up-regulated only GRP78 expression level and the CHOP expression level remained unaltered, which indicates the induction of UPR. Our results are corroborated with the

previous studies which reported the involvement of ER stress in restraint stress model (Pavlovsky et al. 2013; Zhang et al. 2014; Huang et al. 2015a). Moreover, ER stress-related protein levels were also up-regulated in corticosterone-induced depression model, chronic unpredictable mild stress model, and chronic social defeat stress model (Ishisaka et al. 2011; Huang et al. 2013; Tan et al. 2015). The present and all previous experimental findings suggested a potential relationship between stress-induced behavioral anomalies and ER stress. We found that treatment with edaravone and SPB significantly reduced the CRS-induced up-regulation of GRP78 level in HC and PFC regions. In addition, CRS-induced elevated CHOP level was significantly reduced by edaravone and SPB treatment. Previous studies suggested that under ER stress, elevated level of GRP78 leaked into the cytoplasmic region where it directly interacts with NF-κB–IκB complex which triggers NF-κB activation and therefore leads to induction of proinflammatory cytokines (Shkoda et al. 2007). ER stress and ER stress-evoked inflammation form a vicious cycle which ultimately leads to neuronal cell death through apoptosis. In the current study, we showed that edaravone and SPB treatment significantly reduced ER stress proteins and the resulted neuroinflammation in HC and PFC regions.

In our study, oxidative stress parameter evaluation revealed that CRS exposure leads to an increase in the amount of MDA and nitrite levels and a reduction in glutathione level in HC and PFC regions which is accompanied with cognitive dysfunction and anxiety- and depressive-like behavior. Our results are in concordance with the previous experimental studies which suggested the role of oxidative damage in restraint stress-induced behavioral anomalies (Liu et al. Liu et al. 2013b; Pesarico et al. 2015). Edaravone is a free radical scavenger which has previously shown its ameliorating effect against traumatic brain injury and focal cerebral ischemia-induced behavioral deficits through inhibition of oxidative stress (Lu et al. 2012; Ohta et al. 2013). In addition, previous studies demonstrated that edaravone acts as a potent anti-inflammatory agent (Zhang et al. 2005; Yuan et al. 2008). In the present study, our results demonstrated that edaravone treatment significantly ameliorates CRS-evoked oxidative modifications and neuroinflammation in HC and PFC regions. Earlier studies demonstrated the protective potential of SPB against cognitive deficits in different animal models (Ricobaraza et al. 2009; Takuma et al. 2014). Moreover, SPB has exhibited an anti-depressant effect in the mouse (Schroeder et al. 2007). The resulted protective effect of SPB is attributed to its antioxidant and anti-inflammatory properties. It has been shown that SPB inhibits NF-κB and glial cell activation which further inhibits the oxidative stress and proinflammatory cytokines

(Roy et al. 2012). We found that SPB treatment significantly inhibited CRS-evoked oxidative stress and proinflammatory cytokines in the HC region only. It has been shown that SPB reduced the nitrite level in activated mouse microglia and human astroglia via inhibition of iNOS expression (Roy et al. 2012). However, in our study SPB treatment failed to reduce the CRS-induced elevated level of nitrite in HC and PFC regions. The reason behind the divergence among previously reported and the present effect of SPB against nitrite level is not known, but it might be due to the difference in the activity of SPB between previously reported in vitro studies and the present in vivo study. Edaravone and SPB treatment significantly abolished CRS-evoked cognitive deficit and anxiety- and depressive-like behavior. Therefore, the present results are supportive of the hypothesis that edaravone and SPB could inhibit the CRS-induced oxidative stress and neuroinflammation.

