

Curcumin lessens unpredictable chronic mild stress-induced depression and memory deficits by modulating oxidative stress and cholinergic activity

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Abstract: Unpredictable chronic mild stress (UCMS) model is the most established method to study neurobiological mechanisms of depression. This work was intended to explore the efficacy of curcumin to revert the UCMS-induced oxidative burden and associated depression as well as potential of curcumin as an acetyl cholinesterase (AChE) inhibitor. Animals were initially grouped into control and curcumin (200 mg/kg, p.o) and further subdivided into unstressed and stressed groups. Depression and anxiety were evaluated by forced swim test (FST) and light/dark transition (LDT) while memory function was assessed by passive avoidance test (PAT). Effect of curcumin on oxidative stress following UCMS was determined by measuring peroxidation of lipid (LPO) and antioxidant enzyme activities. AChE activity was also determined. Findings showed that curcumin supplementation significantly attenuated the UCMS-induced depression and anxiety like symptoms, decreased the load of UCMS propagated oxidative stress by improving antioxidant enzymes activities. Curcumin also improved the memory function and exhibited inhibitory effect on AChE activity. In conclusion it can be suggested that supplementation of curcumin in daily life can help in combating the stress-induced depression and ever increasing load of oxidative stress. Study also highlights the anti-acetylcholinesterase potential of curcumin which may be responsible for improved memory function following UCMS.

Keywords: Curcumin, oxidative stress, AChE, UCMS, depression

INTRODUCTION

Depression is an undesirable neurological disorder that disrupts the physical and mental functions and affects almost 15-20 % of the population at some stage in life (Nollet *et al.*, 2013). It has been documented that by 2030 depression will become the principal reason of illnesses (Carnevali *et al.*, 2017). Repeated experience to stressful life events is a chief reason for the precipitation of different psycho-behavioral disorders, including depression and anxiety (Radley *et al.*, 2015). Exposure to UCMS protocol in rodents provokes reduced responsiveness to incentive that is anhedonia, major sign of depression (Mizuki *et al.*, 2014). In rodents UCMS increased aggression equally in resident-intruder test as well as between cage-mates which is comparable to annoying aggressive actions observed in patients of depression (Mineur *et al.*, 2006). Impaired learning and cognition have been documented following long term exposure to stress (Abush and Akirav, 2013). Neuroplasticity impairments in rodents have been reported following chronic stress (Kuipers *et al.*, 2013). At first UCMS acts as a stimulant and increases metabolic rate which in turn elevates the reactive oxygen species (ROS) production. Production of ROS is an effective

method to induce adaptation (Parsons, 1996). Although the excess ROS start to damage the cells, tissues and organs if its amount exceeds beyond the body's capacity to counterbalance it, which results in an unwanted outcomes of oxidative stress. Oxidative stress weakens the antioxidant defense and results in oxidative damage by altering the equilibrium between antioxidant and oxidant factors (Liu *et al.*, 2016). It has been stated that oxidative damages induced by UCMS are intricate in the aetiology of numerous disorders, including psychiatric disorders like hypertension, cognitive dysfunctions, disorders of endocrine like impotence, diabetes mellitus, ulcerative colitis and peptic ulceration (Kumar *et al.*, 2011; Bhattacharya and Muruganandam, 2003).

A number of studies have documented that majority of the adverse effects induced by UCMS including memory impairments, anxiety and depression like disorders can be reversed or repaired by long term treatment of antidepressant drugs (Willner, 2017; Li *et al.*, 2016; Tao *et al.*, 2016). Neuroprotective, antidepressant, anti-anxiety, antioxidant roles of curcumin have been well documented. Beneficial effects of curcumin on memory and anxiety like behavior following acute immobilization stress has also been reported from our lab previously (Haider *et al.*, 2015). In animals curcumin improves both the oxidative and energy-restricting effect of stress and

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restoring normal behavior (Rinwa and Kumar, 2012). Recent studies have shown that curcumin can attenuate neuronal structural abnormalities and biomarkers of inflammation and oxidative stress following chronic stress (Fan *et al.*, 2018; Tabrizi *et al.*, 2019). Chronic unpredictable stress adversely affects production of brain-protective compounds. This may account for altered mental behaviors observed in response to stressful situations. Researchers have come to realize that “emotional” or “psychological” stress negatively impacts our physical body, not just our sense of well-being. Therefore, this study was planned to examine the beneficial impact of curcumin on UCMS-induced behavioral deficits and oxidative stress, as uncertainty of stress have more negative impact on an individual’s psyche than a known one, perhaps due to failure to predict the event in the given frame of time. This work also intended to investigate the impact of curcumin on memory function by estimating enzyme AchE activity as increased activity of AchE diminishes the levels of acetylcholine in synapse.

