NeuroMolecular Medicine (2019) 21:250–261
https://doi.org/10.1007/s12017-019-08538-6

ORIGINAL PAPER

Antidepressant and Neuroprotective Effects of Naringenin via Sonic Hedgehog-GLI1 Cell Signaling Pathway in a Rat Model of Chronic Unpredictable Mild Stress

Mohd Tayyab[1] · Shirin Farheen[1] · Mubeena Mariyath P. M[1] · Nabeela Khanam[1] · M. Mobarak Hossain[1,2] · Mehdi Hayat Shahi[1]

Received: 28 January 2019 / Accepted: 23 April 2019 / Published online: 29 April 2019

Abstract

Depression is one of the most prevalent and crucial public health problem connected to significant mortality and co-morbidity. Recently, numerous studies suggested that dietary flavanones exhibit neuroprotective and antidepressant effects against various psycho-physiological conditions including depression. The present study is focused on the antidepressant and neuroprotective effects of naringenin (NAR) and the involvement of sonic hedgehog (Shh) signaling in the chronic unpredictable mild stress (CUMS)-induced depression. Twenty-four male Wistar rats were randomly assigned into four groups: CON group (saline s.c.), NAR group (NAR 50 mg/kg, p.o.), CUMS group (subjected to CUMS along with saline p.o.), and CUMS + NAR group (NAR 50 mg/kg p.o. along with CUMS) for 28 days including 1-week pre-treatment with NAR. The results showed that NAR was found to inhibit behavioral abnormalities including increased despair in force swim test, and reduced locomotor activity caused by CUMS in open field test. Moreover, Morris water maze revealed that NAR also mitigates CUMS-associated cognitive impairment. In addition to the antidepressant-like effect, NAR mitigates morphological anomalies in the hippocampal CA1 region and cortex. Furthermore, we observed brain-derived neurotrophic factor (BDNF), Shh, GLI1, NKX2.2, and PAX6 were downregulated in the hippocampus of CUMS-exposed rats, which can be upregulated by NAR pre-treatment. GLI1 is main downstream signaling component of Shh signaling cascade, which further regulates the expression of homeodomain transcription factors PAX6 and NKX2.2.

Keywords Depression · Hippocampus · Brain-derived neurotrophic factor · Sonic hedgehog · Memory

Introduction

Depression is one of the major contributors to global burden of mental illness (Ferrari et al. 2013) and as per predictions made by World Health Organization (WHO) reports, by 2020 it will be the second leading cause of disability-adjusted life years, having lifetime prevalence rate ranging from 2 to 15% (Moussavi et al. 2007). More than 322 million people are affected globally with depression. It is a significant disease burden not only at national level but also worldwide (Organization 2017). Depression is affecting all ages, genders, and different socioeconomic groups globally. Moreover, depression affects the healthy lifestyle and leads to poor quality of life, which triggers a huge impact on social and economic growth. Chronic stressful episodes in life are the risk factors for the major depressive disorder as stress is the precipitant of depression and can also impair memory and learning ability (Conrad 2010).

It is a well-established fact that stressful events enhance memory to remember the important information (Roozendaal et al. 2009). However, chronic stress negatively affects physiological system and may instigate deleterious effects on brain structures and functions that are involved

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12017-019-08538-6) contains supplementary material, which is available to authorized users.

✉ Mehdi Hayat Shahi
mehdihayat@gmail.com

[1] Interdisciplinary Brain Research Centre, Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh, Uttar Pradesh 202002, India

[2] Department of Physiology, Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh, Uttar Pradesh 202002, India

in cognitive abilities (Lupien et al. 2007). Moreover, several studies that examine the neuro-psychopathology have suggested the decline in cognition in psychiatric disorders including major depressive disorder (Potvin et al. 2016), and also in the animal models, which mimic the depressive behavior (Yan et al. 2017). Interestingly, antidepressant medication improves the cognitive functions that are associated with depression (Lahr et al. 2007). Chronic unpredictable mild stress (CUMS) (Liu et al. 2014; Yazir et al. 2015) has become a widely accepted animal model of depressive behavior (Willner et al. 1992), as it shows face and predictive validity (Czeh et al. 2016). A number of studies reported that CUMS elicits behavioral changes that can be correlated with clinical depression such as reduced locomotor exploratory behavior (Gronli et al. 2005), increased behavioral despair (Nirmal et al. 2008), and the impaired memory and learning. These are the core symptoms of depression (Burt et al. 1995) as CUMS also plays a key role in cognitive deficits (Yan et al. 2017; Cuadrado-Tejedor et al. 2011; Song et al. 2006).

Modern antidepressants have limited applicability due to factors like low efficacy in certain individuals, long time to display therapeutic effects, and side effects like sedation and dependence. According to some national surveys and recent research, complementary and alternative medicines for treating mental illness (Barnes et al. 2008; Hunt et al. 2010) attracts attention because of the belief that natural products are better. Although the underlying mechanism is not clearly understood yet, but intensive research is going on worldwide to find alternative therapies for treating depression (Dwyer et al. 2011).

Numerous studies have suggested that dietary flavanones exhibit a neuroprotective effect against various psychophysiological conditions including depression (Pathak et al. 2013). Naringenin (4,5,7-trihydroxyflavanone, NAR) is a natural flavanone abundantly present in the peel of citrus fruits, possessing anti-neuroinflammatory (Raza et al. 2013), antioxidative (Heo et al. 2004), neuroprotective (Zbarsky et al. 2005), anti-anxiety activity (Chtourou et al. 2015), and antidepressant property (Yi et al. 2012, 2014) and also improves memory and learning (Ghofrani et al. 2015).

There are various lines of clinical and pre-clinical evidence that support the involvement of brain-derived neurotrophic factor (BDNF) in depression (Castren and Rantamaki 2010; Duman and Monteggia 2006). Moreover, alteration in BDNF levels has been implicated in several mental disorders that affect cognitive abilities (Lu et al. 2014). Studies suggested that hippocampal BDNF expression declines in animal model of depressive behavior like CUMS, which can also be correlated with impaired learning and memory due to depressive behavior (Yan et al. 2017). BDNF also plays its role in the neural proliferation, synaptic plasticity, and neurogenesis (Lee and Son 2009). Furthermore, various studies suggested that, hippocampal neurogenesis declines in depression (Duman and Monteggia 2006; Malberg and Duman 2003).

Sonic hedgehog (Shh) is a powerful mitogen of embryonic as well as adult hippocampal neurogenesis (Briscoe and Therond 2013). Shh is a protein found in vertebrates encoded by SHH gene. The hedgehog family consists of sonic hedgehog (Shh), desert hedgehog (dhh), and Indian hedgehog (ihh), while Shh is the most studied signaling pathway of the three. It was reported that combined depletion of serotonin and norepinephrine with para-chlorophenyl-alanine decreased the mRNA expression of Shh receptors smoothened (Smo) and patch (Ptc) in the dentate gyrus of the hippocampus in adult male mice and treatment with monoamine-releasing agent p-chloroamphetamine increased the Smo mRNA expression (Rajendran et al. 2009). Moreover, it was also reported that electroconvulsive seizure (ECS) treatment in depression also regulates the expression of Smo and Ptc in the hippocampus (Banerjee et al. 2005). Furthermore, a recent study suggested that Smo and GLI1 downregulate in CUMS-induced depressive behavior in mice model, and chronic administration of herbal drugs like urtica dioica and hypericum perforatum significantly recovered the low expression of Smo and GLI1 (Patel et al. 2016). Therefore, to further explore this pathway's role, we examine the downstream signaling component of Shh signaling in the hippocampus in depression. GLI1 is a primary downstream signaling zinc finger transcription factor of Shh signaling which further regulates its expression of its potential targets, which are homeodomain transcription factors NKX2.2 and PAX6 (Shahi et al. 2010, 2012).

In present study, we attempted to investigate the potential effect of NAR in depressive behavior, learning, and memory deficits associated with CUMS and possible regulatory role of Shh-GLI1 signaling cascade in depression, also histopathological changes in the hippocampus and cortex regions of the rat brain.