AChE is an enzyme which ceased the acetylcholine activity through its degradation. Acetylcholine is remarkably involved in regulating long-term potentiation (LTP) in the HC region. Thus, AChE plays an important role in cognitive processes such as learning and memory (Jangra et al. 2014b, 2015b). Previous studies demonstrated that elevated as well as reduced level of AChE enzyme has been implicated in the behavioral anomalies (Jangra et al. 2013; Kasbe et al. 2015. In the current study, CRS exposure increased the AChE activity only in the HC region, which was in concordance with previous studies (Liu et al. 2013a). On the other hand, AChE activity was found unaltered in the PFC region of CRS-exposed group as compared to normal control group. However, treatment with edaravone and SPB significantly reduced the elevated AChE level in the HC region as compared to CRS-exposed group.

CRS exposure activates the hypothalamic–pituitary–adrenal (HPA) axis through which corticosterone level increased. Corticosterone also acts as a neuromodulator that can affect endogenous antioxidant level, ROS, and cytokine production, thereby affecting LTP and memory processes. A study has found that subcutaneous administration of corticosterone induces cognitive deficits in rats through elevation of oxidative stress in the HC region (Sato et al. 2010). Corticosteroid receptors predominantly present in the HC region. Therefore, the high level of corticosterone causes greater activation of glucocorticoids receptor and therefore eventually affects the hippocampal plasticity (Kim and Diamond 2002). In the present study, we found that CRS exposure increased the corticosterone level in the serum, which was consistent with previous studies (Liu et al. 2013a, b). However, edaravone treatment significantly reduced the increased level of corticosterone in the CRS-exposed mice. On the other hand, treatment

with SPB reduced the elevated serum corticosterone level to some extent but the result was found to be statistically non-significant. Therefore, it can be concluded that SPB exerts neuroprotective action without affecting the corticosterone level. BDNF is a neurotrophin that is profoundly present in the HC and cerebral cortex regions, where it plays a prominent role in neurogenesis, neural regeneration, neuroprotection, synaptic transmission, and synaptic plasticity (Schmidt-Kastner et al. 1996). A growing body of evidence indicated the key role of BDNF in pathophysiology of anxiety, depression, and cognitive dysfunction (Jangra et al. 2014a, 2015b; Sulakhiya et al. 2014; Sriram et al. 2016). Several studies suggested that neuroinflammation affects the expression of BDNF level in the brain (Guan and Fang 2006; Calabrese et al. 2014). In the current study, we have observed that CRS exposure reduced the BDNF level in both HC and PFC regions. CRS-evoked BDNF depletion was found to be more severe in the HC region. Moreover, proinflammatory cytokine level and ER stress were found to be more prominent in the HC region as compared to PFC region. It indicates that CRS-evoked neuroinflammation and ER stress-generated neuroinflammation may be responsible for more severe BDNF depletion in the HC region. Postmortem studies in depressed humans showed the reduced BDNF signaling in HC and prefrontal cortex regions as compared to control human group (Autry et al. Autry and Monteggia 2012). Thus, we measured the BDNF level in the PFC region along with the HC region. However, treatment with SPB markedly increased the CRS-induced BDNF depletion in HC and PFC regions. Edaravone treatment significantly increased the CRS-induced reduced BDNF level in the HC region but failed to show any effect in the PFC region. The results are in concordance with the previous studies that demonstrated the neuroprotective potential of edaravone and SPB via elevation of BDNF level in the brain (Schroeder et al. 2007; Corbett et al. 2013; Wang et al. 2013; Okuyama et al. 2015). We found that edaravone and SPB alleviated the neuroinflammation and ER stress particularly in HC region. Therefore, our results strongly suggested that the protective action of edaravone and SPB against CRS-evoked BDNF depletion mediated through mitigation of neuroinflammation and ER stress.

Conclusions

In conclusion, the current study suggested that CRS exposure causes ER stress only in the HC region. Therefore, our results revealed that HC region is more prone to CRS as compared to PFC region. CRS-induced behavioral anomalies are the manifestations of oxido-nitrosative stress, neuroinflammation, ER stress, and BDNF depletion

particularly in the HC region. Moreover, the present study revealed possible mechanisms underlying the protective effect of edaravone and SPB against CRS-induced cognitive dysfunction and anxiety- and depressive-like behavior. Thus, our study suggested that edaravone and SPB could be the neurotherapeutic interventions for the treatment of anxiety, depression, and cognitive dysfunction.