MATERIALS AND METHODS

Chemicals and reagents

Curcumin, acetylthiocholine (ATC), dimethylsulfoxide (DMSO), (H₃NO.HCl) hydroxylamine hydrochloride and all supplementary reagents was bought from Sigma Chemical Co. (St. Louis, USA).

Experimental paradigm

In this study 24 male Albino-Wistar rats of weight ranging from 180-200 gm were used. All the investigational techniques were according to National Institute of Health Guide for Care and Use of Laboratory Animals and approved by the institutional ethics and animal care committee (Publication No. 85-23, revised 1996). Curcumin was dissolved in DMSO and administered orally (200 mg/kg) for 10 days whereas the control rats were administered with equal amounts of DMSO (vehicle) daily. Protective effects of curcumin have been documented previously on the selected dose (Hocking *et al.*, 2018; Emoto *et al.*, 2013). After 10 days both groups (16 rats in each group) were separated into groups of unstressed and stressed rats (n=6). Group of stressed rats were exposed to UCMS in separate room whereas the control rats stood kept in their home cages. Drugs were administered to both stressed and unstressed groups 1 h before every stress session.

Protocol for UCMS induction

Methodology of UCMS is basically identical to the procedure as defined by Bondi *et al.*, (2008) with little amendments. The UCMS procedure was applied for consecutive 20 days. Rats were subjected to the following types of stressors (one stressor a day) 1 h shaking/crowding, 3 min period of warm water (25°C)

swim, 3 min period of cold water (12°C) swim, 5 min exposure to electric bell, 30 min restraint stress, 14 h period of 45° cage tilt, overnight soiled cage, high density housing for 24 h, tail pinch, overnight illumination with strong light. Each stressor was repeated two times during the 20 days stress procedure. After 24 h of the last stressor behavioral analysis was performed.

Behavioral analysis

Light/Dark Transition (LDT) Test

Behavioral assessment for anxiety like behavior was done by using light/dark transition test (LDT) according to the method of Khaliq *et al.* (2012). First the rat was placed in the light compartment and the extent of anxiety was determined by monitoring the number of entries and time spent in the light box for a cutoff time of 5 minutes.

Forced swim test (FST)

FST was used for the assessment of depression-like behaviors according to the procedure described by Haider *et al.* (2015). The rat's swimming behavior was monitored for 300 seconds. The struggling time during which the rat struggles to escape from tank was recorded.

Passive avoidance test (PAT)

To assess memory and learning function in rats, passive avoidance test (PAT) was performed and the methodology was basically identical as defined by Tabassum *et al.* (2017). The time taken by the rat to enter the dark compartment was monitored (step-through latency) for 3 minutes (cutoff time). All the behaviors were executed in a closed and quiet room in balance design.

Biochemical estimations

On the same day immediately after behavioral analysis all the animals were guillotined. The brains were taken out from the cranial cavity, dipped in chilled solution of 0.9% NaCl, and weighed. Brain homogenate (10% w/v) was made by centrifugation (12,000 × g, 20 min at 4°C) with phosphate buffer of pH 7.4 (0.1 M) for the examination of LPO, CAT, GPx, and SOD activities. CAT, SOD, GPx and AchE activities were determined according to the methods of Sinha (1972), Flohe and Gunzler (1984), and Chidambara *et al.* (2002) respectively. Activity of AchE was also estimated in accordance to the methods of Ellman *et al.* (1961). Analysis of oxidative stress was done by means of LPO and the methodology was basically identical as defined by Chow and Tappel (1972) with minor amendments (Haider *et al.*, 2015).