Materials and Methods

Reagents

Naringenin (Purity 95%) and ethidium bromide were purchased from Sigma-Aldrich, USA. Dulbecco's phosphate-buffered saline (PBS) was purchased from HIMedia Laboratory Pvt Ltd, India and other routine chemicals were obtained from Merck, India. TRI reagent was purchased from Sigma-Aldrich, USA. M-MLV reverse transcriptase and random hexamer used for cDNA synthesis was purchased from Invitrogen BioServices Pvt. Ltd., India. dNTP mix (10 mM each), RiboLock RNase Inhibitor, PCR Master Mix (2×), and power SYBR Green PCR Master Mix were

purchased from Thermo Scientific, India. Primers were synthesized from Eurofins, India.

Animals

Adult male Wistar rats (12 ± 2 weeks, $n = 24$), weighing 175-200 g, were obtained from central animal house facility, JNMC, Faculty of Medicine, Aligarh Muslim University (AMU), Aligarh, UP, India. Animals were housed under controlled conditions (three rats per cage; 12 h light and dark; temperature at 22 ± 2 °C) and provided food and water ad libitum for 2 weeks prior to begin the experiments. All the behavioral assessments were carried out between 10:00 A.M. and 2:00 P.M. The reported experiments in this study were performed in accordance with the regulation of Institutional Animal Ethics Committee (IAEC), and were approved by IAEC (Registration No. 401/RO/c/2001/CPCSEA), central animal house, JNMC, Faculty of Medicine, AMU, Aligarh, UP, India. All the experiments were done under the guidelines of CPCSEA, India.

Animals were randomly assigned into four groups ($n = 6$ per group). (1) Control (CON) group received physiological saline for 4 weeks; (2) Protectant group received NAR at a dose of 50 mg/kg/day for 4 weeks (including 1 week of pre-treatment) as shown in Fig. 1; (3) CUMS group was given physiological saline; (4) CUMS + NAR group was treated with NAR 50 mg/kg/day for 4 weeks (including 1 week of pre-treatment).

Drug Treatment

The preferred dose of NAR was 50 mg/kg/day as per the previous studies (Umukoro et al. 2018). NAR was dissolved in saline (0.9% NaCl) and was administered orally at pH 7. The rats were treated with NAR for 4 weeks, with 1 week prior as pre-treatment and up to throughout CUMS (3 weeks).

Chronic Unpredictable Mild Stress Procedure

The CUMS was performed as described previously by Dubey et al. (2015) with slight modification in the protocol (Dubey et al. 2015). Briefly, the CUMS paradigm comprises of a variety of mild stressors applied in an unpredictable manner, as listed: (1) food deprivation for 24 h; (2) soiled bedding (~ 150 ml water per cage) for 22 h; (3) cage tilting (~ 45°) for 22 h; (4) crowded housing (12 animals per cage) for 12 h; (5) restraint stress for 2 h; (6) water deprivation for 24 h; (7) forced swimming for 10 min. These stressors were given subsequently for 21 days (Fig. 1), rats received one stressor every day that makes each stressor was given one time every week of the total course of 3 weeks of CUMS period. CON group was kept in separate room and not subjected to contact with CUMS group.

Behavioral Tests

Open field test (OFT) for exploratory behavior and Force swim test (FST) for behavioral despair were carried out to conclude the CUMS model of depressive-like behavior. Morris water maze (MWM) was used to assess memory and learning. All the behavioral tests were recorded with ANY-maze (Stoelting, IL, USA) tracking system.

Open Field Test

The OFT apparatus consisted of a 60 × 60-cm square, black wooden arena surrounded by 30-cm high walls with 25 equal squares divided on the floor by white-colored lines. The total distance traveled and number of lines crossing was measured as the exploratory behavior over a period of 5 min. The exploratory behavior was recorded by the over-head camera attached to ANY-maze (Stoelting, IL, USA) tracking system. The open field arena was cleaned with 70 percent ethanol between successive trials. The data were calculated by two-way ANOVA multiple comparisons by using post hoc Tukey test and are presented in Fig. 2.

Forced Swim Test

The FST was conducted in an open cylindrical glass container (diameter 15 cm, height 45 cm), filled with tap water up to 35 cm high (25 ± 2 °C) so that rat could not climb over

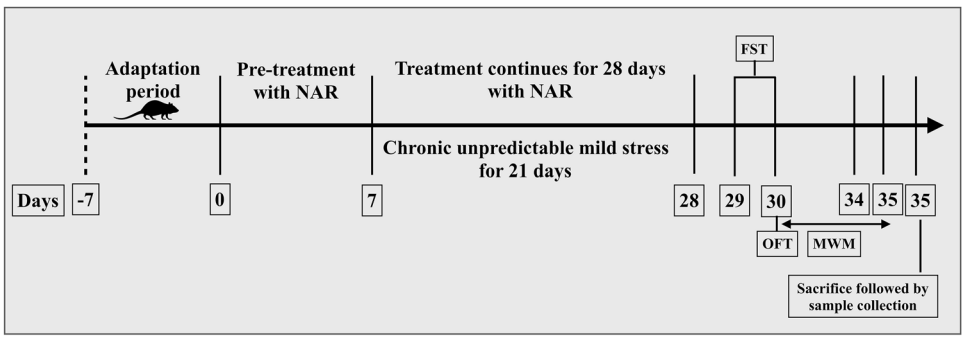

Fig. 1 Schematic representation of the CUMS procedure and treatments in male Wistar rats

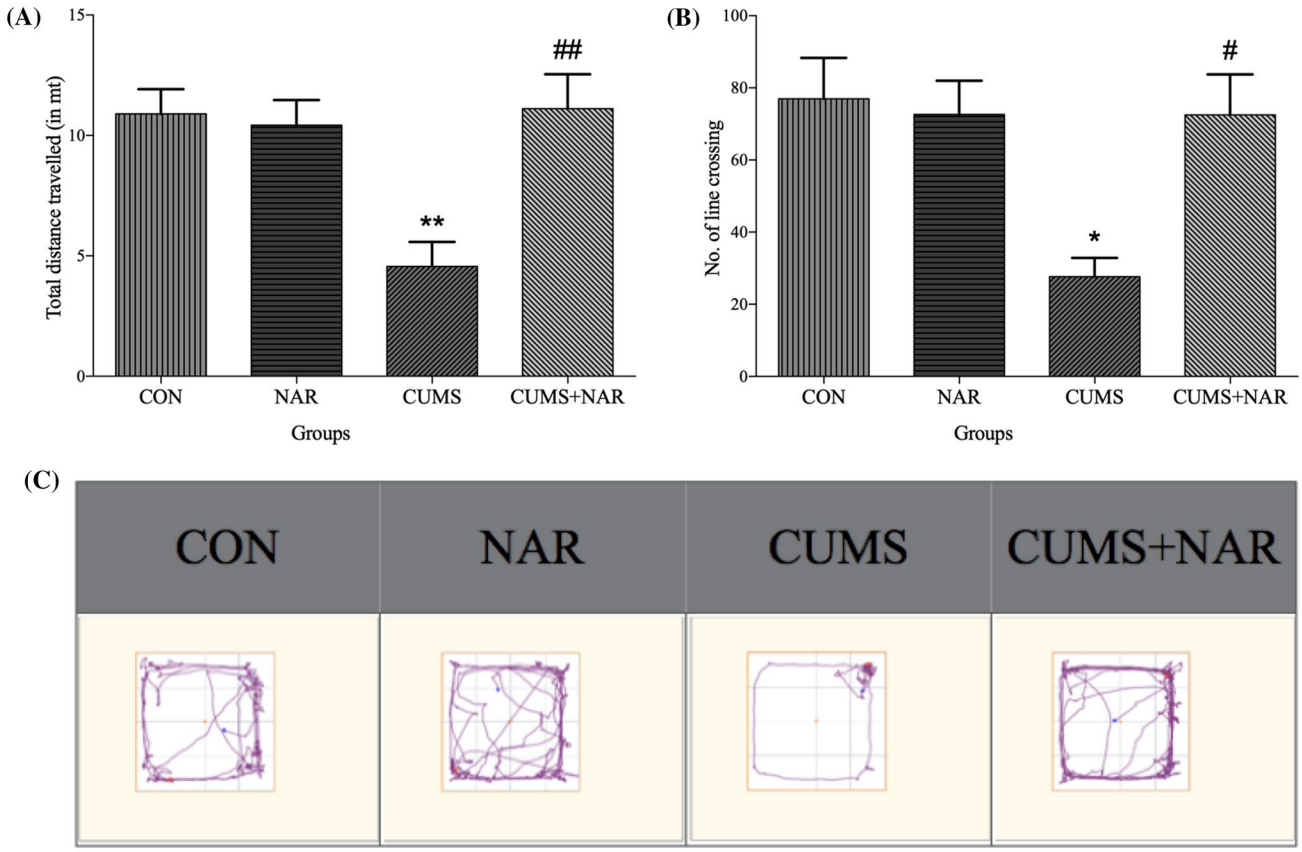

Fig. 2 **a** Total distance traveled in open field test (OFT) monitored for 5 min., results are expressed as Mean ± SE ($n = 6$). Significant difference between CON and CUMS is indicated by $^{**}p < 0.01$ and by $^{\#\#}p < 0.01$ between CUMS and CUMS + NAR group. **b** Number of line crossing, results are expressed as Mean ± SE ($n = 6$). Significant difference between CON and CUMS is indicated by $^{*}p < 0.5$ and by $^{\#}p < 0.05$ between CUMS and CUMS + NAR group. **c** ANY-maze images of open field test