Compliance with Ethical Standards

Conflict of interest None.

References

Autry AE, Monteggia LM (2012) Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol Rev 64(2):238–258

Bannerman DM, Sprengel R, Sanderson DJ, McHugh SB, Rawlins JNP, Monyer H et al (2014) Hippocampal synaptic plasticity, spatial memory and anxiety. Nat Rev Neurosci 15(3):181–192

Bassi GS, Kanashiro A, Santin FM, de Souza GEP, Nobre MJ, Coimbra NC (2012) Lipopolysaccharide-induced sickness behaviour evaluated in different models of anxiety and innate fear in rats. Basic Clin Pharmacol Toxicol 110(4):359–369

Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888

Calabrese F, Rossetti AC, Racagni G, Gass P, Riva MA, Molteni R (2014) Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. Front Cell Neurosci. 8:430

Chaudhari N, Talwar P, Parimisetty A, d'Hellencourt CL, Ravanan P (2014) A molecular web: endoplasmic reticulum stress, inflammation, and oxidative stress. Front Cell Neurosci 8:213

Chiba S, Numakawa T, Ninomiya M, Richards MC, Wakabayashi C, Kunugi H (2012) Chronic restraint stress causes anxiety-and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. Prog Neuropsychopharmacol Biol Psychiatry 39(1):112–119

Cline BH, Anthony DC, Lysko A, Dolgov O, Anokhin K, Schroeter C et al (2015) Lasting downregulation of the lipid peroxidation enzymes in the prefrontal cortex of mice susceptible to stress-induced anhedonia. Behav Brain Res 276:118–129

Corbett GT, Roy A, Pahan K (2013) Sodium phenylbutyrate enhances Astrocytic neurotrophin synthesis via protein kinase C (PKC)-mediated activation of cAMP-response element-binding protein (CREB): implications for Alzheimer disease therapy. J Biol Chem 288(12):8299–8312

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7(2):88–95

García-Bueno B, Caso JR, Leza JC (2008) Stress as a neuroinflammatory condition in brain: damage and protective mechanisms. Neurosci Biobehav Rev 32(6):1136–1151

Gardian G, Browne SE, Choi D-K, Klivenyi P, Gregorio J, Kubilus JK et al (2005) Neuroprotective effects of phenylbutyrate in the N171-82Q transgenic mouse model of Huntington's disease. J Biol Chem 280(1):556–563

Guan Z, Fang J (2006) Peripheral immune activation by lipopolysaccharide decreases neurotrophins in the cortex and hippocampus in rats. Brain Behav Immun 20:64–71

Hayashi A, Kasahara T, Kametani M, Toyota T, Yoshikawa T, Kato T (2009) Aberrant endoplasmic reticulum stress response in lymphoblastoid cells from patients with bipolar disorder. Int J Neuropsychopharmacol 12(1):33–43

Huang GB, Zhao T, Muna SS, Bagalkot TR, Jin HM, Chae HJ et al (2013) Effects of chronic social defeat stress on behaviour, endoplasmic reticulum proteins and choline acetyltransferase in adolescent mice. Int J Neuropsychopharmacol 16(7):1635–1647

Huang P, Li C, Fu T, Zhao D, Yi Z, Lu Q et al (2015a) Flupirtine attenuates chronic restraint stress-induced cognitive deficits and hippocampal apoptosis in male mice. Behav Brain Res 288:1–10

Huang RR, Hu W, Yin YY, Wang YC, Li WP, Li WZ (2015b) Chronic restraint stress promotes learning and memory impairment due to enhanced neuronal endoplasmic reticulum stress in the frontal cortex and hippocampus in male mice. Int J Mol Med 35(2):553–559

Ishisaka M, Kakefuda K, Yamauchi M, Tsuruma K, Shimazawa M, Tsuruta A et al (2011) Luteolin shows an antidepressant-like effect via suppressing endoplasmic reticulum stress. Biol Pharm Bull 34(9):1481–1486

