Revista Brasileira de Farmacognosia (2020) 30:357–366
https://doi.org/10.1007/s43450-020-00020-w

ORIGINAL ARTICLE

Gallic Acid from *Terminalia Bellirica* Fruit Exerts Antidepressant-like Activity

Chandrasekhar Yadavalli[1] · Phani Kumar Garlapati[1] · Anilakumar Kandangath Raghavan[1]

Received: 19 July 2019 / Accepted: 20 September 2019 / Published online: 21 May 2020

Abstract

Terminalia bellirica (Gaertn.) Roxb., Combretaceae, fruits are used in Indian system of medicine for multiple therapeutic applications. The present study was aimed to investigate the effect of gallic acid extract from *T. bellirica* fruits on chronic mild stress-induced depression-like activity in mice model. The results showed that administration of *T. bellirica* ameliorated chronic mild stress-induced depression-like behavior by increased sucrose preference and decreased immobility time. Further, *T. bellirica* treatment has significantly regulated hyperactivity of hypothalamic-pituitary-adrenal axis by decreasing serum corticosterone and acetylcholinesterase. In addition, *T. bellirica* treatment has significantly modulated monoaminergic system by elevating neurotransmitters and inhibiting monoamino oxidases. *Terminalia bellirica* treatment has effectively antagonized the chronic mild stress-induced oxidative stress and apoptotic cell death as evidenced by mRNA or protein expression studies. Thus, the study concluded that *T. bellirica* produces an antidepressant-like activity by regulating hypothalamic-pituitary-adrenal axis, monoaminergic systems, and apoptotic cell death.

Keywords Chronic mild stress · Neuroprotection · HPA-axis · Apoptosis · Monoamines

Introduction

Depression is one of the most important psychological conditions associated with life-threatening behavioral symptoms (Leonard 2010). It is widely accepted that stressful life events often precede a major role in depressive disorders. Chronic mild stress (CMS) is generally acceptable animal model to test depression-like behavior, which employs a series of stressors to imitate the depressive behavior (Shang et al. 2017). Recent studies have suggested that hyperactivity of HPA (hypothalamic-pituitary-adrenal) axis and glucocorticoids over secretion are the major indicators in depression-like disorders (Wang et al. 2015). An alteration in monoamine oxidases (MAO) is responsible in pathophysiology of neuropsychiatric and neurodegenerative disorders via regulation of neurotransmitters, such as serotonin (5-HT), dopamine (DA), epinephrine, and nor-epinephrine (NE) (Duncan et al. 2012). BDNF (brain-derived neurotrophic factor) plays a major role in the physiology of depression by activating series of pathways such as, PI3K-Akt, MEK/ERK, and PLCγ-Ca^{2+} (Numakawa et al. 2013).

Chronic mild stress (CMS) model is a commonly used rodent model to understand the mechanism of depression. Additionally, this model modulates neurotransmitter levels in brain, corticosterone and corticotropin-releasing factor (CRF), expression of tumor necrosis factor-α, BDNF, CREB etc. (Grippo et al. 2005). Recent drug research had recognized that reversible or competitive inhibition of brain monoamine oxidase (MAO) might be more important in depressive patients. Moreover, MAO inhibitors were proven to be beneficiary antidepressant effects by improving brain neurotransmitter levels and reducing MAO activities (Asnis and Henderson 2014).

In recent years, medicinal plants have been given importance as alternative agents to treat depression-like behavior (Kumar et al. 2014). *Terminalia bellirica* (Gaertn.) Roxb., Combretaceae, is well known in Ayurveda for having medicinal properties like anti-spasmodic, anti-asthmatic, anti-tussive, and expectorant (Trivedi et al. 1979). *Terminalia bellirica* contains several triterpenoids

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s43450-020-00020-w) contains supplementary material, which is available to authorized users.

✉ Phani Kumar Garlapati
phanikumar.g@dfrl.drdo.in

[1] Nutrition, Biochemistry and Toxicology Division, Defence Food Research Laboratory, Siddhartha Nagar, Mysore, Karanataka, India

and polyphenols such as chebulagic acid, ellagic acid, gallic acid, phyllembin, and ethyl gallate (Kadian and Parle 2015). Keeping in view of several mechanisms involved in pathophysiology of depression-like disorders, the present study was undertaken to examine the association of neurotransmitters, monoamine oxidases, and antioxidant enzymes in the antidepressant-like activity of *T. bellirica*. Furthermore, we investigated the ability of *T. bellirica* in modulation/regulation of hyperactivity of HPA-axis, oxidative stress, and stress-induced apoptosis.

Materials and Methods

Plant Material and Preparation of Gallic Acid Rich Extract

Terminalia bellirica (Gaertn.) Roxb., Combretaceae, fruits were obtained from local market of Mysore, India, and was authenticated by the Department of Botany, University of Mysore, Mysore, India (voucher specimen no. AND4680). The shade dried fruits were extracted by using a Soxhlet apparatus with alcohol (70%; v/v) for 72 h and lyophilized. Further, the lyophilized powder was subjected to column chromatography (Sephadex LH-20; equilibrated with 95% ethanol). Fraction I (low molecular weight phenolic compounds) was obtained by using mobile phase ethanol. Fraction II (gallic acid rich fraction) was eluted by 50% (v/v) acetone. Gallic acid rich fraction was lyophilized and used for the study, which contained the major component gallic acid and other components in smaller quantities.

HPLC Conditions

The separation of phenolic compounds was performed by using HPLC (JASCO) with UV-VIS detector. The compounds were separated by using RP-C18 column (waters; 250 mm × 4 mm). The mobile phase used for the study was methanol, water, and formic acid (80:19:1) with a flow rate of 0.8 ml min^{-1} in isocratic mode (Adiyaman et al. 2016). Injection volume was 20 μl with a run time of 60 min and detection wavelength was 280 nm.

LC–MS Analysis

Terminalia bellirica extract was analyzed by using Agilent 1260 LC–MS and the chromatographic conditions were DL temperature of 250 °C, scan range of 100–1500 m/z, 2 Hz scan speed, 0.8 kV detector voltages, and mobilizing gas flow of 7 l/min was used for MS analysis of the samples.

Animals and Experimental Design

Animals studies were conducted as per CPCSEA (committee for the purpose of control and supervision of experiments on animals) with approval no. IAEC-2016/AN/11. Forty-two female Balb/c mice (weighing 25–35 g) were divided into six experimental groups viz., control group (CON), chronic mild stress group (CMS), imipramine group (IMP10 mg/kg; *p.o.*), and treatment groups (TB-25, 50 and 100 mg/kg bwt; *p.o.*) (Fig. S1, Supplementary material). CMS protocol was performed as mentioned in our previous study (Chandrasekhar et al. 2017). Various kinds of stressors were applied individually and continuously as mentioned in Fig. S1, Supplementary material. Control mice were housed in a separate room and had no contact with the CMS group. Body weight was recorded on weekly basis and the animals were submitted to the behavioral tests after 24 h of the last stressor.