the ridges or touch its limb or tail at the bottom of the cylindrical chamber. Briefly, the FST was a two-day procedure, 15-min trial was given on first day for training (no data collected), and the next day final tests were conducted for 5 min and the total time immobile was recorded by ANY-maze (Stoelting, IL, USA) tracking system. At the end of test, the wet rats were wiped with towel and kept in their respective home cages. Water was replaced in the cylinder after each test. The data were calculated by two-way ANOVA multiple comparisons by using post hoc Tukey test and are presented as Fig. 3.

Morris Water Maze

In the last week of treatment, the memory and learning was assessed by MWM as previously described (Liu et al. 2014). A circular tank 132 cm in diameter and 60 cm height was filled with opaque water (25 ± 2 °C) and the tank was divided into four virtual quadrants. A 10-cm diameter platform was placed in the center of a target quadrant (south west). On the first day, rats were trained to remember the

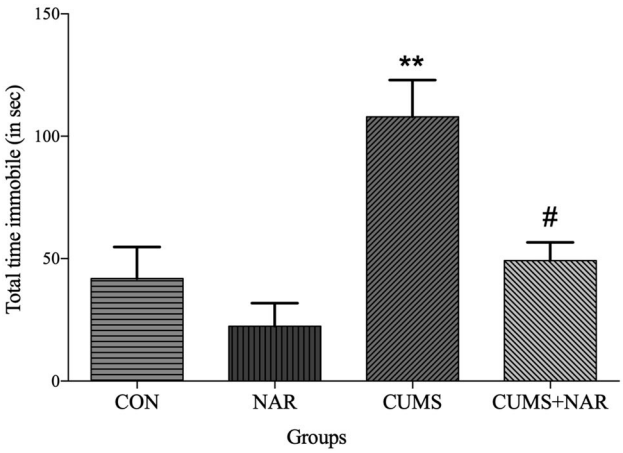

Fig. 3 Total time immobile in forced swim test (FST) monitored for 5 min, results are expressed as Mean ± SE ($n = 6$). Significant difference between CON and CUMS is indicated by $^{**}p < 0.01$ and by $^{\#}p < 0.05$ between CUMS and CUMS + NAR group

visible platform. From the next day, the platform was submerged 2.5 cm below the surface of water so that rats could not see it directly and subjected to three trials (north, east, and west) each day for a period of 5 days. Each trial lasted 60 s only. During trial session, the platform remained hidden to measure escape latency. If the rat could not find the platform itself within 60 s, it was placed on the platform by the investigator to stay there for 30 s. On the sixth day, probe trial was carried out during which the platform was removed and rats were allowed to search the platform for 60 s. The behavior was monitored and time spent in the target quadrant was recorded by ANY-maze (Stoelting, IL, USA) tracking system. After each trial, the rats were gently wiped with a towel and returned to their home cages. The data were calculated by two-way ANOVA multiple comparisons by using post hoc Tukey test and are presented as Fig. 4.

Tissue Sampling

At the end of behavioral experiments, the rats were sacrificed under deep anesthesia by chloral hydrate (400 mg/kg body wt.), the brains were dissected in cold phosphate buffer saline (PBS) and immediately hippocampus and cortex were separated. Samples were snap frozen in liquid nitrogen and stored at − 20 °C. Paraformaldehyde perfusion was done to collect sample for H&E staining.

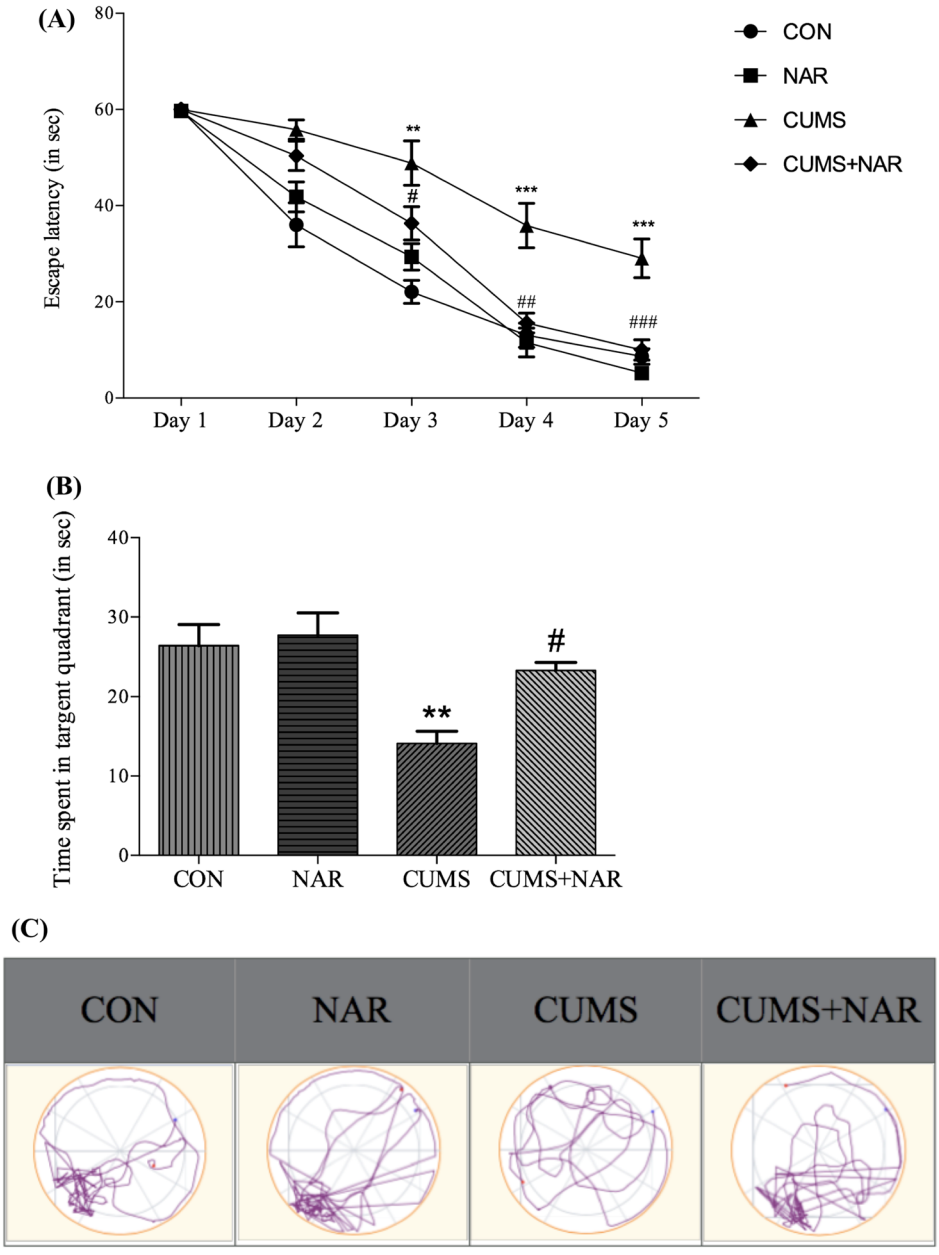

Fig. 4 **a** Escape latency in Morris water maze (MWM), results are expressed as Mean ± SE ($n = 6$). Significant difference between CON and CUMS is indicated by $^{***}p < 0.001$ and by $^{\#\#\#}p < 0.001$ between CUMS and CUMS + NAR group. **b** Time spent in the target quadrant, results are expressed as Mean ± SE ($n = 6$). Significant difference between CON and CUMS is indicated by $^{**}p < 0.01$ and by $^{\#}p < 0.05$ between CUMS and CUMS + NAR group. **c** ANY-maze images of MWM

Histopathological Analysis by Hematoxylin and Eosin (HE) Staining

Histopathological analysis was done as previously described by Gu et al. (2014). The rats were anesthetized by chloral hydrate (400 mg/kg, i.p.) and perfused transcardially with phosphate buffer saline (PBS), followed by ice-cold 4% paraformaldehyde. Brains were harvested and stored in paraformaldehyde. After the dehydration process, brain was embedded in paraffin wax and consecutive coronal sections of 4 mm were collected from hippocampus and cortex according to the rat stereotaxic atlas and stained with HE. The number of cells in the hippocampal and cortex region was counted under a light microscope (×40 magnification, Nikon Eclipse Ci-L microscope). Pyknotic cells or cells that underwent nuclear fragmentation were excluded. The data were calculated by two-way ANOVA multiple comparisons by using post hoc Tukey test and are presented as Fig. 5.