Itoh T, Satou T, Nishida S, Tsubaki M, Imano M, Hashimoto S et al (2010) Edaravone protects against apoptotic neuronal cell death and improves cerebral function after traumatic brain injury in rats. Neurochem Res 35(2):348–355

Jangra A, Datusalia AK, Khandwe S, Sharma SS (2013) Amelioration of diabetes-induced neurobehavioral and neurochemical changes by melatonin and nicotinamide: implication of oxidative stress-"PARP pathway. Pharmacol Biochem Behav 114:43–51

Jangra A, Lukhi MM, Sulakhiya K, Baruah CC, Lahkar M (2014a) Protective effect of mangiferin against lipopolysaccharide-induced depressive and anxiety-like behaviour in mice. Eur J Pharmacol 740:337–345

Jangra A, Datusalia AK, Sharma SS (2014b) Reversal of neurobehavioral and neurochemical alterations in STZ-induced diabetic rats by FeTMPyP, a peroxynitrite decomposition catalyst and 1,5-Isoquinolinediol a poly (ADP-ribose) polymerase inhibitor. Neurol Res 36(7):619–626

Jangra A, Dwivedi S, Sriram CS, Gurjar SS, Kwatra M, Sulakhiya K, Baruah CC, Lahkar M (2015a) Honokiol abrogates chronic restraint stress-induced cognitive impairment and depressive-like behaviour by blocking endoplasmic reticulum stress in the hippocampus of mice. Eur J Pharmacol 770:25–32

Jangra A, Kasbe P, Pandey SN, Dwivedi S, Gurjar SS, Kwatra M et al (2015b) Hesperidin and silibinin ameliorate aluminum-induced neurotoxicity: modulation of antioxidants and inflammatory cytokines level in mice hippocampus. Biol Trace Elem Res 10(7):e0131178

Jiao L, Zhang J, Li Z, Liu H, Chen Y, Xu S (2011) Edaravone alleviates delayed neuronal death and long-dated cognitive dysfunction of hippocampus after transient focal ischemia in Wistar rat brains. Neuroscience 182:177–183

Kasbe P, Jangra A, Lahkar M (2015) Mangiferin ameliorates aluminium chloride-induced cognitive dysfunction via alleviation of hippocampal oxido-nitrosative stress, proinflammatory cytokines and acetylcholinesterase level. J Trace Elem Med Biol 31:107–112

Kikuchi K, Kawahara KI, Uchikado H, Miyagi N, Kuramoto T, Miyagi T et al (2011) Potential of edaravone for neuroprotection in neurologic diseases that do not involve cerebral infarction (Review). Exp Ther Med 2(5):771–775

Kim JJ, Diamond DM (2002) The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 3(6):453–462

Kudo T, Kanemoto S, Hara H, Morimoto N, Morihara T, Kimura R et al (2008) A molecular chaperone inducer protects neurons from ER stress. Cell Death Differ 15(2):364–375

Kumari B, Kumar A, Dhir A (2007) Protective effect of non-selective and selective COX-2-inhibitors in acute immobilization stress-induced behavioral and biochemical alterations. Pharmacol Rep. 59(6):699–707

Liu Y, Gou LS, Tian X, Fu XB, Ling X, Sun LY et al (2013a) Protective effects of luteolin on cognitive impairments induced by psychological stress in mice. Exp Biol Med (Maywood) 238(4):418–425

Liu Y, Zhuang X, Gou L, Ling X, Tian X, Liu L et al (2013b) Protective effects of nizofenone administration on the cognitive impairments induced by chronic restraint stress in mice. Pharmacol Biochem Behav 103(3):474–480

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265–275

Lu F, Nakamura T, Toyoshima T, Liu Y, Hirooka K, Kawai N et al (2012) Edaravone, a free radical scavenger, attenuates behavioral deficits following transient forebrain ischemia by inhibiting oxidative damage in gerbils. Neurosci Lett 506(1):28–32