Behavioral Observations

Behavioral studies, such as sucrose preference test (SPT), tail suspension test (TST), and forced swimming test (FST) were conducted in accordance with previous studies (Chandrasekhar et al. 2018; Kim et al. 2018). Before conducting the SPT, animals were allowed to drink sucrose solution (1%; w/v) to avoid neophobia. Amount of water or sucrose consumed by the mice in a period of 1 h was recorded and calculated in percentage. In TST, mice were individually placed approximately 50 cm above the floor with the help of tape from the tip of the tail. Immobile time was recorded on the basis of mice movements by ANY-maze software. FST was conducted in a transparent glass tank, which is filled with 30-cm high water (25 ± 0.5 °C). The test was conducted for a period of 6 min and the cumulative immobility time during the last 4 min was recorded. The immobile time was considered when the mice remained floating without struggling.

Collection of Hippocampus and Serum

Mice were sacrificed after the behavioral studies under the mild anesthetic condition. Mice brain was immediately dissected and followed by further dissection of hippocampus in a chilled condition. Blood samples were collected into the serum separator and centrifuged for 10 min at 3000×*g* at 4 °C. All the samples were frozen at − 80 °C for further analysis.

Estimation of Serum Acetylcholine Esterase and Corticosterone by ELISA

Serum acetylcholine esterase (AChE) and corticosterone (CORT) were estimated by using ELISA kits as per the manufacturer's protocol (Abcam, USA). The AChE and CORT

concentrations were expressed in nanogram per liter and gram per liter, respectively.

Estimation of Antioxidant Activities

Brain samples were minced and homogenized with 50-mM phosphate buffer (pH 7.4) and centrifuged at 1200×*g* for 10 min at 4 °C. The supernatants were then subjected to the measurement of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidises (GPx), and glutathione reductase (GR) levels according to the assay kit manual (BioVisoin Pvt. Ltd., USA).

Estimation of Monoamine Oxidase

Estimation of MAO was carried according to the previously described procedure (Dhingra and Goyal 2008). The hippocampal tissue was homogenized with isolation buffer (0.25 M sucrose, 10 mM Tris, 0.5 mM EDTA, and pH 7.4), then mitochondrial fraction was obtained by centrifugation at 18,500×*g* for 20 min. MAO-A and MAO-B were measured at 280 nm and 242 nm, respectively.

Monoamine Neurotransmitter Levels

Monoamine neurotransmitters such as dopamine (DA), 5-hydroxytryptamine (5-HT), epinephrine, and norepinephrine (NE) are measured by using HPLC-ECD. The hippocampal tissue was homogenized in ice cold perchloric acid and HPLC conditions were followed as described in our previous study (Chandrasekhar et al. 2017).

Analysis of Quantitative RT-PCR

qRT-PCR studies were followed as per the manufacturer's instruction (SsoFast Eva Green, Bio-Rad). qRT-PCR results were normalized against β-actin (control) and calculated $2^{\Delta\Delta CT}$ values. Primer sequence and amplification conditions of targeted genes are placed in Table 1.

Western Blotting

Hippocampal tissue samples were homogenized in HEPES lysis buffer and the supernatant of the lysates were separated by SDS–PAGE mini gels and transferred onto polyvinylidene fluoride (PVDF) membrane. The membranes were subsequently probed via incubation with Gapdh, Bax, Bcl_2, Caspase-3, Creb, and Bdnf (Santa Cruz, USA; 1:1000). Incubation, blocking, and detection of immune reactivity were followed as mentioned in our previous study (Chandrasekhar et al. 2017).

Statistical Analysis

The results were expressed as mean ± standard deviation (SD). Data were analyzed by using one-way ANOVA followed by Tukey's test and Dunnett's multiple comparisons using GraphPad Prism version 6.03. Differences were considered significant when $p < 0.05$.

Results

Gallic Acid Estimation from *Terminalia Bellirica* by using HPLC and LC–MS

Total tannin content in the *T. bellirica* was 477 mg/g, which is 47% dry weight. HPLC analysis revealed that *T. bellirica* contains gallic acid (36.38%), corilagin (2.14%), chebulinic acid (0.82%), and ellagic acid (3.26%) (Fig. S2, Supplementary Material). Further, LC–MS study was conducted for its confirmation and results are shown in Fig. S2, Supplementary Material.

Effect of *Terminalia Bellirica* on CMS-induced Body Weight Loss

Percentage of body weight gain/loss during 7-week stress protocol is shown in Fig. 1a. CMS exposure induced a significant body weight loss when compared

Table 1 Primer sequences of mice for RT-PCR and amplification conditions

Gene	Primer sequence, 5′-3′	Amplification condition	Size bp
BDNF	F-GTGACTGAAAAAGTTCCACC	Tm: 59.0 ° C	122
	R-GACGTTTACTTCTTTCAT	Cycles: 39	
CREB	F-TCTAATGAAGAACAGGGAGG	Tm: 57.0 ° C	136
	R-GTGCCTTAAGTGCTTTTAGCTC	Cycles: 39	
Bax	F-ATATTGCTGTCCAGTTCATC	Tm: 58.0 ° C	151
	R-CCTTTTTGCTACAGGGTTTC	Cycles: 39	
Bcl2	F-ATGACTGAGTACCTGAACC	Tm: 56.0 ° C	78
	R-ATATAGTTCCACAAAGGCATC	Cycles: 39	
β-actin	F-GATGTATGAAGGCTTTGGTC	Tm: 58.0 ° C	96
	R-TGTGCATTTTATTGGTCTC	Cycles: 39	