Reverse Transcriptase PCR and Real-Time PCR

Total RNA was extracted from the rat hippocampus using TRI reagent following the manufacturer's instructions. cDNA was synthesized from total RNA using M-MLV Reverse Transcriptase. The reverse transcriptase PCR (RT-PCR) and real-time PCR (qRT-PCR) were performed to check the expression of BDNF, Shh, GLI1, NKX2.2, and PAX6 (Primer sequences mentioned in Table 1). RT-PCR was followed by agarose gel electrophoresis and the analysis was carried out by using FlourChem-E System, CA, USA. Thereafter, to determine quantitative mRNA expression, qRT-PCR was performed as described by Schmittgen et al. (Schmittgen and Livak 2008). The qRT-PCR was conducted on LightCycler 480 Instrument (Roche). The data were calculated by one-way ANOVA multiple comparisons by using post hoc Tukey test and are presented as Fig. 6. We also calculated the data by non-parametric test using Kruskal–Wallis test multiple comparisons, presented in supplementary material as Figure S1.

Statistical Analysis

The data of behavioral tests and histopathological analysis were shown as mean ± SEM, analyzed by two-way ANOVA multiple comparisons by using post hoc Tukey test. For escape latency repeated measure, two-way ANOVA was used. For qRT-PCR, one-way ANOVA multiple comparisons by using post hoc Tukey test was used. We also performed the non-parametric analyses of the qRT-PCR data by using Kruskal–Wallis test multiple comparisons, presented in supplementary material. GraphPad Prism 7 (for Mac OSX) was used for the analysis. A value of $p < 0.05$ was regarded as significant.

Results

Effect of Naringenin on Locomotor Activity

In the open field test, as presented in Fig. 2a, the total distance traveled in CUMS group was significantly decreased as compared to CON group [$F(3,15) = 10.21$, $p < 0.01$, two-way ANOVA, Tukey test], which was significantly increased by NAR pre-administration, CUMS versus CUMS + NAR ($p < 0.01$). Moreover, number of line crossing was also decreased significantly in CUMS-exposed rats as compared with CON group [$F(3,15) = 5.351$, $p < 0.05$, two-way ANOVA, Tukey test] as shown in Fig. 2b, which was also increased by NAR pre-administration, CUMS versus CUMS + NAR ($p < 0.05$).

Effect of Naringenin on Forced Swim Test

As shown in Fig. 3 NAR reverses the despair behavior caused by CUMS. Significant increase was observed in total time immobile CON versus CUMS [$F(3,15) = 10.21$, $p < 0.01$, two-way ANOVA, Tukey test] in forced swim test, which was significantly reversed by NAR pre-administration, CUMS versus CUMS + NAR ($p < 0.05$).

Effect of Naringenin on Memory and Learning

In Morris water maze, we observed that CUMS increased learning and memory deficits; however, pre-treating with NAR reversed these effects. As displayed in Fig. 4a, significant decrease was observed in the escape latency [$F(3,15) = 41.67$, $p < 0.001$, two-way ANOVA, Bonferroni's test] between the CON and CUMS group during the 5 days of training period. In the final probe trial (Fig. 4b), there was also significant decrease in time spent in the target quadrant, when comparing CON versus CUMS [$F(3,15) = 8.768$, $p < 0.01$, one-way ANOVA, Tukey test], this was significantly increased in CUMS + NAR as compared to CUMS group ($p < 0.05$). These results suggested that NAR prevented the learning and memory deficit associated with CUMS.

Effect of Naringenin on CUMS Expedited Histopathological Changes

In HE staining, we examined the morphological changes, cell count, and arrangement of hippocampal CA1 cells, and likewise in cortical cells. As shown in Fig. 5a, CUMS triggered morphological deformity in the hippocampal CA1 and cortex region (Fig. 5b). Cells in the CA1 hippocampal region exhibit a regular arrangement, the edges were distinct

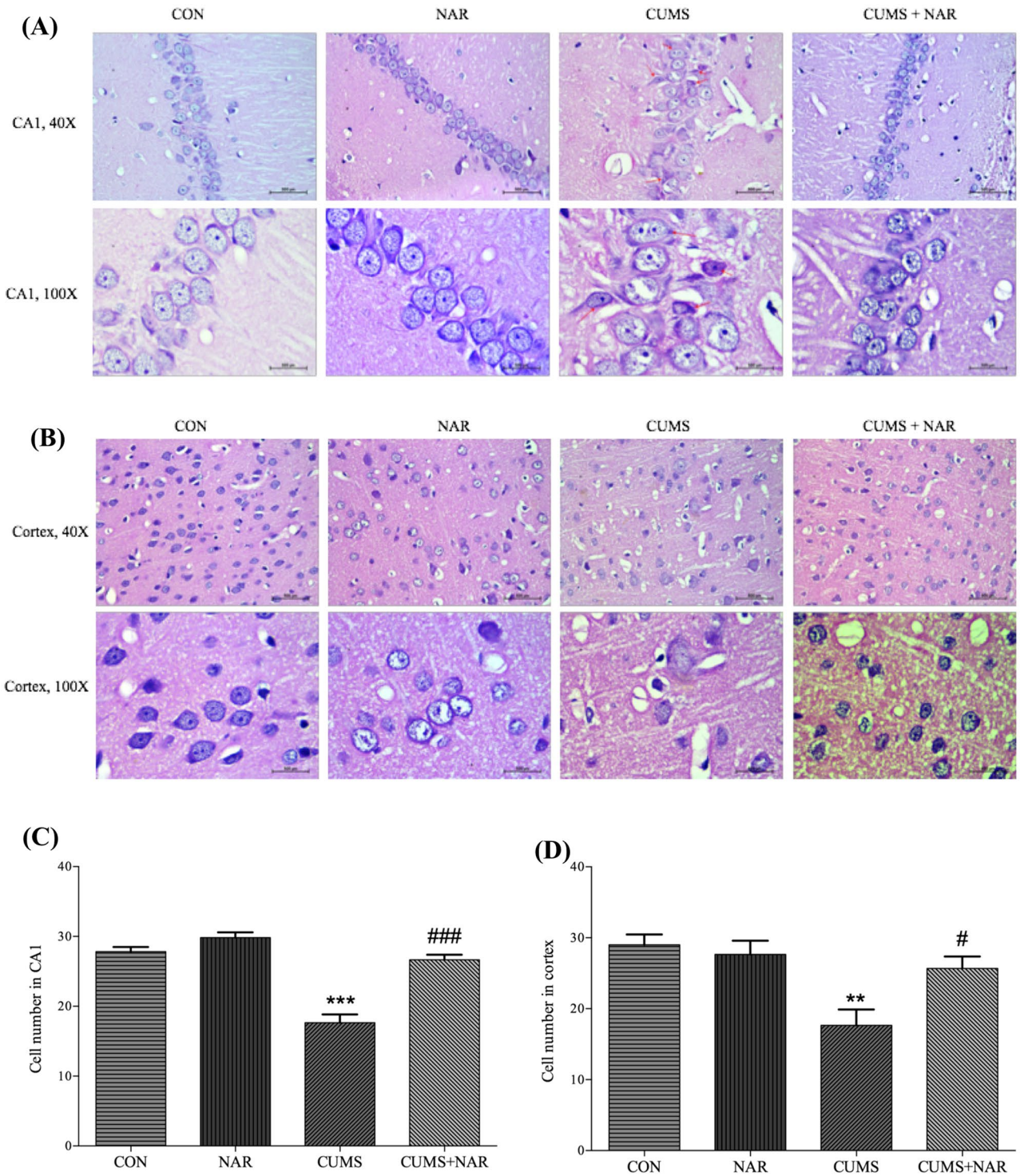

Fig. 5 **a** Representative light micrographs of HE-stained CA1 hippocampal cells at 40× and 100×. **b** Representative light micrographs of HE-stained cortical cells at 40× and 100×. **c** Number of cells in the CA1 region, data are expressed as Mean ± SE ($n = 6$). Significant difference between CON and CUMS is indicated by $^{***}p < 0.001$ and by $^{###}p < 0.001$ between CUMS and CUMS + NAR group. **d** Number of cells in cortical region, data are expressed as Mean ± SE ($n = 6$). Significant difference between CON and CUMS is indicated by $^{**}p < 0.01$ and by $^{#}p < 0.05$ between CUMS and CUMS + NAR group