Maes M (2008) The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Neuro Endocrinol Lett 29(3):287–291

McEwen BS, Magarinos ANA (1997) Stress Effects on Morphology and Function of the Hippocampusa. Ann N Y Acad Sci 821(1):271–284

Nishitoh H (2012) CHOP is a multifunctional transcription factor in the ER stress response. J Biochem 151(3):217–219

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95(2):351–358

Ohta M, Higashi Y, Yawata T, Kitahara M, Nobumoto A, Ishida E et al (2013) Attenuation of axonal injury and oxidative stress by edaravone protects against cognitive impairments after traumatic brain injury. Brain Res 1490:184–192

Okuyama S, Morita M, Sawamoto A, Terugo T, Nakajima M (2015) Furukawa Y. Edaravone enhances brain-derived neurotrophic factor production in the ischemic mouse brain pharmaceuticals (Basel). 8(2):176–185

Oyadomari S, Mori M (2004) Roles of CHOP/GADD153 in endoplasmic reticulum stress. Cell Death Differ 11(4):381–389

Pavlovsky AA, Boehning D, Li D, Zhang Y, Fan X, Green TA (2013) Psychological stress, cocaine and natural reward each induce endoplasmic reticulum stress genes in rat brain. Neuroscience 246:160–169

Pesarico AP, Stangherlin EC, Mantovani AC, Zeni G, Nogueira CW (2015) 7-Fluoro-1, 3-diphenylisoquinoline-1-amine abolishes depressive-like behavior and prefrontal cortical oxidative damage induced by acute restraint stress in mice. Physiol Behav 149:294–302

Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229(2):327–336

Qi X, Hosoi T, Okuma Y, Kaneko M, Nomura Y (2004) Sodium 4-phenylbutyrate protects against cerebral ischemic injury. Mol Pharmacol 66(4):899–908

Radecki DT, Brown LM, Martinez J, Teyler TJ (2005) BDNF protects against stress-induced impairments in spatial learning and memory and LTP. Hippocampus 15(2):246–253

Ricobaraza A, Cuadrado-Tejedor M, Pérez-Mediavilla A, Frechilla D, Del RÃo J, GarcÃa-Osta A (2009) Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's

disease mouse model. Neuropsychopharmacology 34(7):1721–1732

Roy A, Ghosh A, Jana A, Liu X, Brahmachari S, Gendelman HE et al (2012) Sodium phenylbutyrate controls neuroinflammatory and antioxidant activities and protects dopaminergic neurons in mouse models of Parkinson's disease. PLoS One 7(6):e38113

Sato H, Takahashi T, Sumitani K, Takatsu H, Urano S (2010) Glucocorticoid Generates ROS to Induce Oxidative Injury in the Hippocampus, Leading to Impairment of Cognitive Function of Rats. J Clin Biochem Nutr. 47(3):224–232

Schmidt-Kastner R, Wetmore C, Olson L (1996) Comparative study of brain-derived neurotrophic factor messenger RNA and protein at the cellular level suggests multiple roles in hippocampus, striatum and cortex. Neuroscience 74(1):161–183

Schönthal AH (2012) Endoplasmic reticulum stress: its role in disease and novel prospects for therapy. Scientifica 2012:857516

Schroeder FA, Lin CL, Crusio WE, Akbarian S (2007) Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. Biol Psychiatry 62(1):55–64

Shkoda A, Ruiz PA, Daniel H, Kim SC, Rogler G, Sartor RB et al (2007) Interleukin-10 blocked endoplasmic reticulum stress in intestinal epithelial cells: impact on chronic inflammation. Gastroenterology 132(1):190–207

Sokka AL, Putkonen N, Mudo G, Pryazhnikov E, Reijonen S, Khiroug L et al (2007) Endoplasmic reticulum stress inhibition protects against excitotoxic neuronal injury in the rat brain. J Neurosci 27(4):901–908

Srinivasan K, Sharma SS (2011) Sodium phenylbutyrate ameliorates focal cerebral ischemic/reperfusion injury associated with comorbid type 2 diabetes by reducing endoplasmic reticulum stress and DNA fragmentation. Behav Brain Res 225(1):110–116