STATISTICAL ANALYSIS

The statistical evaluation was performed by the two-way ANOVA. Post-hoc investigation was done by Tukey’s test for multiple comparisons using SPSS version 20. Results are stated as mean ± SD. Values *p*<0.05 were considered as significant.

RESULTS

Impact of pre-supplementation of curcumin on immobility time during FST

The effect of curcumin on depression like symptoms produced by UCMS is presented in (fig. 1). Analysis of data revealed a significant impact of stress [$F(1, 20) = 75.44, p < 0.01$], curcumin [$F(1, 20) = 83.36, p < 0.01$], and association between stress and curcumin [$F(1, 20) = 8.93, p < 0.01$] on immobility time in FST. Post-hoc showed that administration of curcumin significantly ($p < 0.01$) decreased the immobility time in comparison to respective control rats, while rats subjected to UCMS exhibited substantial ($p < 0.01$) rise in immobility in comparison to unstressed control rats. UCMS-induced increased immobility was significantly ($p < 0.01$) attenuated by administration of curcumin as compared to respective control rats.

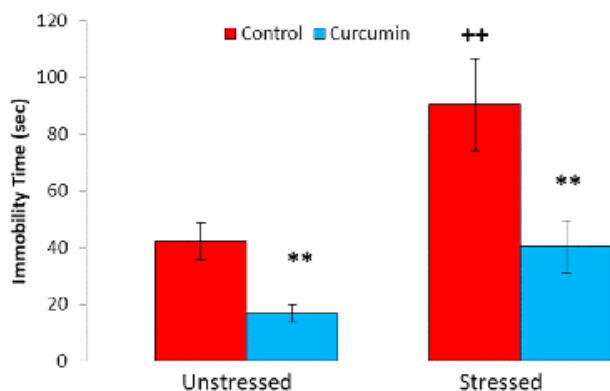


Fig. 1: Effect of curcumin on immobility time in force swim test (FST) in stressed and unstressed rats. Data represented as mean \pm SD; (n=6) rats per group. Significance difference was obtained by Tukey's test. ** $p < 0.01$ versus respective controls and ++ $p < 0.01$ versus unstressed control group.

Impact of pre-supplementation of curcumin on anxiety profile in light/dark transition test (LDT)

Latency to move to dark box

The effect of curcumin on latency to move to dark box following UCMS is shown in (fig. 2a). Data analysis revealed a significant impact of stress [$F(1, 20) = 116.14, p < 0.01$], curcumin [$F(1, 20) = 67.23, p < 0.01$] and association between stress and curcumin [$F(1, 20) = 14.65, p < 0.01$] on latency to move to dark box in LTD. Post-hoc analysis showed that curcumin administration substantially ($p < 0.01$) delayed the latency to move to dark box as compared to respective unstressed control rats indicating anxiolytic action of curcumin. Exposure to UCMS significantly ($p < 0.01$) decreased the latency to move to dark box in comparison to unstressed controls while pre-treatment with curcumin substantially ($p < 0.05$) increased the latency to move to dark box as compared to respective stressed controls.