Table 1 Primers sequences for the semi-quantitative reverse transcriptase PCR

Sr. No.	Symbol	Forward primer	Reverse primer
1	β-Actin	5′TCTTCCAGCCTTCCTTCCTG3′	5′CACACAGAGTACTTGCGCTC3′
2	BDNF	5′GGAGTACATATCGGCCACCA3′	5′GTGCTCAAAAGTGTCAGCCA3′
3	Shh	5′ACTATGAGGGTCGAGCAGTG3′	5′CCCCGGGACTTAGATCCTTC3′
4	GLI1	5′CTTCAAGGCCCAGTACATGC 3′	5′GATCTGTGTAGCGCTTGGTG3′
5	NKX2.2	5′CAACGATGAAGAAGGCTCGG3′	5′CCCTGGGTCTCCTTGTCATT3′
6	PAX6	5′AGCGGTTGGGTATTCAGGAA 3′	5′CAACCACATGAGCCAACACA3′

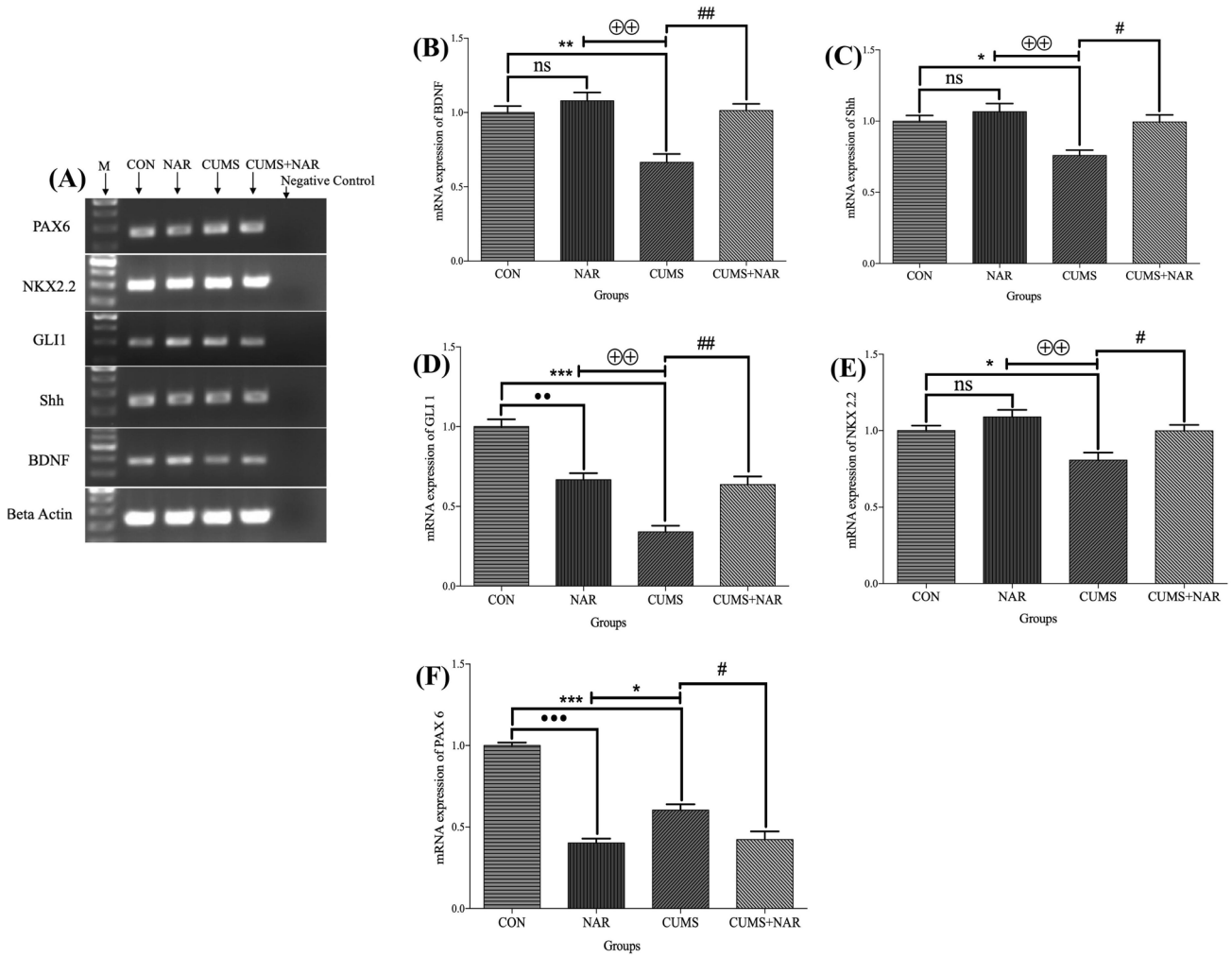

Fig. 6 mRNA expression of genes by RT-PCR and qRT-PCR in hippocampus. **a** RT-PCR mRNA expression of housekeeping gene β-Actin, BDNF, Shh, GLI1, NKX2.2, and PAX6. **b–f** qRT-PCR gene expression results are expressed as Mean ± SE ($n = 3$). **b** BDNF expression; Significant difference between CON and CUMS is $^{**}p < 0.01$, CUMS and CUMS + NAR is $^{\#\#}p < 0.01$, CON and NAR is not significant, and NAR and CUMS is $^{\oplus\oplus}p < 0.01$. **c** Shh expression; Significant difference between CON and CUMS is $^{*}p < 0.05$, CUMS and CUMS + NAR is $^{\#}p < 0.05$, CON and NAR is not significant, and NAR and CUMS is $^{\oplus\oplus}p < 0.01$. **d** GLI1 expression; Significant difference between CON and CUMS is $^{***}p < 0.001$, CUMS and CUMS + NAR is $^{\#\#}p < 0.01$, CON and NAR is $^{\bullet\bullet}p < 0.01$, and NAR and CUMS is $^{\oplus\oplus}p < 0.01$. **e** NKX2.2 expression; Significant difference between CON and CUMS is $^{*}p < 0.05$, CUMS and CUMS + NAR is $^{\#}p < 0.05$, CON and NAR is not significant, and NAR and CUMS is $^{\oplus\oplus}p < 0.01$. (6F) PAX6 expression, Significant difference between CON and CUMS is $^{***}p < 0.001$, CUMS and CUMS + NAR is $^{\#}p < 0.05$, CON and NAR is $^{\bullet\bullet\bullet}p < 0.001$, and NAR and CUMS is $^{\oplus}p < 0.05$. M- Marker/DNA ladder of 1000 bp and ns denotes not significant

and a clear nucleus was seen in the CON and NAR groups. Also the cells of NAR and CUMS + NAR group seem to be more rounded and nourished as compared to CUMS group. For the number of cells in CA1 and cortex region, post hoc comparisons indicated that the CUMS group showed significantly smaller number of cells compared to CON and CUMS + NAR group. The result is expressed as number of cells in CA1 (Fig. 5c) and significant decrease was observed, CON versus CUMS [F (3,15) = 47.57, $p < 0.001$, two-way ANOVA, Tukey test], which was significantly reversed by NAR pre-administration, CUMS versus CUMS + NAR ($p < 0.001$). Moreover, in cortex (Fig. 5d), significant decrease in the cell numbers was also observed in CUMS when compared to CON group [F (3,15) = 8.345, $p < 0.05$, one-way ANOVA, Tukey test], similarly it was significantly reversed by NAR pre-administration, CUMS versus CUMS + NAR ($p < 0.05$). We found that pre-treatment with NAR, protected cell death in hippocampal CA1 region and cortex region caused by CUMS.

