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Curcumin confers neuroprotection against alcohol-induced hippocampal neurodegeneration via CREB-BDNF pathway in rats

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ABSTRACT

Background: Alcohol abuse causes severe damage to the brain neurons. Studies have reported the neuroprotective effects of curcumin against alcohol-induced neurodegeneration. However, the precise mechanism of action remains unclear.
Methods: Seventy rats were equally divided into 7 groups (10 rats per group). Group 1 received normal saline (0.7 ml/rat) and group 2 received alcohol (2 g/kg/day) for 21 days. Groups 3, 4, 5 and 6 concurrently received alcohol (2 g/kg/day) and curcumin (10, 20, 40 and 60 mg/kg, respectively) for 21 days. Animals in group 7 self- administered alcohol for 21 days. Group 8 treated with curcumin (60 mg/kg, i.p.) alone for 21 days. Open Field Test (OFT) was used to investigate motor activity in rats. Hippocampal oxidative, antioxidative and inflammatory factors were evaluated. Furthermore, brain cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) and brain derived neurotrophic factor (BDNF) levels were studied at gene level by reverse transcriptase polymerase chain reaction (RT-PCR). In addition, protein expression for BDNF, CREB, phosphorylated CREB (CREB-P), Bax and Bcl-2 was determined by western blotting.
Result: Voluntary and involuntary administration of alcohol altered motor activity in OFT, and curcumin treatment inhibited this alcohol-induced motor disturbance. Also, alcohol administration augmented lipid peroxidation, mitochondrial oxidized glutathione (GSSG), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and Bax levels in isolated hippocampal tissues. Furthermore, alcohol-induced significant reduction were observed in reduced form of glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) activities and CREB, BDNF and Bcl-2 levels. Also curcumin alone did not change the behavior and biochemical and molecular parameters.
Conclusion: Curcumin can act as a neuroprotective agent against neurodegenerative effects of alcohol abuse, probably via activation of CREB-BDNF signaling pathway.

1. Introduction

In the search for new potential sources of medications, natural herbal compounds have often been considered as valuable and viable options. The use of such compounds as therapeutic supplements has been reported for treating neurodegenerative diseases [1,2]. Recent studies have assessed the protective effects of herbal medications in suppressing apoptosis and oxidative stress [3,4].

Alcohol is a sedative agent with neurodegenerative properties [5,6] and its pharmacological similarity to sedative and hypnotic agents, makes it a potential candidate for abuse [7,8]. Studies have shown that alcohol abuse causes anxiety, depression and cognitive impairments in animal models [8,9]. Alcohol abuse induces oxidative stress and activates neuroapoptotic and neuroinflammatory pathways [10–12]. Long-term alcohol consumption increases the expression of apoptotic proteins such as caspases-3, 9 and 8, and causes DNA fragmentation [13–15]. Furthermore, it decreases Bcl-2 and increases Bax proteins [13–15]. Mitochondria play a crucial role in regulating neurotoxicity

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induced by abuse of alcohol and other addictive substances [14,16]. These substances cause the release of cytochrome c and decrease mitochondrial biogenesis [17]. Alcohol abuse affects some parts of the brain such as hippocampus and amygdala, more than the other brain areas [18]. Alcohol when consumed progressively, causes neurodegeneration in some regions of hippocampus such as Cornu Ammonis (CA1, CA2, CA3) and Dentate gyrus (DG) [19,20].

Curcumin (diferuloylmethane), the most abundant component of turmeric, is extracted from rhizomes of the plant *Curcuma longa* [21]. This non-nutritive yellow pigment is an established nutraceutical dietary phenol and thus, carries a great medicinal significance [1,2]. Studies have shown that curcumin improves blood flow and can reduce anxiety, depression and cognitive impairments in animal models of diabetes and other neurological disorders [22,23]. Curcumin has shown to induce antioxidative, anti-inflammatory, anti-apoptotic, immunomodulatory and neuroprotective effects in hippocampus [24–26]. Furthermore, curcumin potently stimulates neurogenesis and therefore, it has been proposed as a therapeutic agent for treating neurodegenerative disorders and substance abuse [27–30]. The synergistic effect of curcumin on superoxide dismutase and catalase activities has been reported previously [30]. In addition, favorable effects of curcumin on mitochondrial biogenesis and inhibition of oxidative stress are well-evident[31]. Curcumin is also involved in suppressing major proinflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor −alpha (TNF-α) [31,32].

Studies have shown that activated CREB or CREB-P is involved in a variety of functional responses associated with nervous system [33]. In addition, CREB acts as a transcription factor for production of brain derived neurotrophic factor (BDNF). This pathway is important for neurodevelopment, neurogenesis and mood disorders like anxiety and depression [34–36]. Studies have proposed that one of the signaling pathway involved in curcumin induced neuroprotection might be CREB-BDNF pathway [37,38]. However, the exact mechanism for this herb induced neuroprotection is yet to be explained. Therefore, we designed this study to investigate the in vivo role of curcumin in protecting rat hippocampal cells against alcohol-induced neuroapoptosis, oxidative stress, neuroinflammation and reduced cell density. In addition, we aimed to study the potential involvement of phospho-CREB-BDNF signaling in curcumin-mediated neuroprotection.

2. Materials & methods

2.1. Animals

Seventy male adult wistar rats (about 8 weeks old) weighing 200 ± 8.0 g were obtained from Pasteur Institute of Iran (Tehran, Iran) and were transferred to laboratory. Animals were acclimated to experimental conditions (12 h light dark cycle, 24 °C) for 2 weeks and had free access to standard food and tap water. The present study was performed in accordance with the guidelines for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication No.85–23, revised 1996). The experimental protocol was approved by the Research Council of Iran University of Medical Sciences, Tehran, Iran.

2.2. Drug

Curcumin was purchased from Sigma-Aldrich (USA) (Cat. No. G0062890) and dissolved in normal saline for administration. Alcohol obtained from Tehran Shimi (Tehran, Iran). Drugs were freshly prepared just before use and the volume was adjusted to 0.7 ml/rat.

2.3. Experimental design

70 adult male rats were assigned to one of the following groups:

- Group 1 (negative control) treated with normal saline (0.7 ml/rat, i.p.) for 21 days.
- Group 2 (positive control) treated with alcohol (2 g/kg/day by gavage, once daily) for 21 days.
- Groups 3, 4, 5 and 6 treated concurrently with alcohol (2 g/kg/day, by gavage, once daily) and curcumin (10, 20, 40 and 60 mg/kg, i.p., respectively) for 21 days.
- Group 7 (positive control) treated with self-administration of alcohol for 21 days.
- Group 8 treated with curcumin (60 mg/kg, i.p.) alone for 21 days.

On day 22, Open Field Test (OFT) – a standard behavioral method for studying hippocampal degeneration was performed to evaluate the motor activity and depression in experimental animals. Two hour after behavioral test, OFT, oxidative stress, inflammation and apoptosis were also evaluated in hippocampal tissues. Keeping in view the importance of CREB-BDNF signaling pathway in neuroprotection, the involvement of this pathway in curcumin-mediated neuroprotective effects against alcohol-induced disturbances was studied. Real-time reverse transcriptase-PCR (RT-PCR) was performed to study the changes induced by curcumin treatment in BDNF and CREB gene expressions. Furthermore, protein levels for BDNF, CREB, Bax and Bcl-2 were assessed by western blot analysis. Changes in gene and protein levels were studied in dentate gyrus and CA1 areas of rat brain. In addition, crystal violet staining was also performed in these areas to study cell density and neurodegeneration.

Alcohol and curcumin doses were selected from a preliminary study performed at our laboratory and other previous studies [1,39–42].

2.4. Open field test (OFT)

This assay was used to evaluate anxiety and locomotor activity in rodents [43,44]. Four typical behaviors in the OFT were assessed and scored.

1. Line crossing (ambulation) distance: Total distance of the grid lines crossed by each rat.
2. Center square entries: Number of times each rat entered the central red square with all four paws.
3. Center square duration: The time spent by each rat in the central square.
4. Rearing: Frequency with which each rat stands on its hind legs in the maze.