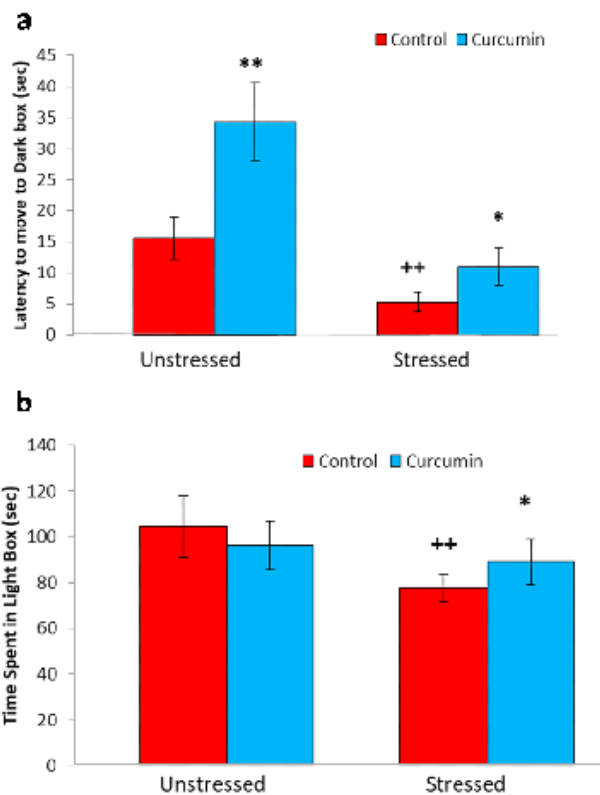


Fig. 2: Effect of curcumin on anxiety profile in light/dark transition test (LDT) in stressed and unstressed rats. Data represented as mean \pm SD; (n=6) rats per group. Significance difference was obtained by Tukey's test. * $p < 0.05$; ** $p < 0.01$ versus respective controls and ++ $p < 0.01$ versus unstressed control group. (a) Latency to move to dark box and (b) time spent in light box.

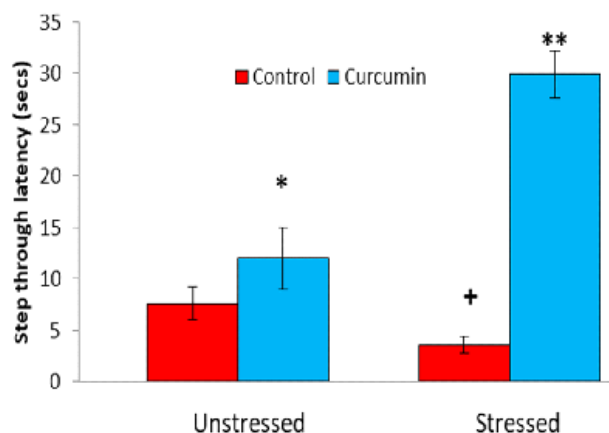


Fig. 3: Effect of curcumin on step-through latency in passive avoidance test (PAT) in stressed and unstressed rats. Data represented as mean \pm SD; (n=6) rats per group. Significance difference was obtained by Tukey's test. * $p < 0.05$; ** $p < 0.01$ versus respective controls and + $p < 0.05$; versus unstressed control group.

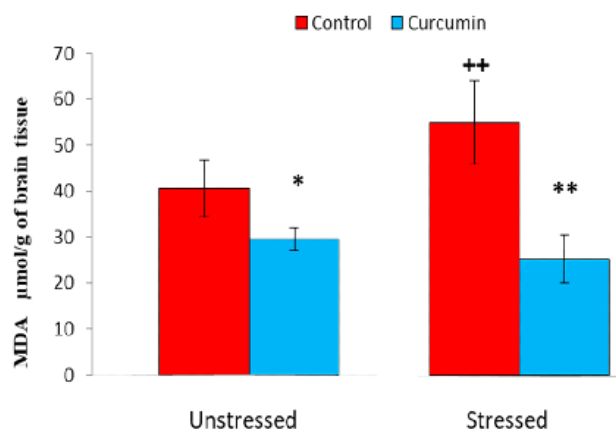


Fig. 4: Effect of curcumin on brain lipid peroxidation in stressed and unstressed rats. Data represented as mean \pm SD; (n=6) rats per group. Significance difference was obtained by Tukey's test. * p <0.05; ** p <0.01 versus respective controls and ⁺⁺ p <0.01 versus unstressed control group.

Time spent in light box

The effect of curcumin on time spent in light box following UCMS is shown in (fig. 2b). Data analysis revealed a significant impact of stress [F (1, 20) = 14.8,

p <0.01] and association between stress and curcumin [F (1, 20) = 9.6, p <0.01] on time spent in light box in LTD. Post-hoc analysis exhibited that rats exposed to UCMS spent significantly (p <0.01) less time in light box as compared to unstressed control rats while rats pre-treated with curcumin spent more time (p <0.01) in light box in comparison to respective stressed controls.