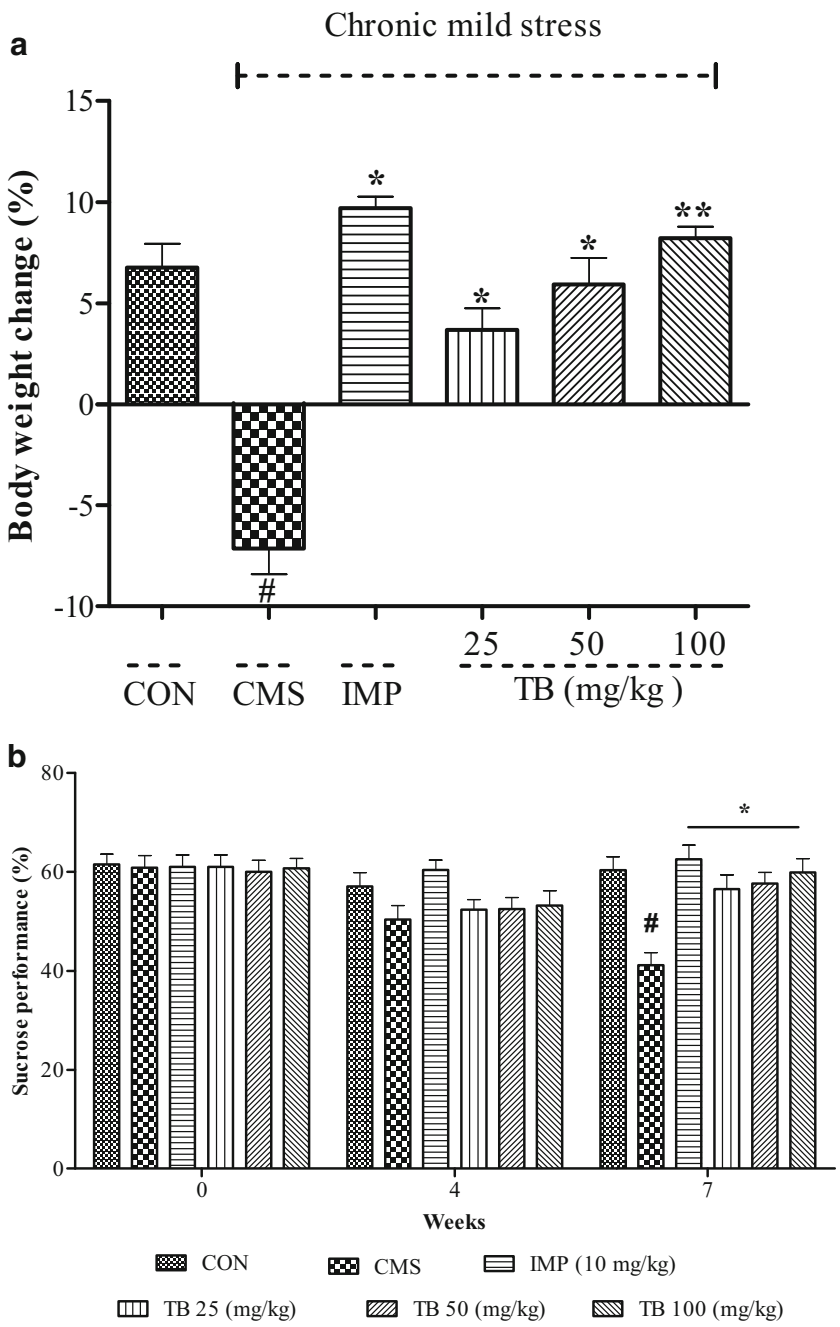

Fig. 1 Effects of *Terminalia bellirica* on body weight (**a**) and sucrose preference (**b**). Control (CON) and chronic mild stress group (CMS) received saline water, imipramine (IMP; 10 mg/kg., *p.o.*), TB-25, TB-50, and TB-100 (extract of *T. bellirica*, 25 mg/kg., *p.o.*, 50 mg/kg., *p.o.* and 100 mg/kg., *p.o.*, respectively). Data were presented as mean ± SD ($n = 7$). For statistical significance: #, $p < 0.05$ vs CON; *, $p < 0.05$; **, $p < 0.01$ vs CMS

with control mice ($p < 0.05$; Fig. 1a). However, TB-25, TB-50, and TB-100 showed a dose-dependent body weight gain. Treatment with *T. bellirica* and IMP significantly reversed ($p < 0.05$) the CMS-induced body weight loss.

Effect of *Terminalia Bellirica* on Sucrose Preference Test

Chronic mild stress exposure for a period of 7 weeks resulted in reduced sucrose consumption in CMS group as compared with control mice ($p < 0.05$). However, treatment of *T. bellirica* (25, 50, 100 mg/kg) enhanced the uptake of sucrose solution when compared with CMS mice ($p < 0.05$; Fig. 1b).

Effect of *Terminalia Bellirica* on CMS-induced on TST and FST

The effect of *T. bellirica* treatment on immobility time of mice subjected to TST and FST is shown in Fig. 2a and b, respectively. There was a significant increase in