Effect of Naringenin on BDNF Expression

The mRNA expression of BDNF was observed using RT-PCR and qRT-PCR in the rat hippocampus. RT-PCR results showed BDNF expression was decreased in the CUMS as compared to CON group (Fig. 6a); however, this declined expression triggered by CUMS was retrieved by NAR pre-administration (CUMS + NAR group). Further quantitative analysis of BDNF mRNA expression by using qRT-PCR confirmed the decline in the CUMS [F (3, 8) = 13.76, $p < 0.01$, one-way ANOVA, Tukey test] compared to CON group and NAR group and this low mRNA expression was recovered by NAR in CUMS + NAR group ($p < 0.01$) as shown in Fig. 6b. BDNF mRNA expression was also increased in NAR group as compared to CON but not significantly, which suggests that NAR shows neuroprotective effect.

Effect of Naringenin on Shh Signaling Pathway

The mRNA expression of Shh, GLI1, NKX2.2, and PAX6 was examined in the hippocampus of the male Wistar rats as shown in Fig. 6a by using RT-PCR and, respectively, by qRT-PCR. Our qRT-PCR results showed significant decrease in Shh mRNA expression (Fig. 6c) in CUMS [F (3, 8) = 8.22, $p < 0.05$, one-way ANOVA, Tukey test], when compared to CON and NAR group ($p < 0.01$), and pre-treatment with NAR recovered this decreased Shh expression ($p < 0.05$) in CUMS rats (CUMS + NAR). There was no significant change in Shh mRNA expression while comparing CON versus NAR groups.

GLI1 mRNA expression (Fig. 6d) was decreased significantly in the CUMS group [F (3, 8) = 36.72, $p < 0.001$, one-way ANOVA, Tukey test] as compared to CON and NAR group ($p < 0.01$), which was significantly increased by NAR pre-administration in CUMS + NAR group ($p < 0.01$). GLI1 expression also decreased in NAR group ($p < 0.01$) as compared to CON group.

Moreover, we also observed NKX2.2 expression (Fig. 6e) decreased in the CUMS group [F (3, 8) = 8.14, $p < 0.05$, one-way ANOVA, Tukey test] compared to CON and NAR group ($p < 0.01$), which was significantly recovered by NAR pre-administration in CUMS + NAR group ($p < 0.05$). We did not observe any significant change in the NKX2.2 expression between CON and NAR group.

The expression level of PAX6 (Fig. 6f) decreased in CUMS group and NAR group as compared to CON group [F (3, 8) = 64.01, $p < 0.001$, one-way ANOVA, Tukey test]. Interestingly, PAX6 mRNA expression was increased in CUMS group compared to NAR ($p < 0.05$) and CUMS + NAR groups, respectively ($p < 0.05$).

Discussion

In the present study, our results showed that oral pre-administration of NAR exhibits antidepressant-like effect in CUMS model of depressive-like behavior in rats. We also found that NAR has the potential to ameliorate memory and learning deficits associated with depressive behavior. Intriguingly, we observed the involvement of canonical Shh-GLI1 signaling in the antidepressant effect of NAR in CUMS-induced depressive behavior.

CUMS is a well-established and the most validated animal model of depressive-like behavior (Willner et al. 1992; Czeh et al. 2016; Boyko et al. 2015). CUMS model has been used for studying underlying mechanisms involved in depression and exploring the novel potential targets for tentative antidepressants (Banasr et al. 2007). Despair behavior is characterized as one of the core criteria in the diagnosis of major depression and is defined as increased immobility time in forced swim test. The animal is placed in a cylindrical vessel filled with water and lack of struggling represents behavioral despair (Pollak et al. 2010). Forced swim test is commonly used to evaluate the efficacy of antidepressant drugs. Exploration-based behavioral test like open field test is also used to evaluate antidepressant drugs as it shows face and construct validity of CUMS (Berton and Nestler 2006), in which reduced locomotor activity corresponds to the depression-like behavior (Boyko et al. 2015).

We observed that 21 days of CUMS significantly increased the immobility time in FST and decreased locomotor activity in OFT, which was successfully reversed by NAR pre-treatment in rats. Our behavioral results are consistent with previous studies in which CUMS rat model shows decreased locomotor activity and increased immobility time.

These antidepressant effects of NAR are in agreement with previous studies in other animal models of depressive behavior (Yi et al. 2012, 2014).

Furthermore, depression is found to be associated with impaired memory and learning in murine model (Conrad 2010) and this is also corroborated by various clinical studies on depressed patients (Burt et al. 1995; Bremner et al. 2000). Depressive effects induced by chronic stress could impair cognitive functions (Cuadrado-Tejedor et al. 2011; Song et al. 2006). We performed MWM test as this is one of the widely used methods to evaluate the hippocampal-dependent spatial learning and memory (Morris 1984). Moreover, as chronic stress alters the spatial memory, MWM has been used to observe memory deficits caused by CUMS-induced depressive behavior in animal models (Yan et al. 2017; Hu et al. 2017). Our results demonstrated that CUMS caused significant memory and learning impairment in rats, which can be reversed by NAR pre-treatment.

Hippocampus is the primary structure necessary for declarative memory (Roozendaal et al. 2009; Phelps 2004), and considering the role of hippocampus CA1 region in the memory and learning, we performed histopathological examination, to assess the strength of the cells and morphological changes in this region. We found that, in the CUMS group, the CA1 cells were irregularly arranged with lower cell number as compared to CON and NAR group. And also we counted the cells in the cortical region, as cortex is also involved in the cognitive functions. We found that in the CUMS group the cells were deformed and nucleus became ambiguous in both brain regions. However, NAR pre-treatment reversed the neuronal damage and displayed hippocampal and cortical cell strength, which corresponds to improved memory and leaning associated with depression-like behavior.

CUMS is known to decrease hippocampal neurogenesis, which can be reversed by antidepressant drug therapies (Patel et al. 2016). Interestingly, BDNF and Shh signaling play an important role in neurogenesis (Malberg and Duman 2003; Tayyab et al. 2017; Yao et al. 2016). Various studies suggest that hippocampal BDNF expression decreases in depression (Castren and Rantamaki 2010; Duman and Monteggia 2006). In our study, we also observed the similar result of BDNF. BDNF expression was decreased in the CUMS and pre-treatment with NAR increased its expression and showed antidepressant activity (Yi et al. 2014; Castren and Rantamaki 2010).

Shh is a powerful regulator of adult hippocampal neurogenesis (Yao et al. 2016). Recently, a study reported that Shh expression downregulates in prenatal stress caused by CUMS in the hippocampus region of brain (Fatima et al. 2019). Similarly, in our study, RT-PCR and qRT-PCR results revealed that Shh expression downregulates in the CUMS group as compared to CON and NAR group (positive control). Interestingly, pre-treatment with NAR restores these depleted Shh levels in CUMS + NAR group and showed neuroprotective effect by NAR.

GLI1 is the main downstream transcription factor of Shh signaling pathway, which further regulates the homeodomain transcription factor PAX6 and NKX2.2 (Shahi et al. 2010, 2012). We observed robust decrease in GLI1 mRNA expression in the CUMS group as compared to CON group and this low expression was upregulated by NAR pre-administration in CUMS + NAR group. This downregulated expression of GLI1 can be potentially correlated with Shh-GLI1 signaling in CUMS.

Furthermore, we also investigate the effect of CUMS and NAR on NKX2.2 and PAX6, which are considered downstream homeodomain transcriptional factors of Shh-GLI1 signaling. Various studies demonstrated the role of these two homeodomain transcription factors at early embryonic development stage as well as in the various brain tumors (Shahi et al. 2010, 2012; Balaskas et al. 2012). We observed significant decrease in the NKX2.2 expression in CUMS compared to CON and NAR group which was significantly reversed by NAR pre-administration in CUMS + NAR group.

Furthermore, PAX6 expression was also significantly decreased in CUMS compared to CON group, which was significantly increased by NAR pre-treatment in CUMS + NAR group. We also observed significantly decreased expression of PAX6 in NAR group compared to CON and CUMS group. There are studies that explain these homeodomain transcription factors are regulated by graded response of Shh signaling (Balaskas et al. 2012). Therefore, this low expression of PAX6 in NAR group might be repressed by NKX2.2 over expression in NAR group compared to CON group.