Impact of pre-supplementation of curcumin on step-through latency in PAT

The effect of curcumin on memory following UCMS is presented in (fig. 3). Analysis of data revealed a significant impact of stress [F (1, 20) = 63.24, p <0.01], curcumin [F (1, 20) = 305.41, p <0.01], and association between stress and curcumin [F (1, 20) = 156.8, p <0.01] on step-through latency in PAT. Analysis by post-hoc revealed that curcumin supplementation to unstressed rats significantly (p <0.01) increased the step-through latency in comparison to respective unstressed controls. Rats exposed to UCMS exhibited significant (p <0.05) decreased step-through latency as compared to unstressed control rats, while this decrease in step-through latency induced by stress was significantly attenuated (p <0.01) by the administration of curcumin as compared to respective stressed control rats.

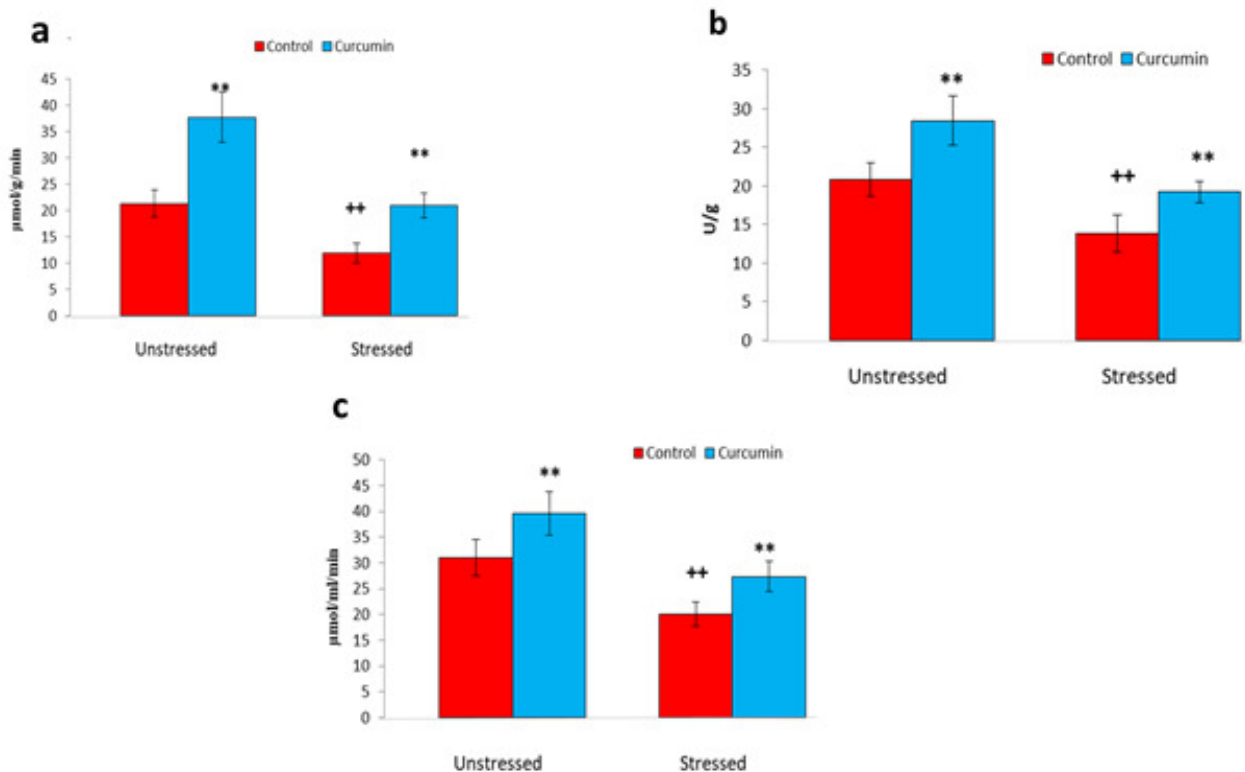


Fig. 5: Effect of curcumin on antioxidant enzymes activities in stressed and unstressed rats. Data represented as mean \pm SD; (n=6) rats per group. Significance difference was obtained by Tukey's test. ** p <0.01 versus respective controls and ⁺⁺ p <0.01 versus unstressed control group. (a) Catalase (CAT), (b) superoxide dismutase (SOD), (c) glutathione peroxidase (GPx).