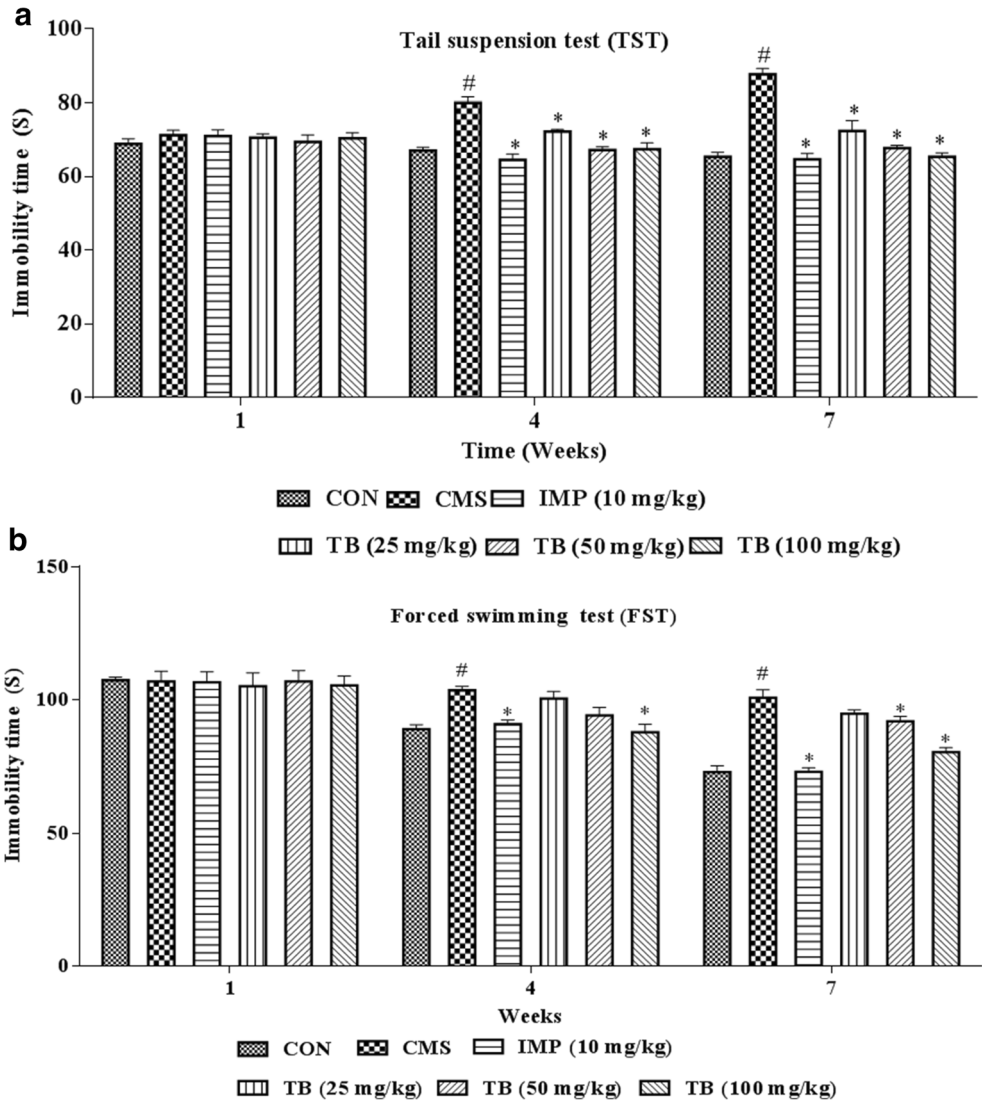

Fig. 2 Effects of *Terminalia bellirica* on tail suspension test. **a** Forced swim test. **b** In CMS-induced mice. Control (CON) and chronic mild stress group (CMS) received saline water, imipramine (IMP; 10 mg/kg., *p.o.*), TB-25, TB-50, and TB-100 (extract of *T. bellirica*, 25 mg/kg., *p.o.*, 50 mg/kg., *p.o.* and 100 mg/kg., *p.o.*, respectively).Data were presented as mean ± SD ($n = 7$). For statistical significance: #, $p < 0.05$ vs CON; *, $p < 0.05$ vs CMS

immobility time by CMS exposure in mice when compared with control animals ($p < 0.05$). Mice treated with *T. bellirica* along with stress protocol showed a reduced immobility time in TST and FST as compared with CMS group ($p < 0.05$).

Effect of *Terminalia Bellirica* on CMS-induced Serum CORT and AChE Levels

The results indicated that continuous stress protocol of the study significantly elevated the levels of serum CORT and serum AChE (56% and 14%, respectively) as compared with the control mice ($p < 0.05$). However, treatment with *T. bellirica* significantly reduced these serum CORT and AChE levels ($p < 0.05$; Fig. 3a, b).

Effect of *Terminalia Bellirica* on CMS-induced Oxidative Stress

Continuous stress protocol of the study significantly downregulated the levels of antioxidant enzymes such as SOD (11.05 ± 0.8 to 4.68 ± 0.38 unit g/protein), CAT (66.28 ± 1.6 to 37.29 ± 1.92 unit g/protein), GPx (15.11 ± 1.06 to 5.67 ± 0.94 unit g/protein), and GR (18.26 ± 1.24 to 6.73 ± 0.86 unit g/protein) as compared with control animals ($p < 0.05$). In contrast, *T. bellirica* treatment significantly increased these antioxidant enzymes (Fig. 4; $p < 0.05$).

Effect of *Terminalia Bellirica* on CMS-induced Monoamine Oxidase Activities

CMS exposure significantly increased the hippocampal MAO-A by 2.5-fold (Fig. 5a) and MAO-B by 4-fold (Fig. 5b) as

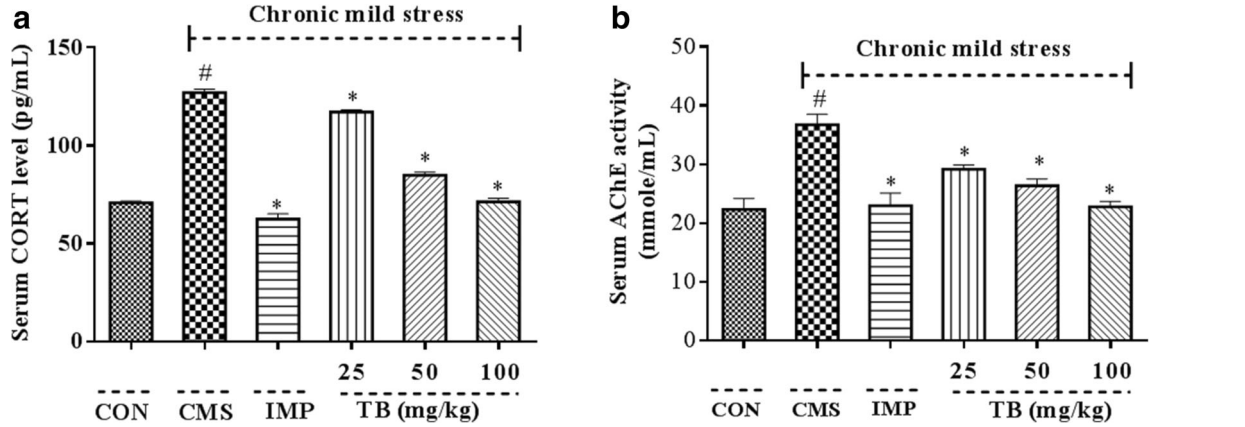

Fig. 3 Effects of *Terminalia bellirica* on serum corticosterone levels (**a**) and acetylcholine esterase levels (**b**) in CMS-treated mice. Control (CON) and chronic mild stress group (CMS) received saline water; imipramine (IMP; 10 mg/kg., *p.o.*), TB-25, TB-50, and TB-100 (extract of *T. bellirica*, 25 mg/kg., *p.o.*, 50 mg/kg., *p.o.*, and 100 mg/kg., *p.o.*, respectively). Data were presented as mean ± SD ($n = 6$). For statistical significance: #, $p < 0.05$ vs CON; *, $p < 0.05$ vs CMS

compared with unstressed animals ($p < 0.05$). In contrast, these CMS-induced MAO activities were significantly reduced by the treatment of *T. bellirica* in a dose-dependent manner from $p < 0.05$.

Effect of *Terminalia Bellirica* on CMS-induced Monoamine Levels

Figure 6 revealed that the CMS procedure was able to induce a significant decrease on epinephrine, nor-epinephrine, dopamine, and serotonin levels when compared with control mice ($p < 0.01$). However, these monoamine levels, such as nor-epinephrine (108.5 ± 2.9 and 225.9 ± 2.8 ng/100 mg), epinephrine (78.2 ± 1.0 and 92.3 ± 3.4 ng/100 mg), dopamine (83.6 ± 1.2 and 101.5 ± 1.5 ng/100 mg), and serotonin (56.4 ± 3.8 and 74.4 ± 1.2 ng/100 mg) were significantly increased by TB-50 and TB-100 treatments, respectively. In contrast, CMS-induced monoamine levels were not affected by low dose of *T. bellirica*, *i.e.*, TB-25.