Srinivasan K, Sharma SS (2012) Edaravone offers neuroprotection in a diabetic stroke model via inhibition of endoplasmic reticulum stress. Basic Clin Pharmacol Toxicol 110(2):133–140

Sriram CS, Jangra A, Gurjar SS, Hussain MI, Borah P, Lahkar M et al (2015) Poly (ADP-ribose) polymerase-1 inhibitor, 3-aminobenzamide pretreatment ameliorates lipopolysaccharide-induced neurobehavioral and neurochemical anomalies in mice. Pharmacol Biochem Behav 133:83–91

Sriram CS, Jangra A, Gurjar SS, Mohan P, Bezbaruah BK (2016) Edaravone abrogates LPS-induced behavioral anomalies, neuroinflammation and PARP-1. Physiol Behav 154:135–144

Sulakhiya K, Kumar P, Jangra A, Dwivedi S, Hazarika NK, Baruah CC et al (2014) Honokiol abrogates lipopolysaccharide-induced depressive like behavior by impeding neuroinflammation and oxido-nitrosative stress in mice. Eur J Pharmacol 744:124–131

Tabas I, Ron D (2011) Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. Nat Cell Biol 13(3): 184–190

Takuma K, Hara Y, Kataoka S, Kawanai T, Maeda Y, Watanabe R et al (2014) Chronic treatment with valproic acid or sodium butyrate attenuates novel object recognition deficits and hippocampal dendritic spine loss in a mouse model of autism. Pharmacol Biochem Behav 126:43–49

Tan H, Zou W, Jiang J, Tian Y, Xiao Z, Bi L et al (2015) Disturbance of hippocampal H_2S generation contributes to CUMS-induced depression-like behavior: involvement in endoplasmic reticulum stress of hippocampus. Acta Biochim Biophys Sin (Shanghai) 2015:gmv009

Tse YC, Montoya I, Wong AS, Mathieu A, Lissemore J, Lagace DC et al (2014) A longitudinal study of stress-induced hippocampal volume changes in mice that are susceptible or resilient to chronic social defeat. Hippocampus 24(9):1120–1128

Vorhees CV, Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 1(2):848–858

Wang M, Wey S, Zhang Y, Ye R, Lee AS (2009) Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. Antioxid Redox Signal 11(9):2307–2316

Wang G, Su J, Li L, Feng J, Shi L, He W et al (2013) Edaravone alleviates hypoxia-acidosis/reoxygenation-induced neuronal injury by activating ERK1/2. Neurosci Lett 543:72–77

Yuan WJ, Yasuhara T, Shingo T, Muraoka K, Agari T, Kameda M et al (2008) Neuroprotective effects of edaravone-administration on 6-OHDA-treated dopaminergic neurons. BMC neurosci 9(1):75

Zhang N, Komine-Kobayashi M, Tanaka R, Liu M, Mizuno Y, Urabe T (2005) Edaravone reduces early accumulation of oxidative products and sequential inflammatory responses after transient focal ischemia in mice brain. Stroke 36(10):2220–2225

Zhang Y, Liu W, Zhou Y, Ma C, Li S, Cong B (2014) Endoplasmic reticulum stress is involved in restraint stress-induced hippocampal apoptosis and cognitive impairments in rats. Physiol Behav 131:41–48

Zhao T, Huang G-B, Muna SS, Bagalkot TR, Jin H-M, Chae H-J et al (2013) Effects of chronic social defeat stress on behavior and choline acetyltransferase, 78-kDa glucose-regulated protein, and CCAAT/enhancer-binding protein (C/EBP) homologous protein in adult mice. Psychopharmacology 228(2):217–230

Zhao Y, Yan Y, Zhao Z, Li S, Yin J (2015) The dynamic changes of endoplasmic reticulum stress pathway markers GRP78 and CHOP in the hippocampus of diabetic mice. Brain Res Bull 111:27–35