Impact of pre-supplementation of curcumin on brain LPO

The effect of curcumin on brain LPO is presented in (fig. 4). Data analysis revealed a significant impact of curcumin [$F(1, 20) = 62.84, p < 0.01$], association between stress and curcumin [$F(1, 20) = 13.25, p < 0.01$] and non-significant impact of stress [$F(1, 20) = 3.72, NS$] on brain lipid peroxidation levels. Post-hoc analysis showed that curcumin substantially ($p < 0.05$) lessened the rate of lipid peroxidation in comparison to respective unstressed controls. Exposure to UCMS substantially ($p < 0.01$) increased the LPO in comparison to unstressed controls while stress-induced increase in LPO was substantially ($p < 0.01$) declined by curcumin administration in stressed rats in comparison to respective stressed controls.

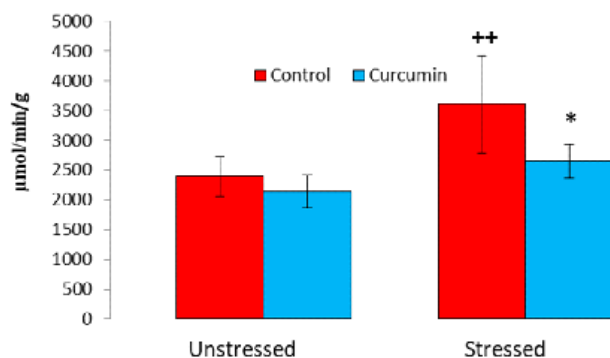


Fig. 6: Effect of curcumin on acetylcholinesterase activity in stressed and unstressed rats. Data represented as mean \pm SD; (n=6) rats per group. Significance difference was obtained by Tukey's test. * $p < 0.05$; versus respective controls and ++ $p < 0.01$ versus unstressed control group.

Impact of pre-supplementation of curcumin on antioxidant enzyme activities

The impact of curcumin on antioxidant enzyme activities i.e., CAT, GPx and SOD are presented in (fig. 5a, 5b and 5c) respectively. Analysis of data revealed a significant effect of stress [$F(1, 20) = 68.54, p < 0.01$], curcumin [$F(1, 20) = 64.33, p < 0.01$], and non-significant effect of association between stress and curcumin [$F(1, 20) = 0.8, NS$] on CAT activity. Data also revealed a significant effect of stress [$F(1, 20) = 69.64, p < 0.01$], curcumin [$F(1, 20) = 32.86, p < 0.01$], and non-significant effect of association between curcumin and stress [$F(1, 20) = 0.8, NS$] on GPx activity and a significant effect of curcumin [$F(1, 20) = 43.64, p < 0.01$], stress [$F(1, 20) = 67.79, p < 0.01$] and non-significant effect of association between stress and curcumin [$F(1, 20) = 1.2, NS$] has been observed on SOD activity. Post-hoc analysis revealed that curcumin supplementation substantially ($p < 0.01$) improved the antioxidant enzymes activities in comparison to respective unstressed control rats. Rats subjected to UCMS displayed a substantial ($p < 0.01$) decline in CAT, SOD and GPx activities as compared to unstressed control rats, while supplementation of curcumin attenuated ($p < 0.01$) stress-induced decrease in

antioxidant enzyme activities as compared to respective stressed controls.

Impact of pre-supplementation of curcumin on AchE activity

The effect of curcumin on the activity of AchE is presented in (fig. 6). Analysis of data revealed a significant effect of stress [$F(1, 20) = 68.54, p < 0.01$] and curcumin [$F(1, 20) = 64.33, p < 0.01$] on AchE activity while association of stress and curcumin did not exhibit any significant effect. Post-hoc analysis showed that exposure to UCMS substantially ($p < 0.01$) increased the AchE enzyme activity in comparison to unstressed control rats while supplementation of curcumin significantly ($p < 0.05$) attenuated the stress-induced rise in AchE activity in comparison to respective stressed controls.