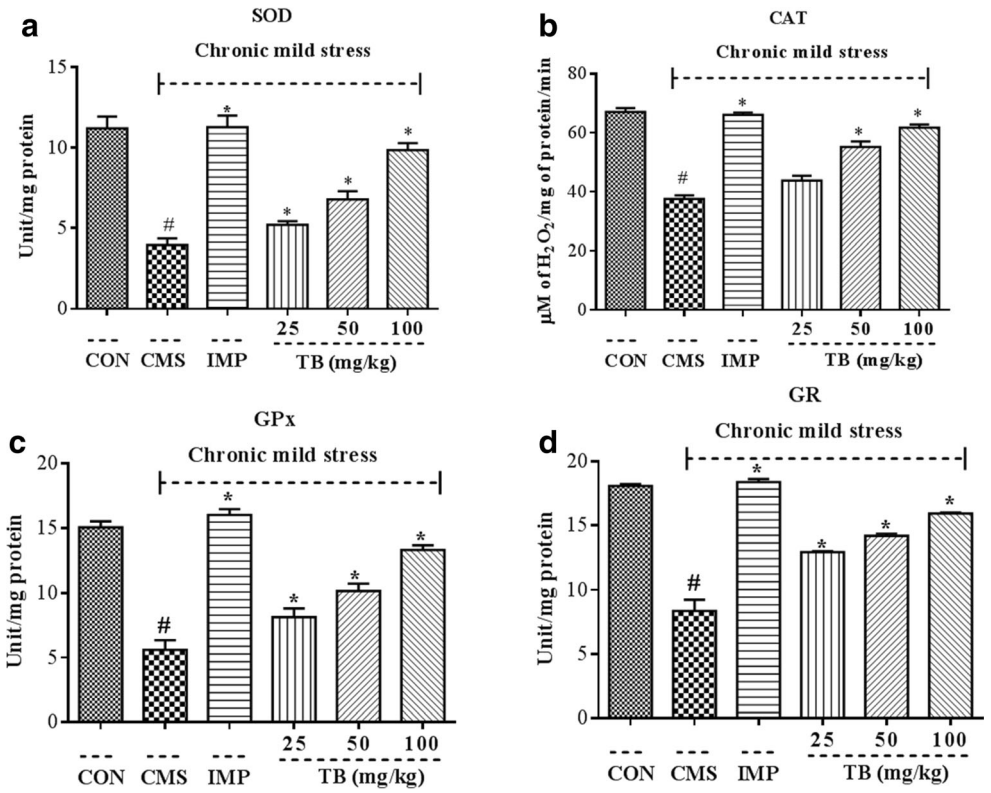

Fig. 4 Effects of *Terminalia bellirica* on antioxidant levels in CMS-induced mice. **A** *SOD*, superoxidase dismutase; **b** *CAT*, catalase; **c** *GPx*, glutathione peroxidase; **d** *GR*, glutathione reductase. Control (CON) and chronic mild stress group (CMS) received saline water; imipramine (IMP; 10 mg/kg., *p.o.*), TB-25, TB-50, and TB-100 (extract of *T. bellirica*, 25 mg/kg., *p.o.*, 50 mg/kg., *p.o.*, and 100 mg/kg., *p.o.*, respectively).Data were presented as mean ± SD ($n = 3$). For statistical significance: #, $p < 0.05$ vs CON; *, $p < 0.05$ vs CMS

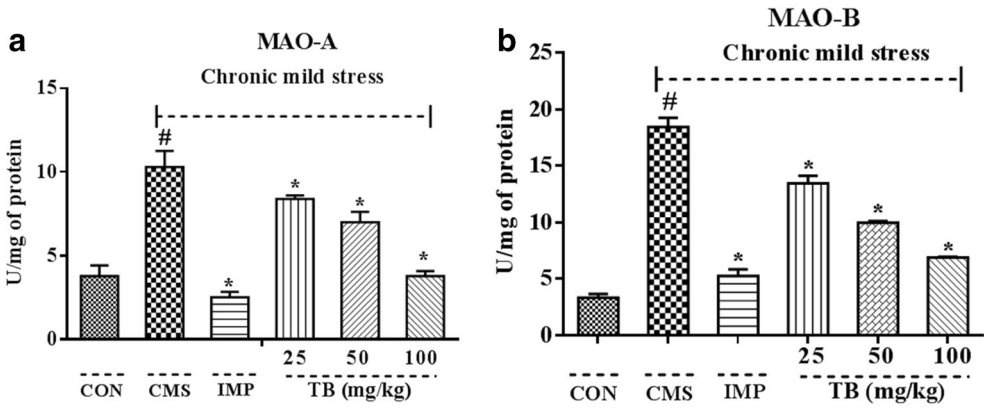

Fig. 5 Effects of *Terminalia bellirica* on the levels of MAO-A and MAO-B. Control (CON) and chronic mild stress group (CMS) received saline water; imipramine (IMP; 10 mg/kg., *p.o.*), TB-25, TB-50, and TB-100 (extract of *T. bellirica*, 25 mg/kg., *p.o.*, 50 mg/kg., *p.o.*, and 100 mg/kg., *p.o.*, respectively). Data were presented as mean ± SD ($n = 3$). For statistical significance: #, $p < 0.05$ vs CON; *, $p < 0.05$ vs CMS

Effect of *Terminalia Bellirica* on CMS-induced Bcl_2 and Bax Expression

Chronic mild stress induced apoptotic cell death by increasing Bax/Bcl_2 ratio in mice hippocampus (35:1). CMS-induced Bax transcript levels were downregulated by the *T. bellirica* treatment (Fig. 7). Mice groups treated with IMP and TB-100 could significantly increase the transcript levels of Bcl_2 by 86% and 72%, respectively, when compared with CMS (10%) exposed mice. Western blot analysis further confirmed the protective effect of *T. bellirica* on CMS-induced cell death by downregulated Bax levels and upregulated Bcl_2 levels (Fig. 8a, b).

Effect of *Terminalia Bellirica* on CMS-induced BDNF, CREB, and Caspase-3 Expression

Figure 8c illustrated that CMS significantly induces upregulation of caspase-3 expression by twofold when compared with control animals ($p < 0.05$). In contrast, treatment with *T. bellirica* and IMP normalized the CMS-induced caspase-3 expression. RT-PCR and western blot analysis (Figs. 7 and 8, respectively) illustrated that CMS exposure significantly downregulated BDNF and CREB expressions in hippocampus ($p < 0.05$). CMS-induced BDNF downregulation and increased CREB phosphorylation were significantly reverted by *T. bellirica* and IMP treatments ($p < 0.05$; Fig. 8d, e).

Discussion

Chronic mild stress plays a key role in the pathophysiology of depression, in which mice were exposed to various stressors for certain period to mimic chronic stressful life events of human (Auriacombe et al. 1997). There are multiple mechanisms that have been associated in the pathogenesis of depressive disorder, such as change in neurotransmitter systems, over activation of monoamine oxidases, oxidative stress, dysregulation of HPA-axis, inflammatory cytokines, and apoptotic pathway (Messay et al. 2012).