2.5. Mitochondrial preparations

Animals were anesthetized using sodium thiopental (50 mg/kg, i.p) and the hippocampus was isolated from each rat. The isolated tissues were homogenized in cold homogenization buffer (25 mM 4-morpholinepropanesulfonic acid, 400 mM sucrose, 4 mM magnesium chloride ($MgCl_2$), 0.05 mM ethylene glycol tetra acetic acid (EGTA), pH 7.3) and the homogenized tissues were centrifuged at 450 × *g* for 10 min. The supernatants obtained were re-centrifuged at 12000 × *g* for 10 min. Finally, the sediments were re-suspended in homogenization buffer and stored at 0 °C. Total mitochondrial proteins in tissues were determined using Dc protein assay kit (Bio-Rad, California, United States). Briefly, Bradford reagent (1 part Bradford: 4 parts dH_2O) was added to serial dilution series (0.1-1.0 mg/ml) of a known protein sample concentration; e.g.,

bovine serum albumin (BSA), dissolved in homogenization buffer. These serial dilution series were prepared and used for providing a standard curve. On the other hand, 10, 15, 20, 25 and 30 μl of the protein extract (homogenized cell solutions) were added to multiple wells. Bradford reagent was also added to each well. Densities of colors of all wells were read by plate reader at 630 nm. Finally, by using the standard curve, protein quantity in extracts were obtained. These homogenized cell solutions were analyzed for the measurement of oxidative stress and inflammatory markers [1,45,46].

2.6. Measurement of oxidative stress parameters

2.6.1. Determination of lipid peroxidation

For assessment of lipid peroxidation, malondialdehyde (MDA), a natural by-product, was measured. Briefly, 100 μl of SDS lysis solution was added to wells containing 100 μl sample solution or MDA standard. After shaking and incubation of these wells, 250 μl of thiobarbituric acid (TBA) reagent was added to each well and incubated at 95 °C for 45–60 min. Next, tubes were centrifuged at 1000 × *g* for 15 min and 300 μl of *n*-Butanol was added to 300 μl of supernatant. Then, the tubes were centrifuged for 5 min at 10,000 × *g*. Finally, the absorbance was read at 532 nm and the results obtained were expressed as nmol/mg of protein [27,45,47].

2.6.2. Determination of reduced (GSH) and oxidized (GSSG) glutathione

For measurement of GSH and GSSG levels, 25 μl of 1× glutathione reductase solution and 25 μl of 1× nicotinamide adenine dinucleotide phosphate (NADPH) solution was added to a 96-well plate, containing standard solution of glutathione or sample of homogenized solution. Then, 50 μl of the 1× Chromogen was added to each well and mixed vigorously. Finally, the absorbance was read at 405 nm for each GSSG/GSH standard and sample. Using the standard curve, amount of GSSG/GSH was determined and the results obtained were expressed as nmol/mg of protein [1,45,48].

2.6.3. Determination of manganese superoxide dismutase (MnSOD) activity

The previously described method was used to assess SOD activity [1,45,49]. SOD activity was calculated using the following equation: SOD activity = {[(A blank 1 – A blank 3) – (A sample – A blank 2)]/(A blank 1 − A blank 3)} × 100. Results were reported as U/ml/mg protein [1,45,46].

2.6.4. Determination of glutathione peroxidase (GPx) activity

GPx activity was assessed as described previously [1,45,50]. It was measured based on change in absorbance [ΔA340/min] by the following equation:

ΔA340/min = A340 nm (Start) − A340 nm (Stop)/Reaction time (min), any change in the absorbance is directly proportional to GPx activity.

GPx activity: ΔA340/min × Reaction volume (ml) × Dilution factor of the original sample/Extinction coefficient for NADPH at 340 nm × Volume of the tested sample. Results were expressed as mU/mg protein [1,45,47].

2.6.5. Determination of glutathione reductase (GR) activity

GR activity was assessed as described previously [1,45]. Results were expressed as mU/mg protein [1,45,49].

2.7. Measurement of inflammatory parameters

2.7.1. Determination of IL-1β and TNF-α level

Briefly, wells containing sheep anti-rat IL-1β and TNF-α polyclonal antibody (Sigma Chemical Co., Poole, and Dorset, UK) were washed three times with washing buffer (0.5 M of sodium chloride (NaCl), 2.5 mM sodium dihydrogen phosphate (NaH_2PO_4), 7.5 mM Na_2HPO_4, 0.1% Tween 20, pH 7.2). Then, 100 ml of 1% (w/v) ovalbumin (Sigma Chemical Co., Poole, Dorset, UK) solution was added to each well and incubated at 37 °C for 1 h. Following three washes, 100 ml of samples and standards were added to each well and incubated at 48 °C for 20 h. After three washes, 100 ml of the biotinylated sheep anti-rat IL-1β or TNF-α antibody (1:1000 dilutions in washing buffer containing 1% sheep serum, Sigma Chemical Co., Poole, and Dorset, UK) was added to each well. Next, after 1 h incubation and three washes, 100 ml avidin-HRP (Dako Ltd, UK) (1:5000 dilution in wash buffer) was added to each well and the plate were incubated for 15 min. After washing three times, 100 ml of TMB substrate solution (Dako Ltd., UK) was added to each well and then incubated for 10 min at room temperature. Then, 100 ml of 1 M H_2SO4 was added and absorbance was read at 450 nm. Results were expressed as ng IL-1β/ml or TNF-α/ml [1,45,51].

2.8. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted from 200 μg of hippocampal tissue by using ONE STEP-RNA reagent (Bio Basic, Canada inc.) according to the manufacturer's instructions. Extracted RNA was assessed for quantity and quality using a nanodrop (ND-1000, Thermo Scientific Fisher, US) and gel electrophoresis respectively. To eliminate genomic contamination, RNA was treated with DNase I (Qiagen, Hilden, Germany) as described by the manufacturer. Next, complementary DNA (cDNA) was synthesized using 1 μg of total RNA. The integrity and quality of cDNA was examined using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primer as housekeeping. Real-time reverse transcriptase-PCR (RT-PCR) was carried out to evaluate the differences in expression patterns for BDNF and CREB genes among samples of each group. Primers were designed using Primer 3 software version 0.4 (frodo.wi.mit.edu) and are as follows:

BDNF Forward: 5′-GGAGGCTAAGTGGAGCTGAC-3′
Reverse: 5′-GCTTCCGAGCCTTCCTTTAG-3′
CREB1 Forward: 5′-CAGACAACCAGCAGAGTGGA-3′
Reverse: 5′-CTGGACTGTCTGCCCATTG-3′
GAPDH Forward: 5′-AGACAGCCGCATCTTCTTGT-3′
Reverse: 5′-CCGTTCACACCGACCTTCA-3′

Real time RT-PCR was performed in 20 μl reactions containing 1 μl cDNA target, 100 nM forward and reverse primers and 1 × SYBR® Premix Ex Taq™ II (Takara, Tokyo, Japan). Experiments were carried out in triplicate using a CFX96™ Real-Time System (C1000TM Thermal Cycler; Bio-Rad, Hercules, CA, USA). Amplification conditions were as follow: initial denaturation at 95 °C for 10 min, followed by 40 cycles (denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 1 min). The relative value of the mRNA expression of CREB and BDNF genes were calculated by comparing the cycle thresholds (CTs) of the target gene with that of the housekeeping gene (GAPDH) using the $2^{-\Delta\Delta ct}$ method and REST 2009 software [52]. Serial dilutions of cDNAs were used for calculation of the primer sets efficiencies in real-time PCR. In this regard, the efficiencies of various primer sets were found to be similar [48,53].

2.9. Protein expression determination using western blot analysis

We studied the immunoreactivity of CREB, CREB-P, BDNF, Bax and Bcl-2 contents in the hippocampal tissues by western blotting. Electrotransfer of the resolved bands from gel to polyvlinylidene difluoride (PVDF) membrane (Millipore, Bedford, USA) was performed in 90 min at 0.7 mA/cm^2 using a semi-dry transfer apparatus (PeQlab). After the transferring step, the membrane was weakly stained for 3 min with Coomassie blue G-250 (1 μg/100 ml) (Sigma Aldrich, UK). Then, the membrane was dried and cut into 2 mm wide strips. After destaining with methanol, the stripes were washed and blocked with 2% BSA overnight at 4 °C. Next day, bovine serum (dilution 1:100) was added and was incubated in room temperature (RT) for 2 h on a shaker. The membranes were then washed with PBS-T (three washing steps) and were incubated with the following conjugated polyclonal anti-rabbit antibody: BDNF and CREB (total and phosphorylated; 1:500 dilutions in BSA, 360 min, RT; Sigma Aldrich, Germany) Bax and Bcl-2 (1:1000 dilutions in BSA, 240 min, RT; Sigma Aldrich, Germany). Next, all stripes were incubated with secondary HRP conjugated polyclonal rabbit anti-sheep antibody (1:5000 dilution in BSA, 120 min, RT; Sina Biotech, Iran). The stripes were washed and incubated with chemiluminescent substrate [(Luminol and hydrogen peroxide (H_2O_2)] for 2 min at RT. Finally, the reactive bands were detected on X-ray film within 10–20 s under safe light condition [53,54].