These results suggested the involvement of Shh-GLI1 signaling cascade in CUMS-induced depressive behavior and in providing the antidepressant and neuroprotective effects by NAR. Moreover, the role of NKX2.2 and PAX6 in animal model of depressive behavior has been reported for the first time in our study.

We believe that we would need to further explore the interaction of Shh, GLI1, PAX6, and NKX2.2 for identification of potential therapeutic targets to treat depressive-like behavior. The present study suggests the possible antidepressant effect of NAR in CUMS model of male Wistar rats, and its ability to ameliorate learning and memory alterations caused by CUMS. In addition, this study suggested the role of Shh-GLI1 signaling pathway in depressive rat model, which further regulates NKX2.2 and PAX6.

Acknowledgements This work was supported by Interdisciplinary Brain Research Centre (IBRC), Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. We are thankful to the Coordinator and all members of IBRC.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Ethical Approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

References

Balaskas, N., Ribeiro, A., Panovska, J., Dessaud, E., Sasai, N., Page, K. M., et al. (2012). Gene regulatory logic for reading the Sonic Hedgehog signaling gradient in the vertebrate neural tube. *Cell, 148*(1–2), 273–284. https://doi.org/10.1016/j.cell.2011.10.047.

Banasr, M., Valentine, G. W., Li, X. Y., Gourley, S. L., Taylor, J. R., & Duman, R. S. (2007). Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. *Biological Psychiatry, 62*(5), 496–504. https://doi.org/10.1016/j.biopsych.2007.02.006.

Banerjee, S. B., Rajendran, R., Dias, B. G., Ladiwala, U., Tole, S., & Vaidya, V. A. (2005). Recruitment of the Sonic hedgehog signalling cascade in electroconvulsive seizure-mediated regulation of adult rat hippocampal neurogenesis. *European Journal of Neuroscience, 22*(7), 1570–1580. https://doi.org/10.1111/j.1460-9568.2005.04317.x.

Barnes, P. M., Bloom, B., & Nahin, R. L. (2008). Complementary and alternative medicine use among adults and children: United States, 2007. *National Health Statiatics Report, 12,* 1–23.

Berton, O., & Nestler, E. J. (2006). New approaches to antidepressant drug discovery: Beyond monoamines. *Nature Reviews Neuroscience, 7*(2), 137–151. https://doi.org/10.1038/nrn1846.

Boyko, M., Kutz, R., Grinshpun, J., Zvenigorodsky, V., Gruenbaum, S. E., Gruenbaum, B. F., et al. (2015). Establishment of an animal model of depression contagion. *Behavioural Brain Research, 281,* 358–363. https://doi.org/10.1016/j.bbr.2014.12.017.

Bremner, J. D., Narayan, M., Anderson, E. R., Staib, L. H., Miller, H. L., & Charney, D. S. (2000). Hippocampal volume reduction in major depression. *American Journal of Psychiatry, 157*(1), 115–118. https://doi.org/10.1176/ajp.157.1.115.

Briscoe, J., & Therond, P. P. (2013). The mechanisms of Hedgehog signalling and its roles in development and disease. *Nature Reviews Molecular Cell Biology, 14*(7), 416–429. https://doi.org/10.1038/nrm3598.

Burt, D. B., Zembar, M. J., & Niederehe, G. (1995). Depression and memory impairment: a meta-analysis of the association, its pattern, and specificity. *Psychological Bulletin, 117*(2), 285–305.

Castren, E., & Rantamaki, T. (2010). The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. *Developmental Neurobiology, 70*(5), 289–297. https://doi.org/10.1002/dneu.20758.

Chtourou, Y., Slima, A. B., Gdoura, R., & Fetoui, H. (2015). Naringenin mitigates iron-induced anxiety-like behavioral impairment, mitochondrial dysfunctions, ectonucleotidases and acetylcholinesterase alteration activities in rat hippocampus. *Neurochemical Research, 40*(8), 1563–1575. https://doi.org/10.1007/s11064-015-1627-9.

Conrad, C. D. (2010). A critical review of chronic stress effects on spatial learning and memory. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 34*(5), 742–755. https://doi.org/10.1016/j.pnpbp.2009.11.003.

Cuadrado-Tejedor, M., Ricobaraza, A., Del Rio, J., Frechilla, D., Franco, R., Perez-Mediavilla, A., et al. (2011). Chronic mild stress in mice promotes cognitive impairment and CDK5-dependent tau hyperphosphorylation. *Behavioural Brain Research, 220*(2), 338–343. https://doi.org/10.1016/j.bbr.2011.01.005.

Czeh, B., Fuchs, E., Wiborg, O., & Simon, M. (2016). Animal models of major depression and their clinical implications. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 64,* 293–310. https://doi.org/10.1016/j.pnpbp.2015.04.004.

Dubey, V. K., Ansari, F., Vohora, D., & Khanam, R. (2015). Possible involvement of corticosterone and serotonin in antidepressant and antianxiety effects of chromium picolinate in chronic unpredictable mild stress induced depression and anxiety in rats. *Journal of Trace Elements in Medicine and Biology, 29,* 222–226. https://doi.org/10.1016/j.jtemb.2014.06.014.

Duman, R. S., & Monteggia, L. M. (2006). A neurotrophic model for stress-related mood disorders. *Biological Psychiatry, 59*(12), 1116–1127. https://doi.org/10.1016/j.biopsych.2006.02.013.

Dwyer, A. V., Whitten, D. L., & Hawrelak, J. A. (2011). Herbal medicines, other than St. John's Wort, in the treatment of depression: a systematic review. *Alternative Medicine Review, 16*(1), 40–49.

Fatima, M., Srivastav, S., Ahmad, M. H., & Mondal, A. C. (2019). Effects of chronic unpredictable mild stress induced prenatal stress on neurodevelopment of neonates: Role of GSK-3beta. *Scientific Reports, 9*(1), 1305. https://doi.org/10.1038/s41598-018-38085-2.

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J., et al. (2013). Burden of depressive disorders by country, sex, age, and year: Findings from the global burden of disease study 2010. *PLoS Medicine, 10*(11), e1001547. https://doi.org/10.1371/journal.pmed.1001547.

Ghofrani, S., Joghataei, M. T., Mohseni, S., Baluchnejadmojarad, T., Bagheri, M., Khamse, S., et al. (2015). Naringenin improves learning and memory in an Alzheimer's disease rat model: Insights into the underlying mechanisms. *European Journal of Pharmacology, 764,* 195–201. https://doi.org/10.1016/j.ejphar.2015.07.001.

Gronli, J., Murison, R., Fiske, E., Bjorvatn, B., Sorensen, E., Portas, C. M., et al. (2005). Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. *Physiology & Behavior, 84*(4), 571–577. https://doi.org/10.1016/j.physbeh.2005.02.007.

Gu, H. F., Nie, Y. X., Tong, Q. Z., Tang, Y. L., Zeng, Y., Jing, K. Q., et al. (2014). Epigallocatechin-3-gallate attenuates impairment of learning and memory in chronic unpredictable mild stress-treated rats by restoring hippocampal autophagic flux. *PLoS ONE, 9*(11), e112683. https://doi.org/10.1371/journal.pone.0112683.

Heo, H. J., Kim, D. O., Shin, S. C., Kim, M. J., Kim, B. G., & Shin, D. H. (2004). Effect of antioxidant flavanone, naringenin, from Citrus junoson neuroprotection. *Journal of Agriculture and Food Chemistry, 52*(6), 1520–1525. https://doi.org/10.1021/jf035079g.

Hu, C., Luo, Y., Wang, H., Kuang, S., Liang, G., Yang, Y., et al. (2017). Re-evaluation of the interrelationships among the behavioral tests in rats exposed to chronic unpredictable mild stress. *PLoS ONE, 12*(9), e0185129. https://doi.org/10.1371/journal.pone.0185129.

Hunt, K. J., Coelho, H. F., Wider, B., Perry, R., Hung, S. K., Terry, R., et al. (2010). Complementary and alternative medicine use in England: Results from a national survey. *International Journal of Clinical Practice, 64*(11), 1496–1502. https://doi.org/10.1111/j.1742-1241.2010.02484.x.

Lahr, D., Beblo, T., & Hartje, W. (2007). Cognitive performance and subjective complaints before and after remission of major depression. *Cognitive Neuropsychiatry, 12*(1), 25–45. https://doi.org/10.1080/13546800600714791.