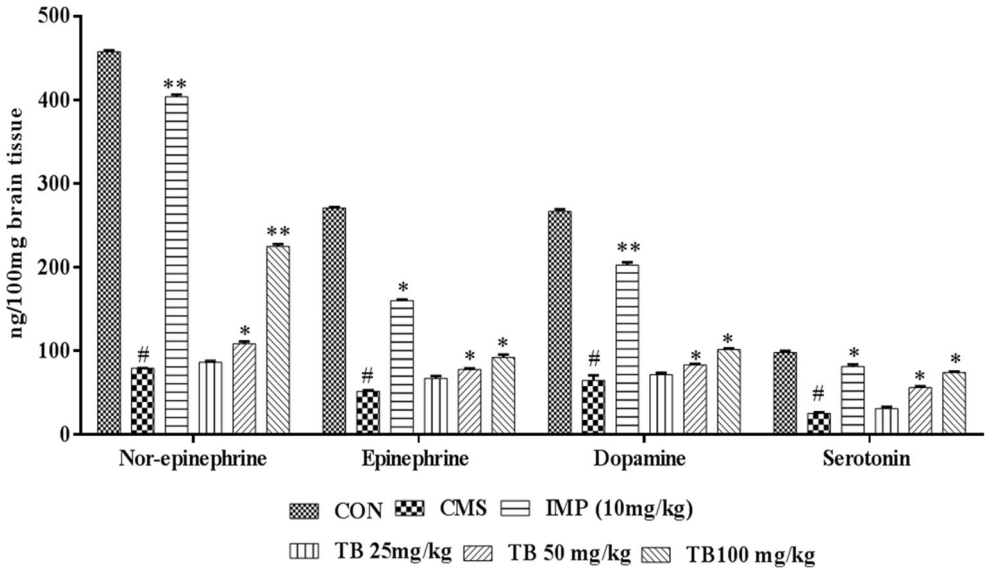

Fig. 6 Effects of *Terminalia bellirica* on the levels of monoamine neurotransmitters in hippocampus of CMS-stressed mice. Control (CON) and chronic mild stress group (CMS) received saline water; imipramine (IMP; 10 mg/kg., *p.o.*), TB-25, TB-50, and TB-100 (extract of *T. bellirica*, 25 mg/kg., *p.o.*, 50 mg/kg., *p.o.*, and 100 mg/kg., *p.o.*, respectively). Data were presented as mean ± SD ($n = 6$). For statistical significance:*, $p < 0.05$; **, $p < 0.01$ vs CMS; #, $p < 0.01$ vs CON

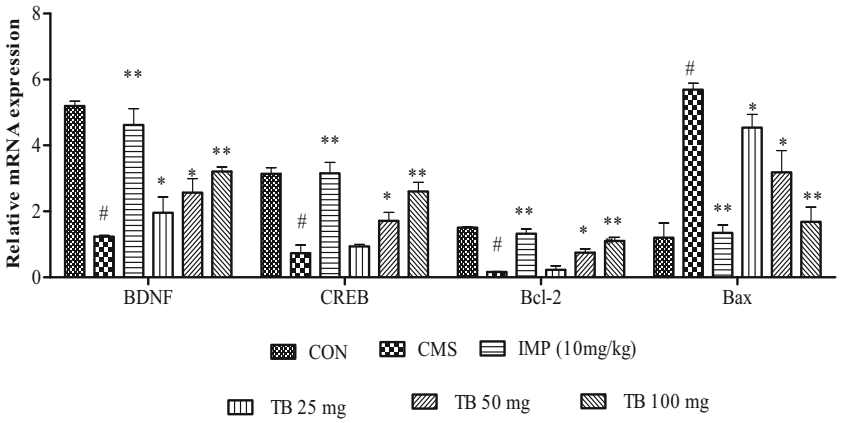

Fig. 7 Effects of *Terminalia bellirica* on the mRNA expression of CREB, BDNF, Bcl_2, and Bax in hippocampus of CMS-stressed mice. Control (CON) and chronic mild stress group (CMS) received saline water; imipramine (IMP; 10 mg/kg., *p.o.*), TB-25, TB-50, and TB-100 (extract of *T. bellirica*, 25 mg/kg., *p.o.*, 50 mg/kg., *p.o.*, and 100 mg/kg., *p.o.*, respectively). Data were presented as mean ± SD ($n = 6$). For statistical significance: *, $p < 0.05$; **, $p < 0.01$ vs CMS; #, $p < 0.01$ vs CON

The sucrose preference is an index of anhedonia-like behavioral changes. In the present study, mice exposed to CMS showed reduced sucrose preference as compared with control animals at week 7. *T. bellirica* treatment significantly improved the sucrose preference, which showed the antidepressant-like action of *T. bellirica*. FST and TST have been widely used as behavioral parameters to assess the locomotor activity in depression models. CMS exposure significantly increased the immobility time of the mice in the TST and FST; these results are in consistent with previous studies by Mao et al. (2009). However, *T. bellirica* treatment in the present study reversed the CMS-induced behavioral despair by reducing the immobility time.

In depression-like disorders, BDNF plays a crucial role in regulating synaptic plasticity and neuronal survival (Popova and Naumenko 2019). As mentioned in the previous studies, BDNF transcription was downregulated in the pathogenesis of depression and lead to hippocampal atrophy, and the same was reversed by antidepressant drug treatment (Mao et al. 2012). Present RT-PCR and western blot studies confirmed that CMS exposure induced a downregulated hippocampal BDNF expression, while *T. bellirica* treatment significantly reversed it. In addition, BDNF has been proved to have neuroprotective property by activating serotonergic and dopaminergic neurotransmission (Huang and Reichardt 2001). Based on these results, we can speculate that gallic acid from *T. bellirica* can increase the levels of neurotransmitters and signaling to promote the expression of BDNF.

It has been postulated that over production of glucocorticoids may eventually lead to atrophy and decreased hippocampal neurogenesis, which results in neuro-anatomical, molecular and behavioral changes (Lanfumey et al. 2008). It has been reported that release of serum CORT activates glutamate receptor; it may lead to open Ca^{2+} channel by NMDA receptor (*N*-methyl-D-aspartate) binding activity, resulting an overload of calcium influx, induces neuronal cell death (Roozendaal 2002). In the present study, *T. bellirica* treatment suppressed the CMS-induced serum CORT levels, indicating the antidepressant-like effects of *T. bellirica*. Furthermore, overload of calcium influx may affect the activity of CREB expression (Hardingham and Bading 2002), which directly affects the regulation of BDNF. Present study confirmed that treatment with *T. bellirica* regulates hyperactivity of HPA-axis via modulating overload of calcium influx and its consequent upregulation in the hippocampal BDNF and CREB expressions. Over production of AchE dysregulates cholinergic neurotransmission in the nervous system (Dang et al. 2009). The present study suggested that CMS induced AChE levels in mice. Moreover, *T. bellirica* treatment significantly reversed the stress-dependent AChE levels.