2.10. Histological studies

Isolated hippocampus was fixed in formalin and washed with physiologic serum. After dehydrating the tissues using ethanol, tissues were embedded in paraffin wax and 5 μm thick sections were prepared. Next, the sections were stained with crystal violet stain and images of 20 sections per group were captured (400 × magnification). Images were analyzed using morphometeric software (Optikavision pro, Italy). The cell density was determined in an area of 1.30 mm of hippocampal subfield in all sections [49,55].

2.11. Statistical analysis

All data were statistically analyzed using Graph Pad PRISM software (version 6). The data were expressed as mean ± standard error of the mean (SEM). Differences between control and treatment groups were evaluated using one way ANOVA. Differences between the behaviors in groups were evaluated by Tukey's post-hoc test. $P < 0.05$ or $P < 0.001$ was considered statistically significant.

3. Results

3.1. The effects of various doses of curcumin on alcohol-induced disturbances in open field test (OFT) measurements

The animals in negative control group entered central square more frequently and spent more time in the central square region of the open field as compared to animals in self-administered and forcefully-administered (2 g/kg/day) alcohol groups ($p < 0.05$) (Table 1).

On the other hand, curcumin at all doses increased the frequency of central square entries and time spent in the central region of the OFT. However, this increase was statistically significant in groups treated with 40 and 60 mg/kg of curcumin as compared to self-administered and forcefully-administered (2 g/kg/day) alcohol groups ($p < 0.05$) (Table 1). Furthermore, both alcohol treated groups demonstrated less ambulation distance in OFT as compared to negative control animals. Curcumin treatment increased ambulation distance in OFT in comparison to both alcohol treated groups ($p < 0.05$) (Table 1). We also found a significant difference in frequency of central square entries, time spent in the central region, rearing number and ambulation distance between the group treated with 60 mg/kg curcumin in combination with alcohol (2 g/kg/day) and the group treated with 10 mg/kg curcumin in combination with alcohol (2 g/kg/day) ($p < 0.05$) (Table 1).The treatment of animals by curcumin (60 mg/kg) alone did not change the ambulation distance, frequency of central square entries, time spent in the central region and rearing number in OFT, and results were similar to control group and significantly different from both alcohol treated groups ($P < 0.05$) (Table 1).

3.2. The effects of various doses of curcumin on alcohol-induced lipid peroxidation in mitochondria

Both self-administered and forcefully-administered (2 g/kg/day) alcohol groups showed markedly elevated mitochondrial MDA levels in comparison with the negative control group ($P < 0.05$) (Table 2). Various doses of curcumin (40 and 60 mg/kg) diminished this alcohol-induced increase in the MDA level and the results were found to be significant in comparison with both alcohol treated groups ($P < 0.05$) (Table 2). Furthermore, we found a significant difference in the MDA level between the group treated with 60 mg/kg curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg curcumin in combination with alcohol (2 g/kg/day) ($p < 0.05$) (Table 2) The results obtained with curcumin (60 mg/kg) alone were similar to

Table 1
The effects of alcohol consumption and alcohol in combination with various doses of curcumin (10, 20, 40 and 60 mg/kg) and curcumin only on open field exploratory activity and anxiety-like behavior in rats.

Groups	Ambulation distance (cm)	Central square entries (number)	Time spent in central square (sec)	Number of rearing
Control	384 ± 11	19 ± 1.3	155 ± 10	16 ± 1.5
Alcohol (self administration)	301 ± 12[a]	13 ± 2[a]	129 ± 15[a]	12 ± 3[a]
Alcohol (2 mg/kg/day)	330 ± 10[a]	11 ± 1[a]	116 ± 12[a]	11 ± 3[a]
Alcohol (2 mg/kg/day) + Curcumin (10 mg/kg)	341 ± 9	13 ± 1.1	124 ± 19	12 ± 0.7
Alcohol (2 mg/kg/day) + Curcumin (20 mg/kg)	354 ± 13	15 ± 3.1	133 ± 11	13.5 ± 1.5
Alcohol (2 mg/kg/day) + Curcumin (40 mg/kg)	364 ± 11[b]	18 ± 1.8[b]	140 ± 8[b]	14.5 ± 3[b]
Alcohol (2 mg/kg/day) + Curcumin (60 mg/kg)	370 ± 10[b,c]	19 ± 3[b,c]	147 ± 13[b,c]	15 ± 3[b,c]
Curcumin (60 mg/kg)	382 ± 9[b]	20 ± 1.1[b]	152 ± 12[b]	16 ± 1[b]

Data are represented as mean ± SEM (n = 8, each group).
[a] $p < 0.05$ vs. control group.
[b] $p < 0.05$ vs. alcohol (2 g/kg/day) and alcohol self-administration groups.
[c] $p < 0.05$ vs. Alcohol in combination with curcumin (10 mg/kg) group.

Table 2
The effects of various doses of curcumin on alcohol-induced oxidative stress in rats.

Groups	MDA (nmol/mg of protein)	SOD (U/ml/mg of protein)	GPx (mU/mg of protein)	GR (mU/mg of protein)
Control	6.9 ± 1.4	74 ± 7.9	133 ± 6	135 ± 7
Alcohol (self administration)	15 ± 1.2[a]	58 ± 6[a]	109 ± 8[a]	128 ± 5[a]
Alcohol (2 g/kg/day)	17 ± 1.9[a]	52 ± 4[a]	102 ± 6[a]	122 ± 7[a]
Alcohol (2 mg/kg/day) + Curcumin (10 mg/kg)	15 ± 1.8	55 ± 7	105 ± 6	125 ± 8
Alcohol (2 mg/kg/day) + Curcumin (20 mg/kg)	14 ± 1.4	60 ± 8	110 ± 7	130 ± 7
Alcohol (2 mg/kg/day) + Curcumin (40 mg/kg)	10 ± 1.9[b]	68 ± 9[b]	128 ± 9[b]	146 ± 8[b]
Alcohol (2 mg/kg/day) + Curcumin (60 mg/kg)	8 ± 1.5[b,c]	72 ± 9[b,c]	130 ± 10[b,c]	151 ± 8[b,c]
Curcumin (60 mg/kg)	7.6 ± 1.4[b]	75 ± 6.3[b]	129 ± 9[b]	142 ± 8[b]

Data are represented as mean ± SEM (n = 8, each group).
MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase, GR: glutathione reductase.
[a] $p < 0.05$ vs. control group.
[b] $p < 0.05$ vs. Alcohol (2 g/kg/day) and alcohol self-administered groups.
[c] $p < 0.05$ vs. Alcohol (2 g/kg/day) in combination with curcumin (10 or 20 mg/kg) group.

control group and significantly different from both alcohol treated groups ($P < 0.05$) (Table 2).

3.3. The effects of various doses of curcumin on alcohol-induced reduction in superoxide dismutase (SOD) activity in mitochondria

Both, self-administered and forcefully-administered (2 g/kg/day) alcohol groups showed markedly reduced SOD activity in comparison with the negative control group ($P < 0.05$)(Table 2). Administration of curcumin (40 and 60 mg/kg) diminished this alcohol-induced reduction in the SOD activity and the results were found to be significant in comparison with both alcohol treated groups ($P < 0.05$)(Table 2). Furthermore, we found a significant difference in the SOD activity between the group treated with 60 mg/kg curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg curcumin in combination with alcohol (2 g/kg/day) ($p < 0.05$) (Table 2), while curcumin (60 mg/kg) alone did not change the SOD activity, and was similar to control group and significantly different from both alcohol treated groups ($P < 0.05$) (Table 2).