Lee, E., & Son, H. (2009). Adult hippocampal neurogenesis and related neurotrophic factors. *BMB Reports, 42*(5), 239–244.

Liu, D., Wang, Z., Gao, Z., Xie, K., Zhang, Q., Jiang, H., et al. (2014). Effects of curcumin on learning and memory deficits, BDNF, and ERK protein expression in rats exposed to chronic unpredictable stress. *Behavioural Brain Research, 271,* 116–121. https://doi.org/10.1016/j.bbr.2014.05.068.

Lu, B., Nagappan, G., & Lu, Y. (2014). BDNF and synaptic plasticity, cognitive function, and dysfunction. *Handbook of Experimental Pharmacology, 220,* 223–250. https://doi.org/10.1007/978-3-642-45106-5_9.

Lupien, S. J., Maheu, F., Tu, M., Fiocco, A., & Schramek, T. E. (2007). The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. *Brain and Cognition, 65*(3), 209–237. https://doi.org/10.1016/j.bandc.2007.02.007.

Malberg, J. E., & Duman, R. S. (2003). Cell proliferation in adult hippocampus is decreased by inescapable stress: Reversal by fluoxetine treatment. *Neuropsychopharmacology, 28*(9), 1562–1571. https://doi.org/10.1038/sj.npp.1300234.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods, 11*(1), 47–60.

Moussavi, S., Chatterji, S., Verdes, E., Tandon, A., Patel, V., & Ustun, B. (2007). Depression, chronic diseases, and decrements in health: Results from the World Health Surveys. *Lancet, 370*(9590), 851–858. https://doi.org/10.1016/S0140-6736(07)61415-9.

Nirmal, J., Babu, C. S., Harisudhan, T., & Ramanathan, M. (2008). Evaluation of behavioural and antioxidant activity of Cytisus scoparius Link in rats exposed to chronic unpredictable mild stress. *BMC Complementary Alternative Medicine, 8,* 15. https://doi.org/10.1186/1472-6882-8-15.

Organization, W. H. (2017). *Depression and other common mental disorders: Global health estimates*. Geneva: World Health Organization.

Patel, S. S., Mahindroo, N., & Udayabanu, M. (2016). Urtica dioica leaves modulates hippocampal smoothened-glioma associated oncogene-1 pathway and cognitive dysfunction in chronically stressed mice. *Biomedicine & Pharmacotherapy, 83,* 676–686. https://doi.org/10.1016/j.biopha.2016.07.020.

Pathak, L., Agrawal, Y., & Dhir, A. (2013). Natural polyphenols in the management of major depression. *Expert Opinion on Investigational Drugs, 22*(7), 863–880. https://doi.org/10.1517/13543784.2013.794783.

Phelps, E. A. (2004). Human emotion and memory: Interactions of the amygdala and hippocampal complex. *Current Opinion in Neurobiology, 14*(2), 198–202. https://doi.org/10.1016/j.conb.2004.03.015.

Pollak, D. D., Rey, C. E., & Monje, F. J. (2010). Rodent models in depression research: classical strategies and new directions. *Annals of Medicine, 42*(4), 252–264. https://doi.org/10.3109/07853891003769957.

Potvin, S., Charbonneau, G., Juster, R. P., Purdon, S., & Tourjman, S. V. (2016). Self-evaluation and objective assessment of cognition in major depression and attention deficit disorder: Implications for clinical practice. *Comprehensive Psychiatry, 70,* 53–64. https://doi.org/10.1016/j.comppsych.2016.06.004.

Rajendran, R., Jha, S., Fernandes, K. A., Banerjee, S. B., Mohammad, F., Dias, B. G., et al. (2009). Monoaminergic regulation of Sonic hedgehog signaling cascade expression in the adult rat hippocampus. *Neuroscience Letters, 453*(3), 190–194. https://doi.org/10.1016/j.neulet.2009.02.034.

Raza, S. S., Khan, M. M., Ahmad, A., Ashafaq, M., Islam, F., Wagner, A. P., et al. (2013). Neuroprotective effect of naringenin is mediated through suppression of NF-kappaB signaling pathway in experimental stroke. *Neuroscience, 230,* 157–171. https://doi.org/10.1016/j.neuroscience.2012.10.041.

Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature Reviews Neuroscience, 10*(6), 423–433. https://doi.org/10.1038/nrn2651.

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C(T) method. *Nature Protocols, 3*(6), 1101–1108. https://doi.org/10.1038/nprot.2008.73.

Shahi, M. H., Afzal, M., Sinha, S., Eberhart, C. G., Rey, J. A., Fan, X., et al. (2010). Regulation of sonic hedgehog-GLI1 downstream target genes PTCH1, Cyclin D2, Plakoglobin, PAX6 and NKX2 and their epigenetic status in medulloblastoma and astrocytoma. *BMC Cancer, 10,* 614. https://doi.org/10.1186/1471-2407-10-614.

Shahi, M. H., Rey, J. A., & Castresana, J. S. (2012). The sonic hedgehog-GLI1 signaling pathway in brain tumor development. *Expert Opinion on Therapeutic Targets, 16*(12), 1227–1238. https://doi.org/10.1517/14728222.2012.720975.

Song, L., Che, W., Min-Wei, W., Murakami, Y., & Matsumoto, K. (2006). Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacology, Biochemistry and Behavior, 83*(2), 186–193. https://doi.org/10.1016/j.pbb.2006.01.004.

Tayyab, M., Shahi, M. H., Farheen, S., Mariyath, M. P. M., Khanam, N., Castresana, J. S., et al. (2017). Sonic hedgehog, Wnt, and brain-derived neurotrophic factor cell signaling pathway crosstalk: potential therapy for depression. *Journal of Neuroscience Research*. https://doi.org/10.1002/jnr.24104.

Umukoro, S., Kalejaye, H. A., Ben-Azu, B., & Ajayi, A. M. (2018). Naringenin attenuates behavioral derangements induced by social defeat stress in mice via inhibition of acetylcholinesterase activity, oxidative stress and release of pro-inflammatory cytokines. *Biomedicine & Pharmacotherapy, 105,* 714–723. https://doi.org/10.1016/j.biopha.2018.06.016.

Willner, P., Muscat, R., & Papp, M. (1992). Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neuroscience and Biobehavioral Reviews, 16*(4), 525–534. https://doi.org/10.1016/S0149-7634(05)80194-0.

Yan, T., He, B., Wan, S., Xu, M., Yang, H., Xiao, F., et al. (2017). Antidepressant-like effects and cognitive enhancement of *Schisandra chinensis* in chronic unpredictable mild stress mice and its related mechanism. *Scientific Reports, 7*(1), 6903. https://doi.org/10.1038/s41598-017-07407-1.

Yao, P. J., Petralia, R. S., & Mattson, M. P. (2016). Sonic hedgehog signaling and hippocampal neuroplasticity. *Trends in Neurosciences, 39*(12), 840–850. https://doi.org/10.1016/j.tins.2016.10.001.

Yazir, Y., Utkan, T., Gacar, N., & Aricioglu, F. (2015). Resveratrol exerts anti-inflammatory and neuroprotective effects to prevent memory deficits in rats exposed to chronic unpredictable mild stress. *Physiology & Behavior, 138,* 297–304. https://doi.org/10.1016/j.physbeh.2014.10.010.

Yi, L. T., Li, J., Li, H. C., Su, D. X., Quan, X. B., He, X. C., et al. (2012). Antidepressant-like behavioral, neurochemical and neuroendocrine effects of naringenin in the mouse repeated tail suspension test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 39*(1), 175–181. https://doi.org/10.1016/j.pnpbp.2012.06.009.

Yi, L. T., Liu, B. B., Li, J., Luo, L., Liu, Q., Geng, D., et al. (2014). BDNF signaling is necessary for the antidepressant-like effect of naringenin. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 48,* 135–141. https://doi.org/10.1016/j.pnpbp.2013.10.002.

Zbarsky, V., Datla, K. P., Parkar, S., Rai, D. K., Aruoma, O. I., & Dexter, D. T. (2005). Neuroprotective properties of the natural phenolic antioxidants curcumin and naringenin but not quercetin and fisetin in a 6-OHDA model of Parkinson's disease. *Free Radical Research, 39*(10), 1119–1125. https://doi.org/10.1080/10715760500233113.