Previous neurobehavioral studies proved the serotonin, dopamine, epinephrine, and nor-epinephrine involvement in many neurological and psychiatric diseases (Naughton et al. 2000). In the present study, neurochemical analysis revealed that treatment with *T. bellirica* elevated the levels of neurotransmitters. Previous reports on antidepressant drugs like serotonin reuptake inhibitors (SRI's) and nor-epinephrine reuptake inhibitors (NRI's) are in line with the present study, which were found to have effects on the treatment of depression by elevating monoamine levels through the inhibition of monoamine reuptake (Piñeyro and Blier, 1999).

Monoamino oxidases (MAO-A and MAO-B) are responsible for metabolic degradation of catecholamines and monoamines. MAO-A catalyzes nor-epinephrine, dopamine, and serotonin, whereas MAO-B catalyzes dopamine more specifically (Bortolato et al. 2008). Gallic acid has been reported with MAO's inhibition and hippocampal 5-HT receptor elevation (Chhillar and Dhingra 2013). In the present study, treatment with *T. bellirica* has shown a significant inhibition of CMS-induced hippocampal MAO-A and MAO-B levels.

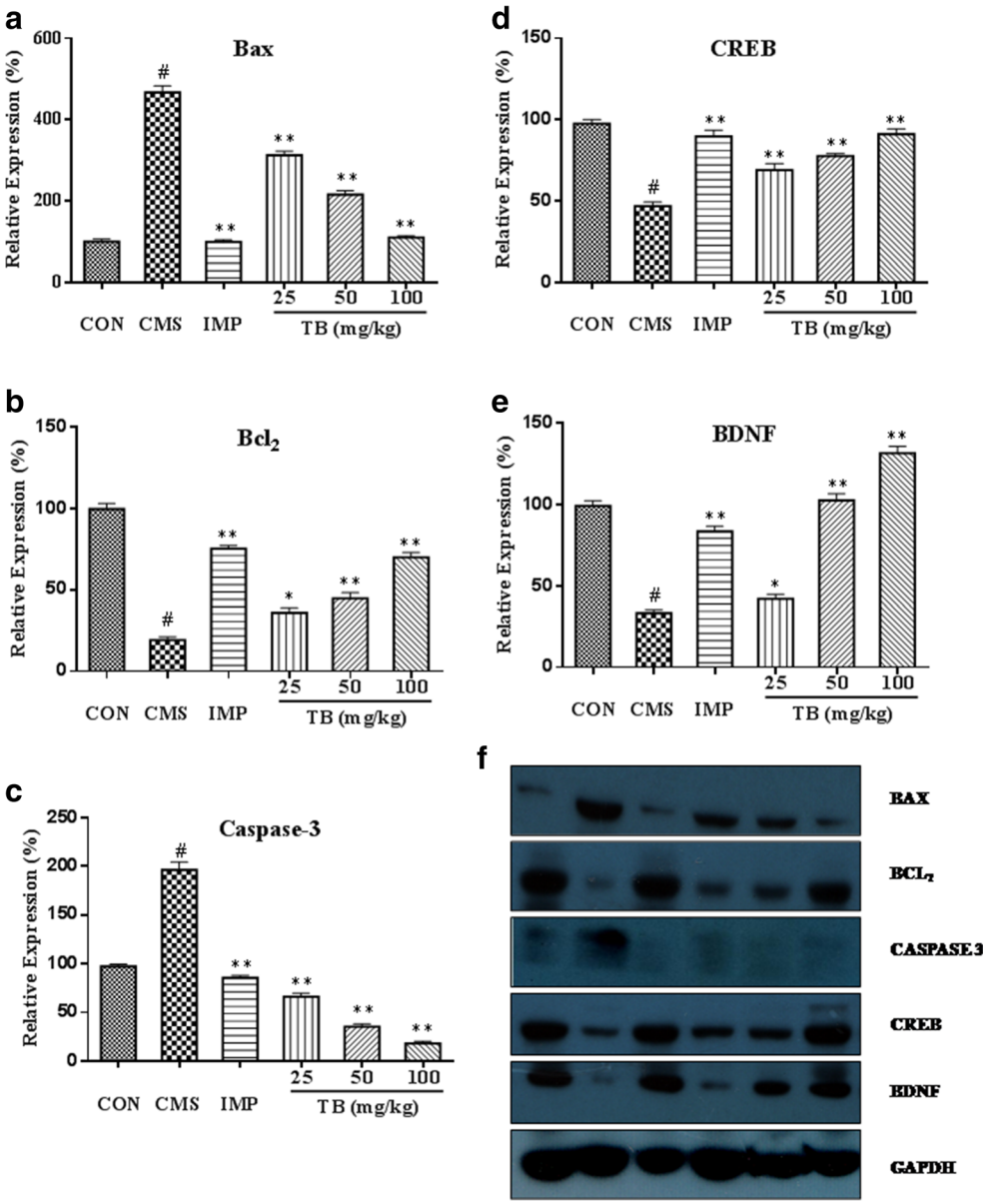

Fig. 8 Effect of *Terminalia bellirica* on protein expression levels. Bax (**a**), Bcl2 (**b**), caspase-3 (**c**), CREB (**d**), BDNF (**e**), and western blot images (**f**) in the hippocampus of CMS-treated mice. Control (CON) and chronic mild stress group (CMS) received saline water; imipramine (IMP; 10 mg/kg., *p.o.*), TB-25, TB-50, and TB-100 (extract of *T. bellirica*, 25 mg/kg., *p.o.*, 50 mg/kg., *p.o.*, and 100 mg/kg., *p.o.*, respectively). Data are represented as the mean ± SD ($n = 6$). #, $p < 0.05$ vs CON; *, $p < 0.05$, **, $p < 0.01$ vs CMS

Moreover, the study demonstrated that *T. bellirica* treatment has an ameliorative effect on CMS-induced metabolic degradation of catecholamines and monoamines.

Previous studies have demonstrated that depression was accompanied by oxidative stress (Liu et al. 2015). Present study suggested that CMS not only induced depressive-like behavior but also stimulated oxidative stress and decreased antioxidant enzyme activities. Further findings of the study demonstrated that *T. bellirica* treatment demolishes CMS-induced oxidative stress via upregulation of antioxidant enzymes viz., SOD, CAT, GPx, and GR.

Neuroprotective effect of gallic acid has been previously studied and demonstrated that gallic acid can reduce the neuronal apoptosis via regulating the Bax/Bcl2 ratio (Chhillar and Dhingra 2013). In the present investigation, CMS exposure elevated Bax expression levels and induced the levels of Bcl2. Further, the study confirms that *T. bellirica* treatment reversed CMS-induced neuronal apoptosis via regulating the Bax/Bcl2 ratio. Therefore, *T. bellirica* can be considered anti-apoptotic agent in controlling depressive-like behavior.