3.4. The effects of various doses of curcumin on alcohol-induced changes in the glutathione peroxidase (GPx) activity in mitochondria

Both, self-administered and forcefully-administered (2 g/kg/day) alcohol groups showed significantly attenuated GPx activity in comparison with the negative control group ($P < 0.05$) (Table 2). In contrast, curcumin (40 and 60 mg/kg) treatment markedly improved the GPx activity when compared to both alcohol treated groups ($P < 0.05$) (Table 2). Furthermore, we found a significant difference in the GPx activity between the groups treated with 40 and 60 mg/kg of curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg curcumin in combination with alcohol (2 g/kg/day) ($p < 0.05$) (Table 2).While curcumin (60 mg/kg) alone did not change the GPx activity, when compared to control group but showed significant difference from both alcohol treated groups ($P < 0.05$) (Table 2).

3.5. The effects of various doses of curcumin on alcohol-induced changes in glutathione reductase (GR) activity in mitochondria

Both, self-administered and forcefully-administered (2 g/kg/day) alcohol groups showed significantly attenuated GR activity in comparison with the negative control group ($P < 0.05$) (Table 2). Administration of curcumin (40 and 60 mg/kg) improved this alcohol-induced reduction in the GR activity when compared to both alcohol treated groups ($P < 0.05$) (Table-2). Furthermore, we found a significant difference in the GR activity between the group treated with 40 and 60 mg/kg of curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg curcumin in combination with alcohol (2 g/kg/day) ($p < 0.05$) (Table 2). Curcumin (60 mg/kg) alone increased the GR activity, but this increase was not significant in comparison to control group. This increase was significantly different in comparison to both alcohol treated groups ($P < 0.05$) (Table 2).

3.6. The effects of various doses of curcumin on alcohol-induced GSH/GSSG alterations in mitochondria

Forceful (2 g/kg/day) and self administration of alcohol markedly decreased the GSH content in isolated mitochondria in comparison with the negative control group ($P < 0.05$) (Table 3).

Table 3
The effects of various doses of curcumin on alcohol-induced mitochondrial GSH/GSSG alterations in rats.

Groups	GSH (nmol/mg of protein)	GSSG (nmol/mg of protein)	GSH/GSSG
Control	75.7 ± 2.1	0.98 ± 0.3	76
Alcohol (self administration)	56.1 ± 4.2[a]	3.1 ± 1.2[a]	18[a]
Alcohol (2 g/kg/day)	52.5 ± 2.1[a]	3.5 ± 1.5[a]	14[a]
Alcohol (2 mg/kg/day) + Curcumin (10 mg/kg)	60.3 ± 3.6	3.2 ± 0.05	18.7
Alcohol (2 mg/kg/day) + Curcumin (20 mg/kg)	61.3 ± 4.6	3.00 ± 0.13	20.04
Alcohol (2 mg/kg/day) + Curcumin (40 mg/kg)	69.2 ± 4.7[b,c]	1.7 ± 0.14[b,c]	40.7[b,c]
Alcohol (2 mg/kg/day) + Curcumin (60 mg/kg)	70.3 ± 1.6[b,c]	1.5 ± 0.30[b,c]	46.8[b,c]
Curcumin (60 mg/kg)	77.1 ± 3.1[b]	1.1 ± 0.2[b]	77[b]

Data are represented as mean ± SEM (n = 8, each group).
[a] $p < 0.05$ vs. control group.
[b] $p < 0.05$ vs. alcohol (2 g/kg/day) and alcohol self-administered groups.
[c] $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (10 or 20 mg/kg) groups.

In contrast, animals treated with 40 and 60 mg/kg of curcumin showed noticeably higher amount of GSH in comparison to the both alcohol treated groups ($P < 0.05$) (Table 3).

GSSG levels in both alcohol treated groups were found to be significantly higher than the negative control group ($P < 0.05$) (Table 3). Curcumin at doses 40 and 60 mg/kg prevented this alcohol-induced increase in the GSSG level when compared to both alcohol treated groups ($P < 0.05$) (Table 3). Furthermore, we found a significant difference in GSH and GSSG levels between the groups treated with 40 and 60 mg/kg of curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg of curcumin in combination with alcohol (2 g/kg/day) ($P < 0.05$) (Table 3). Curcumin (60 mg/kg) alone did not change significantly the GSH and GSSG contents when compared to control group, but these changes were significant in comparison to both alcohol treated groups ($P < 0.001$) (Table 3).

3.7. The effects of various doses of curcumin on alcohol-induced elevated IL-1β and TNF-α level

Forceful (2 g/kg/day) and self administration of alcohol markedly increased IL-1β and TNF-α levels as compared to the negative control group ($P < 0.001$) (Figs. 1 and 2). Furthermore, curcumin (40 and 60 mg/kg) reduced alcohol-induced rise in the inflammatory cytokines and this effect was statistically significant when compared to both alcohol treated groups ($P < 0.001$) (Figs. 1 and 2). In addition, we observed a significant difference in TNF-α and IL-1β levels between the group treated with 40 and 60 mg/kg curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg curcumin in combination with alcohol (2 g/kg/day) ($P < 0.05$) (Figs. 1 and 2). Group under treatment with curcumin (60 mg/kg) alone did not showe significant difference in IL-1β and TNF-α levels, in comparison to control group,while shows significant decrease in IL-1β and TNF-α levels when compared to both alcohol treated groups ($P < 0.001$) (Figs. 1 and 2).

3.8. The effects of various doses of curcumin on alcohol-induced reduced CREB gene expression

Both self-administered and forcefully-administered alcohol (2 g/kg/day) groups demonstrated significantly reduced CREB gene expression in the DG and CA1 regions of hippocampus in comparison to the negative control group ($P < 0.001$) (Fig. 3A and B). In contrast, curcumin (40 and 60 mg/kg) improved the CREB gene expression in the DG and CA1 regions of hippocampus when compared to both alcohol treated groups ($P < 0.001$) (Fig. 3A and B). In addition, we observed a significant difference in the CREB expression between the groups treated with 40 and 60 mg/kg of curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg of curcumin in combination with alcohol (2 g/kg/day) ($P < 0.05$) (Fig. 3A and B). However, curcumin (60 mg/kg) alone did not change the CREB gene expression in the DG and CA1 regions when compared to control group while these changes were significant in comparison to both alcohol treated groups ($P < 0.001$) (Fig. 3A and B).

3.9. The effects of various doses of curcumin on alcohol-induced reduced BDNF gene expression

Forceful (2 g/kg/day) and self administration of alcohol markedly reduced the BDNF gene expression in both the DG and CA1 regions of hippocampus when compared to the negative control group ($P < 0.001$) (Fig. 4A and B). In contrast, curcumin (40 and 60 mg/kg) improved the BDNF gene expression in DG and CA1 regions when compared to both alcohol treated groups ($P < 0.001$) (Fig. 3A and B). Furthermore, our data showed a significant difference in the BDNF gene expression between the group treated with 60 mg/kg curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg curcumin in combination with alcohol (2 g/kg/day) ($P < 0.05$) (Fig. 4A and B). Curcumin (60 mg/kg) only treated group did not change BDNF gene expression in both DG and CA1 regions, when compared to control group, while this change was significant in comparison to both alcohol treated groups ($P < 0.001$) (Fig. 4A and B).