DISCUSSION

The outcomes of the present work indicated that exposure to UCMS for 20 days significantly induced anxiety and depression-like symptoms as indicated by decline in time spent in light box, decrease in latency to move to dark box and increased immobility in FST. Previously, it has been stated that chronic unpredictable stress decreased neurogenesis in the brain of rats and contributed to depression-like symptoms (Hsieh and Eisch, 2010; Koo *et al.*, 2010). Decreased struggling time in FST and decreased exploratory activity in open field test following UCMS have also been reported (Mineur *et al.*, 2006; Zhang *et al.*, 2014; Cui *et al.*, 2014).

In the previous study pre-treatment with curcumin attenuated acute immobilization stress-induced anxiety, hyperactivity and increased immobility in FST (Haider *et al.*, 2015). It has been reported that UCMS-induced depression-like symptoms can be reverted by the treatment with antidepressants (Kudryashov *et al.*, 2015; Zhang *et al.*, 2014). The results of the present study exhibited anxiolytic and antidepressant like effects of curcumin evident by increased time spent in light box and increased latency to move to dark box as compared to respective stressed controls. Decreased immobility time in FST was also observed. Current findings are consistent with the previous work in which daily supplementation of curcumin at 40 mg/kg decreased immobility time in comparison to UCMS group (Zhang *et al.*, 2014).

Memories are strengthened in a stressful condition (Haider *et al.*, 2015), but subjection to aversive stimuli impair the acquisition, learning, storage and reclamation of information (Lupien and Lepage, 2001; Alvarez *et al.*, 2003). Unpredictability of chronic mild stress serves as an aversive stimulus. In the present study exposure to UCMS severely impaired the memory as evident by decreased step-through latency in UCMS exposed rats. The finding is consistent with the previous study in which UCMS

impaired long-term potentiation in rat brain (Alvarez *et al.*, 2003). Altered hippocampal morphology has also been reported after chronic stress (McLaughlin *et al.*, 2007) and produces impairments in cognitive abilities (Xu *et al.*, 2015, Yu *et al.*, 2016). Pre-treatment of curcumin to UCMS exposed rats attenuated stress-induced impairment in memory indicating neuroprotective and memory enhancing effects of curcumin. The present work also revealed that pre-administration of curcumin to UCMS exposed rats normalized stress-induced increase in AChE activity. Acetylcholine is a major neurotransmitter involved in learning and memory (Papandreou *et al.*, 2011). Stimulation of AChE results in rapid degradation of acetylcholine and further down stimulation of acetylcholine receptors exert detrimental impact on cognitive functions (Soreq and Seidman 2001). According to the outcomes of present study it can be proposed that the decrease in AChE activity due to pre-treatment with curcumin leads to an increase in cholinergic neurotransmission and acetylcholine levels in the synapse, hence contributing towards progressive cognitive improvement.

Most living organisms undergo stressful events in daily life (Linnemann *et al.*, 2015). The failure to cope up during stressful conditions results in stress-induced diseases such as depression (Schlotz *et al.*, 2011). ROS may also play a crucial role in the development of neurological and psychiatric ailments including major depression and bipolar disorder (Bakunina *et al.*, 2015). UCMS animal model has been regarded very close to the unforeseen stressors of daily life (Willner *et al.*, 1997). In the present study 20 days exposure to UCMS increased the rate of brain LPO and suppressed the activity of antioxidant enzymes. UCMS-induced increase in LPO and decreased SOD activity have been reported earlier (Lucca *et al.*, 2009). Pre-supplementation of curcumin significantly alleviated the brain LPO and enhanced the antioxidant enzyme activities in UCMS exposed rats, clearly demonstrating antioxidant potential of curcumin against UCMS-induced oxidative stress. Antioxidant role of curcumin following UCMS in the present work is in agreement with the previous research (Cui *et al.*, 2014).

CONCLUSION

The present study concluded that curcumin possesses the capability of restoring UCMS-induced behavioral and cognitive deficits. Apart from this curcumin reinstated the UCMS-induced oxidative burden by restoring the antioxidant enzymes activities. Curcumin also showed strong anti-acetylcholinesterase potential and improved memory function. In view of neuroprotective properties of curcumin it can be suggested that curcumin is a potential therapeutic agent that can be used to treat UCMS-induced behavioral and psychiatric disorders with no side effects unlike any other synthetic antidepressants.

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