Conclusion

The present study showed that CMS induced behavioral, biochemical, and neurochemical changes. These findings of the study demonstrated that gallic acid from *T. bellirica* fruits exhibited antidepressant-like effects as evidenced by increased sucrose preference and decreased immobility time in behavioral observations. Also, *T. bellirica* ameliorated CMS-

induced depression in mice through reduced hyperactivity of HPA-axis, enhancement of antioxidant defense system, normalization of neurotransmitter disturbances, and modulating proteins at molecular levels by which reduced hippocampal neuronal apoptosis. Thus, *T. bellirica* could be considered to be an alternative medical therapy to treat depressant-like disorders. However, additional studies involving its toxicity and clinical studies are necessary to prove the potential application of *T. bellirica* as a therapeutic agent.

Acknowledgments We would like to thank Director Dr. Anil Dutt Semwal, DFRL (DRDO), for providing the experimental facilities.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Protection of Human and Animal Subjects The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of Data The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to Privacy and Informed Consent The authors declare that no patient data appear in this article.

References

Adiyaman P, Kanchana S, Usharani T, Ilaiyaraja N, Kalaiselvan A, Anilakumar KR (2016) Identification and quantification of polyphenolic compounds in underutilized fruits (Star fruit and Egg fruit) using HPLC. Indian J Tradit Know 15:487–493

Asnis GM, Henderson MA (2014) EMSAM (deprenyl patch): how a promising antidepressant was underutilized. Neuropsychiatr Dis Treat 10:1911–1923

Auriacombe M, Reneric JP, Le Moal M (1997) Animal models of anhedonia. Psychopharmacol 134:337–338

Bortolato M, Chen K, Shih JC (2008) Monoamine oxidase inactivation: from pathophysiology to therapeutics. Adv Drug Deliv Rev 60: 1527–1533

Chandrasekhar Y, Ramya EM, Navya K, Phani Kumar G, Anilakumar KR (2017) Antidepressant like effects of hydrolysable tannins of *Terminalia catappa* leaf extract via modulation of hippocampal plasticity and regulation of monoamine neurotransmitters subjected to chronic mild stress (CMS). Biomed Pharmacother 86:414–425

Chandrasekhar Y, Phani Kumar G, Navya K, Ramya EM, Anilakumar KR (2018) Tannins from Terminalia chebula fruits attenuates GABA antagonist-induced anxiety-like behaviour via modulation of neurotransmitters. J Pharm Pharmacol. https://doi.org/10.1111/jphp.13007

Chhillar R, Dhingra D (2013) Antidepressant-like activity of gallic acid in mice subjected to unpredictable chronic mild stress. Fundam Clin Pharmacol 27:409–418

Dang H, Sun L, Liu X, Peng B, Wang Q, Jia W, Chen Y, Pan A, Xiao P (2009) Preventive action of Kai Xin San aqueous extract on depressive-like symptoms and cognition deficit induced by chronic mild stress. Exp Biol Med 234:785–793

Dhingra D, Goyal PK (2008) Inhibition of MAO and GABA: probable mechanisms for antidepressant-like activity of *Nardostachys jatamansi* DC. in mice. Indian J Exp Biol 46:212–218

Duncan J, Johnson S, Ou XM (2012) Monoamine oxidases in major depressive disorder and alcoholism. Drug Discov Ther 6:112–122

Grippo AJ, Francis J, Beltz TG, Felder RB, Johnson AK (2005) Neuroendocrine and cytokine profile of chronic mild stress-induced anhedonia. Physiol Behav 84:697–706

Hardingham GE, Bading H (2002) Coupling of extrasynaptic NMDA receptors to a CREB shut-off pathway is developmentally regulated. Biochim Biophys Acta Protein Proteomics 1600:148–153

Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24:677–736

Kadian R, Parle M (2015) Evaluation of *Terminalia bellerica* for its antipsychotic potential. Int J Pharm Sci Rev Res 30:247–252

Kim S, Shou J, Abera S, Ziff EB (2018) Sucrose withdrawal induces depression and anxiety-like behavior by Kir2.1 upregulation in the nucleus accumbens. Neuropharmacol 130:10–17

Kumar GP, Anilakumar KR, Naveen S (2014) Phytochemicals having neuroprotective properties from dietary sources and medicinal herbs. Pharm J 7:1–17

Lanfumey L, Mongeau R, Cohen-Salmon C, Hamon M (2008) Corticosteroid-serotonin interactions in the neurobiological mechanisms of stress-related disorders. Neurosci Biobehav Rev 32:1174–1184

Leonard BE (2010) The concept of depression as a dysfunction of the immune system. Curr Immunol Rev 6(3):205–212

Liu T, Zhong S, Liao X, Chen J, He T, Lai S, Jia Y (2015) A meta-analysis of oxidative stress markers in depression. PLoS One. https://doi.org/10.1371/journal.pone.0138904

Mao QQ, Huang Z, Ip SP, Xian YF, Che CT (2012) Peony glycosides reverse the effects of corticosterone on behavior and brain BDNF expression in rats. Behav Brain Res 227:305–309

Mao QQ, Ip SP, Ko KM, Tsai SH, Che CT (2009) Peony glycosides produce antidepressant-like action in mice exposed to chronic unpredictable mild stress: effects on hypothalamic-pituitary-adrenal function and brain-derived neurotrophic factor. Prog Neuro-Psychopharmacol Biol Psychiatry 33:1211–1216

Messay B, Lim A, Marsland AL (2012) Current understanding of the bi-directional relationship of major depression with inflammation. Biol Mood Anxiety Disord 2:1–4. https://doi.org/10.1186/2045-5380-2-4

Naughton M, Mulrooney JB, Leonard BE (2000) A review of the role of serotonin receptors in psychiatric disorders. Hum Psychopharmacol 15:397–415

Numakawa T, Adachi N, Richards M, Chiba S, Kunugi H (2013) Brain-derived neurotrophic factor and glucocorticoids : reciprocal influence on the central nervous system. Neuroscience 239:157–172

Piñeyro G, Blier P (1999) Autoregulation of serotonin neurons: role in antidepressant drug action. Pharmacol Rev 51:533–591

Popova NK, Naumenko VS (2019) Neuronal and behavioral plasticity: the role of serotonin and BDNF systems tandem. Expert Opin Ther Targets 23:227–239

Roozendaal B (2002) Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. Neurobiol Learn Mem 78:578–595

Shang X, Shang Y, Fu J, Zhang T (2017) Nicotine significantly improves chronic stress-induced impairments of cognition and synaptic plasticity in mice. Mol Neurobiol 54:4644–4658

Trivedi VP, Nesamany N, Sharma VK (1979) A clinical study of the antitussive and anti-asthmatic effects of Vibhitakphal Churna (*Terminalia bellerica* Roxb.) in the cases of Kasa-Swasa. J Res Ayurveda Siddha 3:1–8

Wang JM, Yang LH, Zhang YY, Niu CL, Cui Y, Feng WS, Wang GF (2015) BDNF and COX-2 participate in anti-depressive mechanisms of catalpol in rats undergoing chronic unpredictable mild stress. Physiol Behav 15:360–368