3.10. The effects of various doses of curcumin on alcohol-induced decreased protein expression of CREB

Both, self-administered and forcefully-administered (2 g/kg/day) alcohol groups demonstrated reduced CREB and CREB-P protein expression in the DG and CA1 regions of hippocampus when compared to the negative control group ($P < 0.001$) (Figs. 5 and 6A and B). In contrast, curcumin (40 and 60 mg/kg) improved

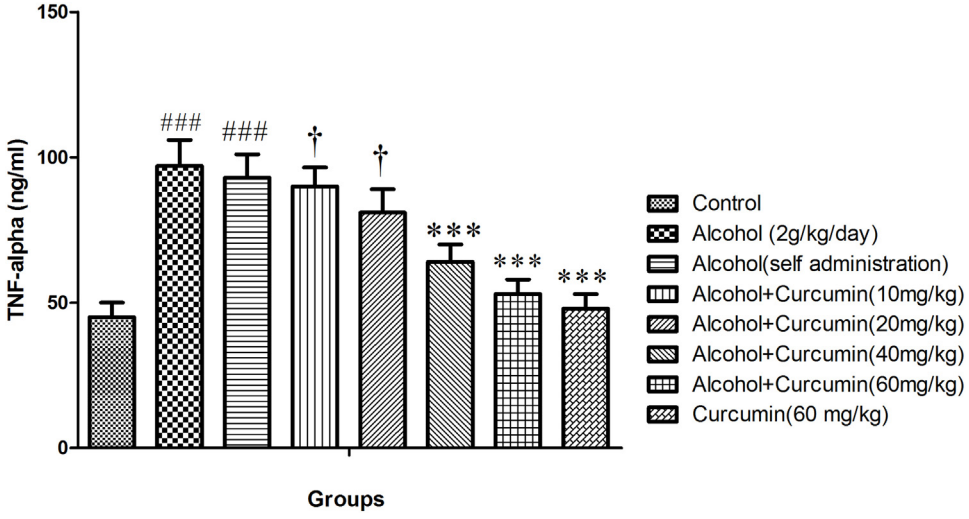

Fig. 1. The effects of alcohol consumption and alcohol in combination with various doses of curcumin (10, 20, 40 and 60 mg/kg) on hippocampal TNF-α levels in rats. *** $p < 0.001$ vs. alcohol (2 g/kg/day) and alcohol self-administration groups. ### $p < 0.001$ vs. control group. † $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (60 mg/kg) group.

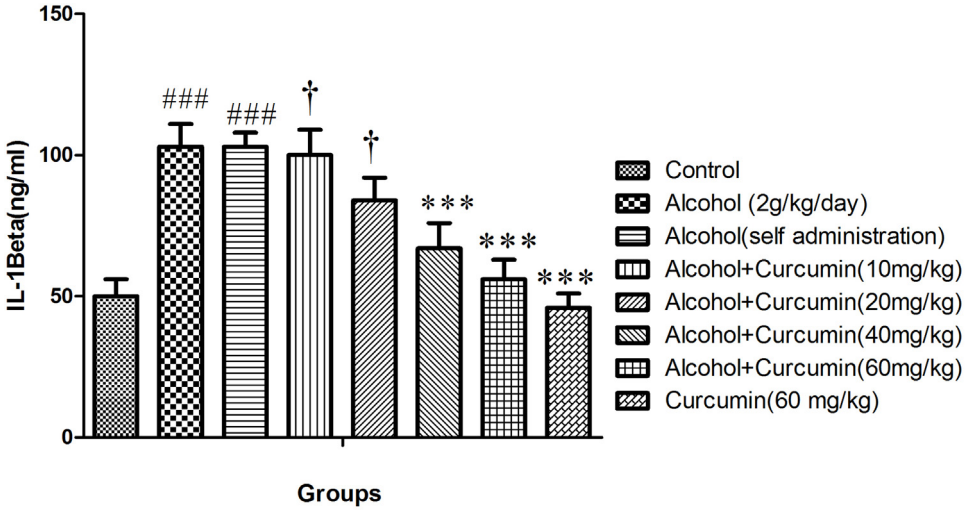

Fig. 2. The effects of alcohol consumption and alcohol in combination with various doses of curcumin (10, 20, 40 and 60 mg/kg) on hippocampal IL-β level in rats. *** $p < 0.001$ vs. alcohol (2 g/kg/day) and alcohol self-administration groups. ### $p < 0.001$ vs. control group. † $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (60 mg/kg) group.

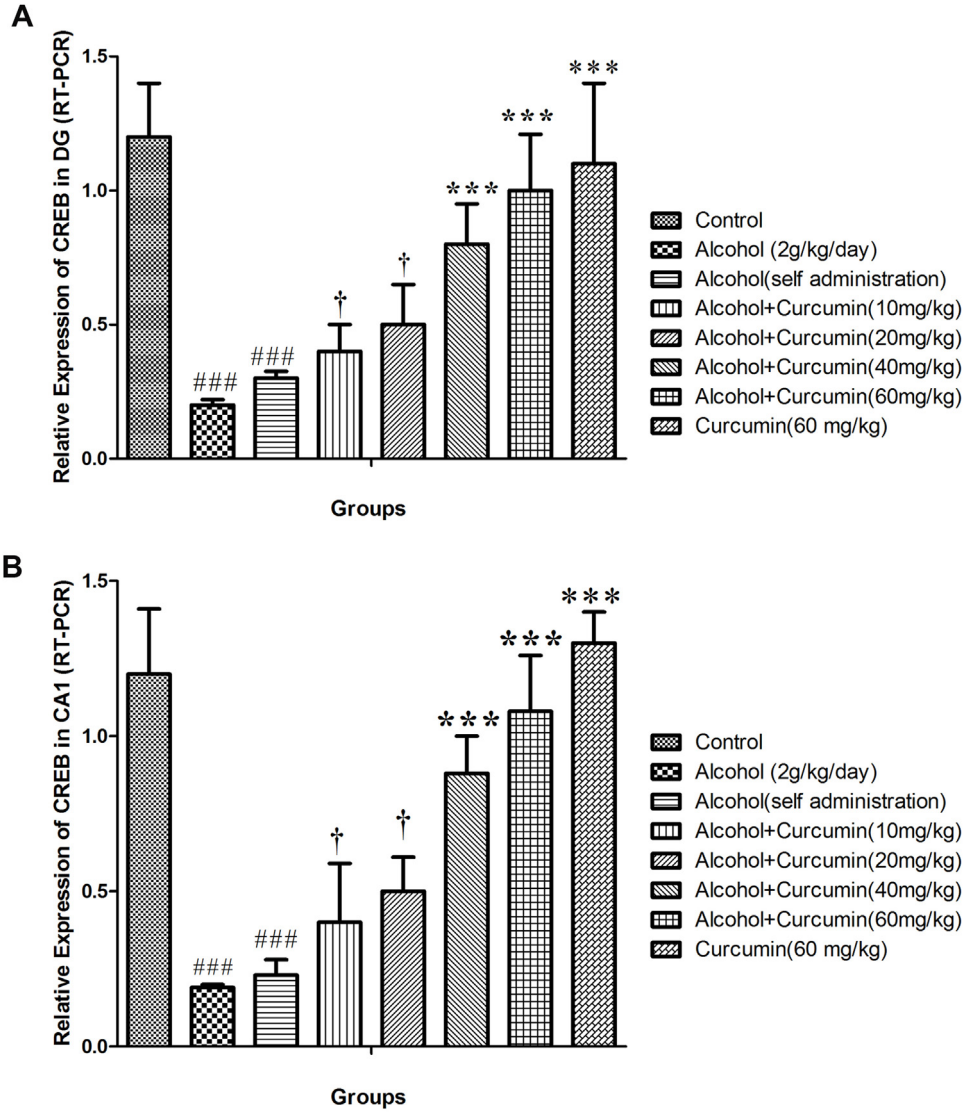

Fig. 3. The effects of various doses of curcumin (10, 20, 40 and 60 mg/kg) on alcohol-induced reduction in CREB gene expression in DG (A) and CA1 (B) regions of hippocampus. *** $p < 0.001$ vs. alcohol (2 g/kg/day) and alcohol self-administration groups. ### $p < 0.001$ vs. control group. † $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (40 and 60 mg/kg) groups.

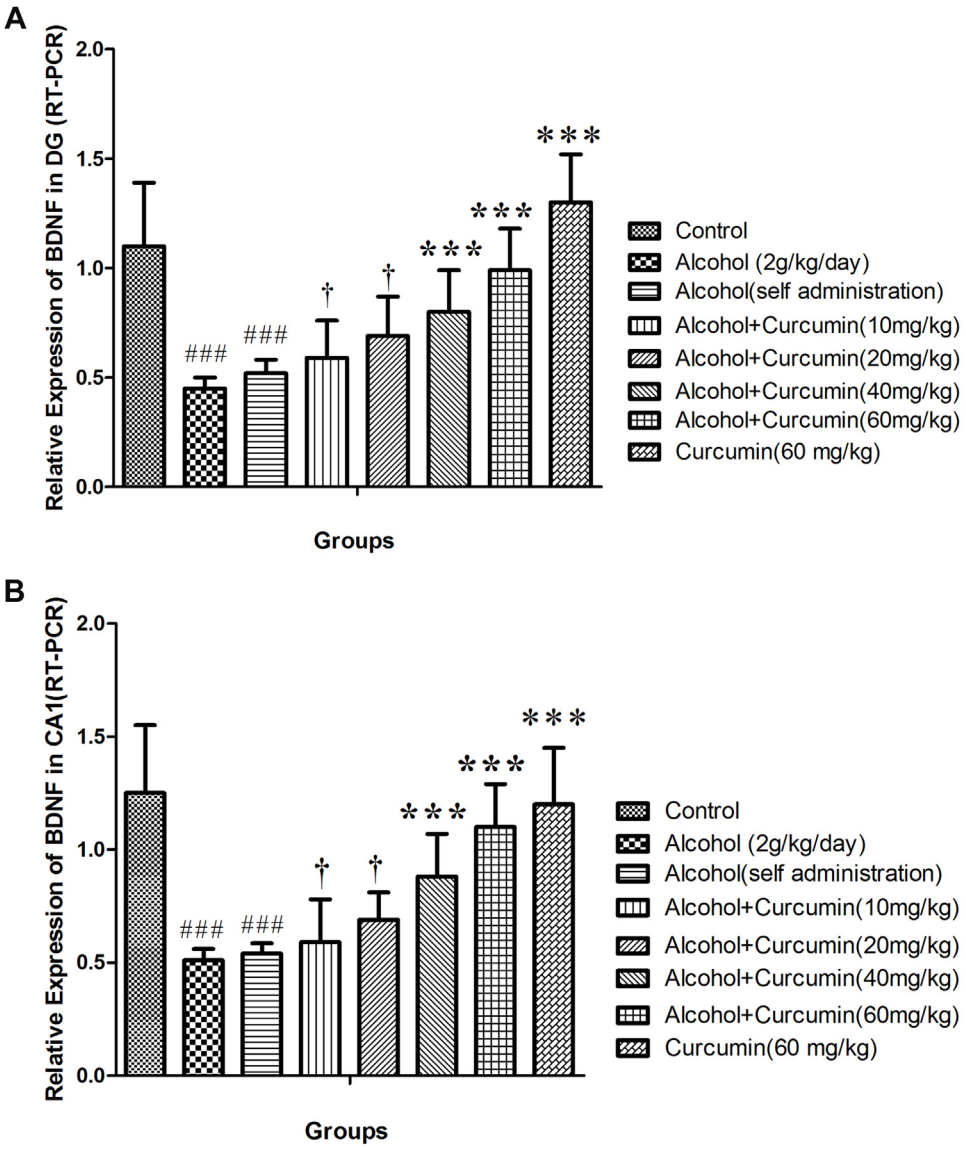

Fig. 4. The effects of various doses of curcumin (10, 20, 40 and 60 mg/kg) on alcohol-induced reduction in BDNF gene expression in DG (A) and CA1 (B) regions of hippocampus. *** $p < 0.001$ vs. alcohol (2 g/kg/day) and alcohol self-administration groups. ### $p < 0.001$ vs. control group. † $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (60 mg/kg) group.

the CREB protein expression in DG and CA1 regions of hippocampus in comparison to both alcohol treated groups ($P < 0.001$) (Figs. 5 and 6A and B). In addition, our data showed a significant difference in the total CREB protein expression in hippocampus between the group treated with 60 mg/kg curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg curcumin in combination with alcohol (2 g/kg/day) ($P < 0.05$) (Fig. 5A and B). However, CREB-P protein expression was significantly different between the groups treated with 40 and 60 mg/kg of curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg of curcumin in combination with alcohol (2 g/kg/day) ($P < 0.05$) (Fig. 5A and B), Curcumin (60 mg/kg) only treated group in comparison to control group did not change significantly the CREB and CREB-P protein expressions and also ratio of CREB-P to CREB in the DG and CA1 regions, these changes were significant in comparison to both alcohol treated groups ($P < 0.001$) (Figs. 5 and 6A and B). On the other hand both self-administered and forcefully-administered (2 g/kg/day) alcohol groups caused decreases in CREB-P/CREB ratio in DG and CA1 regions of hippocampus when compared to the negative control group ($P < 0.001$) (Fig. 7A and B). In contrast, curcumin (40 and 60 mg/kg) increased the CREB-P/CREB ratio of protein expression in DG and CA1 regions of hippocampus in comparison to both alcohol treated groups ($P < 0.001$) (Fig. 7A and B). Differences of CREB-P/CREB ratio in DG and CA1 regions group under treatment by 40 and 60 mg/kg of curcumin in combination with alcohol (2 g/kg/day)was significant when compared to the groups treated with 10 and 20 mg/kg of curcumin in combination with alcohol (2 g/kg/day) ($P < 0.05$) (Fig. 7A and B).Curcumin (60 mg/kg) alone did not change CREB-P/CREB ratio in DG and CA1 regions of hippocampus when compared to the negative control, while these changes were significant when compared to both alcohol treated groups ($P < 0.001$) (Fig. 7A and B).

3.11. The effects of various doses of curcumin on alcohol induced decrease in BDNF in western blot

Both self-administered and forcefully-administered (2 g/kg/day) alcohol groups demonstrated reduced protein expression of

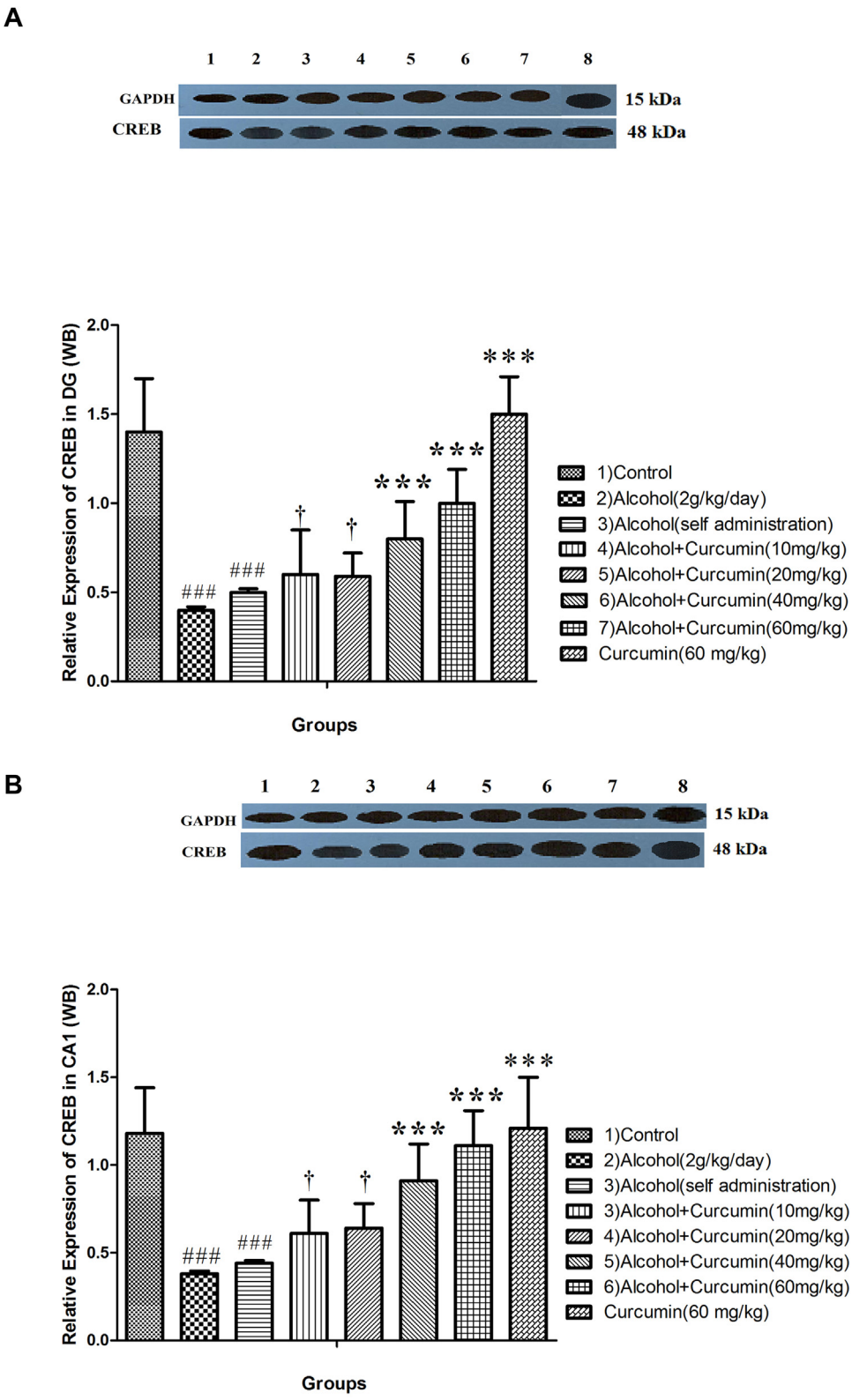

Fig. 5. The effects of various doses of curcumin (10, 20, 40 and 60 mg/kg) on alcohol-induced reduction in CREB protein expression in DG (A) and CA1 (B) regions of hippocampus. *** $p < 0.001$ vs. alcohol (2 g/kg/day) and alcohol self-administration groups. ### $p < 0.001$ vs. control group. † $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (60 mg/kg) group.

BDNF in the DG and CA1 field of hippocampus in comparison with the negative control group ($P<0.001$) (Fig. 8A and B). In contrast, curcumin (40 and 60 mg/kg) improved BDNF protein expression in the DG and CA1 regions of hippocampus in comparison to both alcohol treated groups ($P < 0.001$) (Fig. 8A and B). Furthermore, we observed a significant difference in BDNF protein expression in the

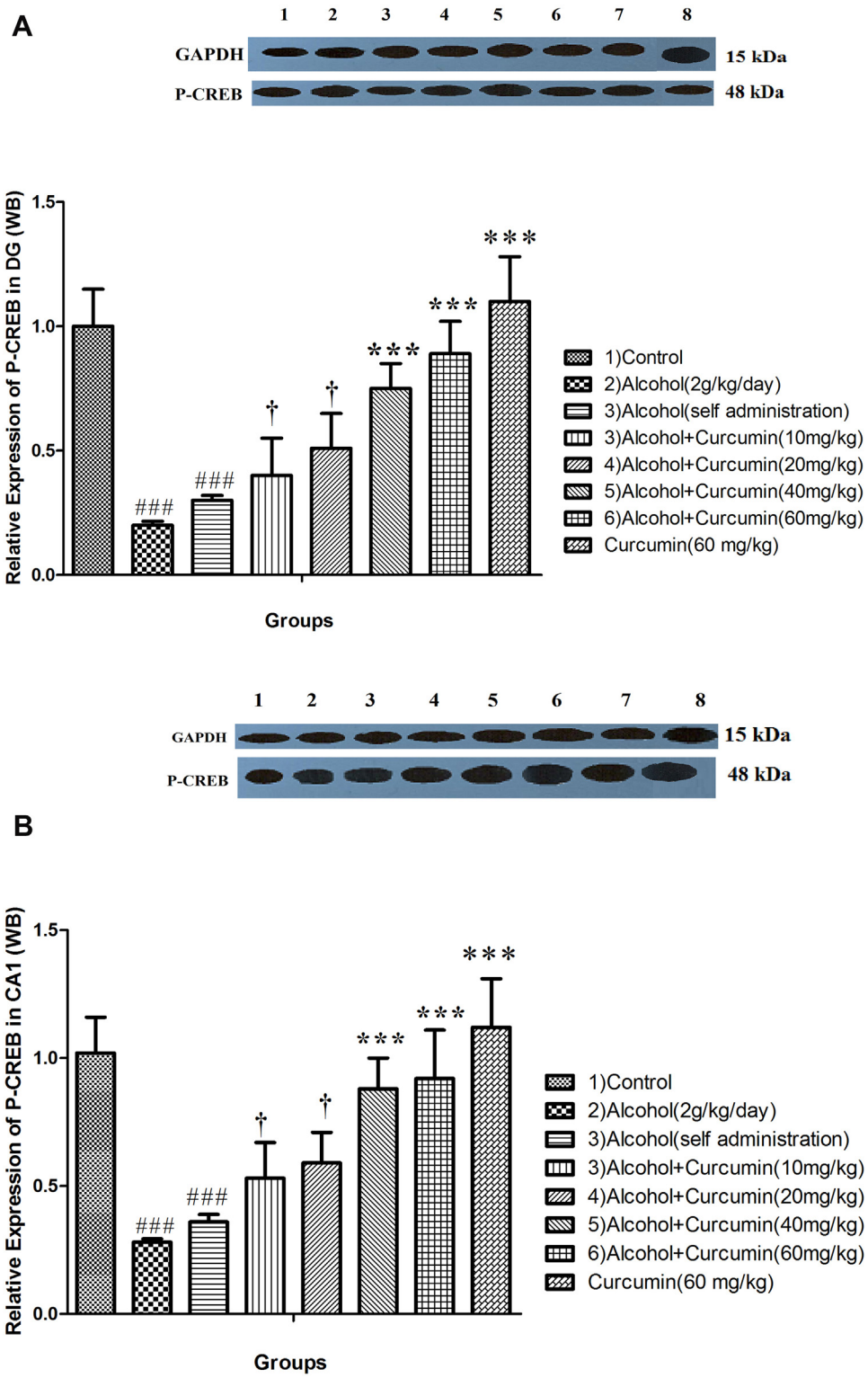

Fig. 6. The effects of various doses of curcumin (10, 20, 40 and 60 mg/kg) on alcohol-induced reduction in phosphorylated-CREB (P-CREB) protein expression in DG (A) and CA1 (B) regions of hippocampus. *** $p < 0.001$ vs. alcohol (2 g/kg/day) and alcohol self-administration groups. ### $p < 0.001$ vs. control group, † $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (40 and 60 mg/kg) groups.

hippocampus between the group treated with 60 mg/kg curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg curcumin in combination with alcohol (2 g/kg/day) ($P < 0.05$) (Fig. 8A and B).Curcumin (60 mg/kg) only treated group did not change BDNF protein expression in both DG and CA1 regions, when compared to control group although these changes were significant in comparison to both alcohol treated groups ($P < 0.001$) (Fig. 8A and B).

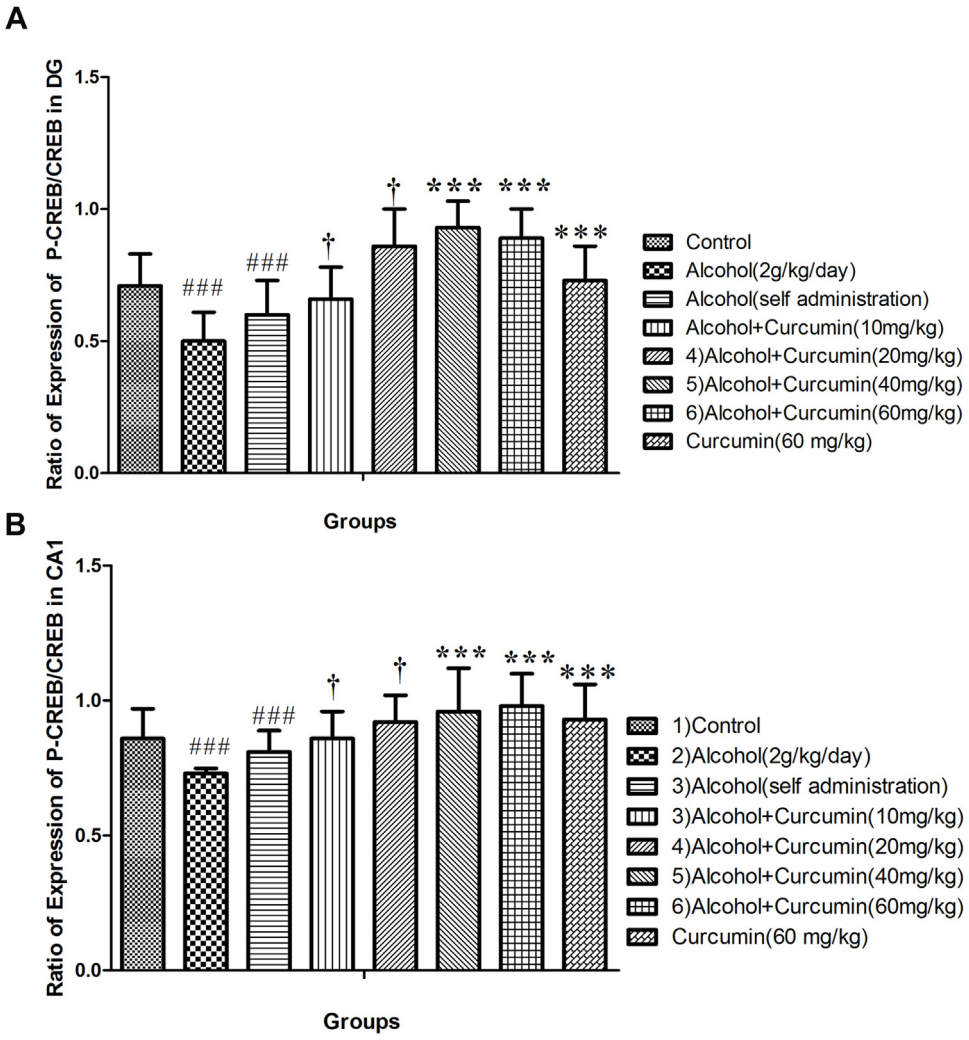

Fig. 7. The effects of various doses of curcumin (10, 20, 40 and 60 mg/kg) on alcohol-induced reduction P-CREB/CREB protein expression ratio in DG (A) and CA1 (B) regions of hippocampus. *** $p < 0.001$ vs. alcohol (2 g/kg/day) and alcohol self-administration groups. ### $p < 0.001$ vs. control group. † $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (40 and 60 mg/kg) group.

3.12. The effects of various doses of curcumin on alcohol-induced changes in protein expression of bax and bcl-2

Forceful (2 g/kg/day) and self administration of alcohol significantly increased Bax and Bax/Bcl-2 ratio and decreased Bcl-2 protein expression in the hippocampus when compared to the negative control group ($P < 0.001$) (Fig. 9A, B and C). In contrast, curcumin (40 and 60 mg/kg) improved Bcl-2 and reduced Bax and Bax/Bcl-2 ratio protein expression in the hippocampus in comparison to the both alcohol treated groups ($P < 0.001$) (Fig. 9A, B and C). In addition, we found a significant difference in Bax, Bax/Bcl-2 ratio and Bcl-2 protein expression in hippocampus between the groups treated with 40 and 60 mg/kg of curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg of curcumin in combination with alcohol (2 g/kg/day) ($P < 0.05$) (Fig. 9A, B and C), Curcumin (60 mg/kg) alone did not change Bcl-2 and Bax protein expressions and also did not affect the ratio of Bax/Bcl-2 in both DG and CA1 regions, and these differences were not significant in comparison to control group while were significant compared to both alcohol treated groups ($P < 0.001$) (Fig. 9A, B and C).

3.13. Histological studies

Tissue sections of both self-administered and forcefully-administered (2 g/kg/day) alcohol groups had dramatic loss of the granular cells in DG and pyramidal cells in CA1 regions as compared to control group ($p < 0.05$) (Table 4). In contrast, curcumin (40 and 60 mg/kg) treatment significantly improved neuronal cell density in the hippocampus ($p < 0.05$) (Table 4). Degenerated shrunken dark cells with condensed nucleus were observed in granular cells and pyramidal cells in the groups treated with 2 g/kg/day of alcohol (Figs. 10 and 11). Curcumin (40 and 60 mg/kg) treatment effectively preserved the neuronal architecture (Figs. 10 and 11). Furthermore, we observed significant differences in cell number, cell shape and histomorphological changes in the hippocampus between the groups treated with 40 and 60 mg/kg of curcumin in combination with alcohol (2 g/kg/day) and the groups treated with 10 and 20 mg/kg of curcumin in combination with alcohol (2 g/kg/day) ($P < 0.05$) (Table 4) (Figs. 10 and 11). Group under treatment by curcumin (60 mg/kg) only did not significantly change the cells' density and shape in both DG and CA1 regions when compared to control group, but these changes were significant in comparison to both alcohol treated groups ($P < 0.001$) (Table 4) (Figs. 10 and 11).

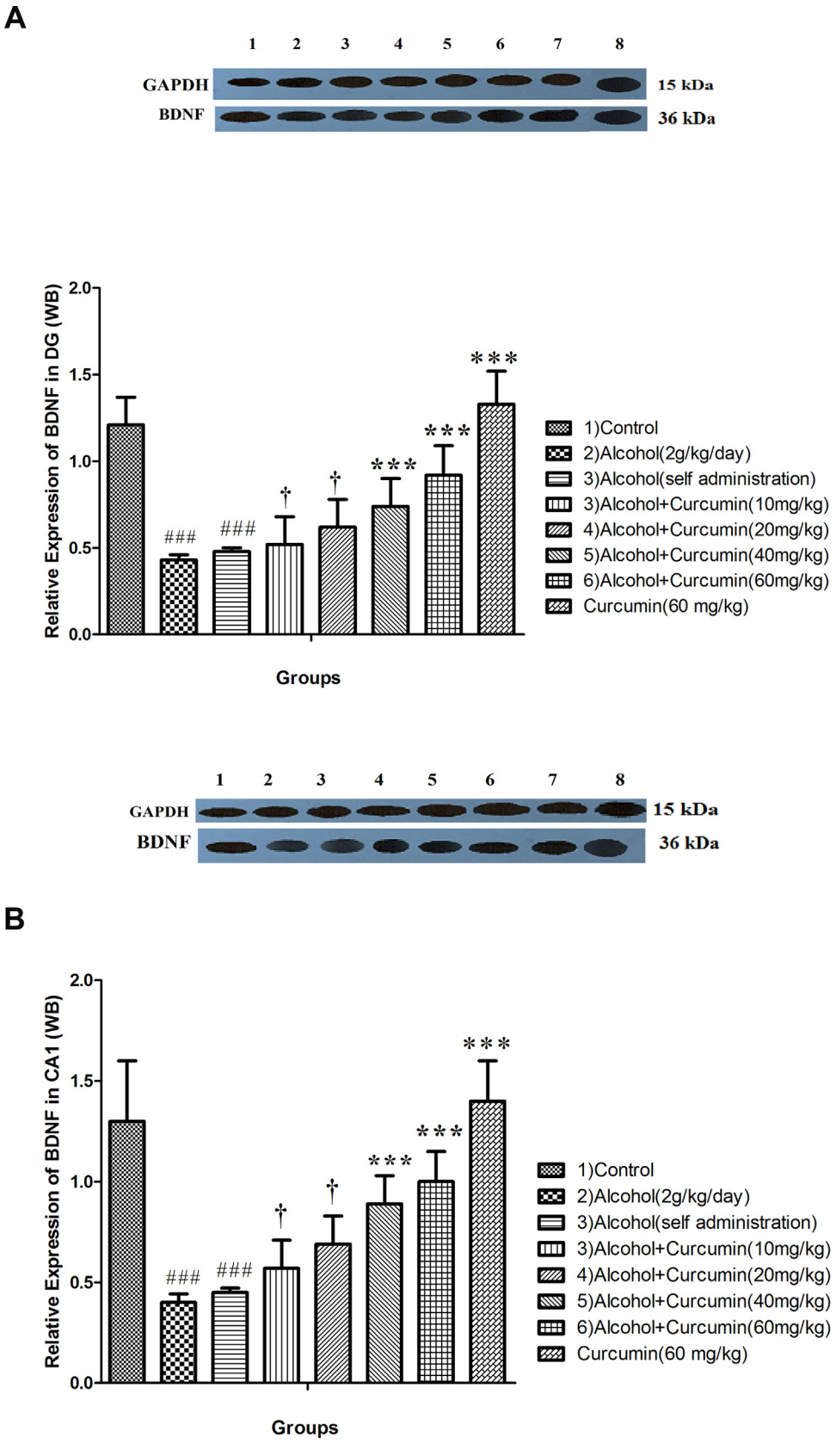

Fig. 8. The effects of various doses of curcumin (10, 20, 40 and 60 mg/kg) on alcohol-induced reduction in BDNF protein expression in DG (A) and CA1 (B) regions of hippocampus. *** $p < 0.001$ vs. alcohol (2 g/kg/day) and alcohol self-administration groups. ### $p < 0.001$ vs. control group. † $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (60 mg/kg) group.

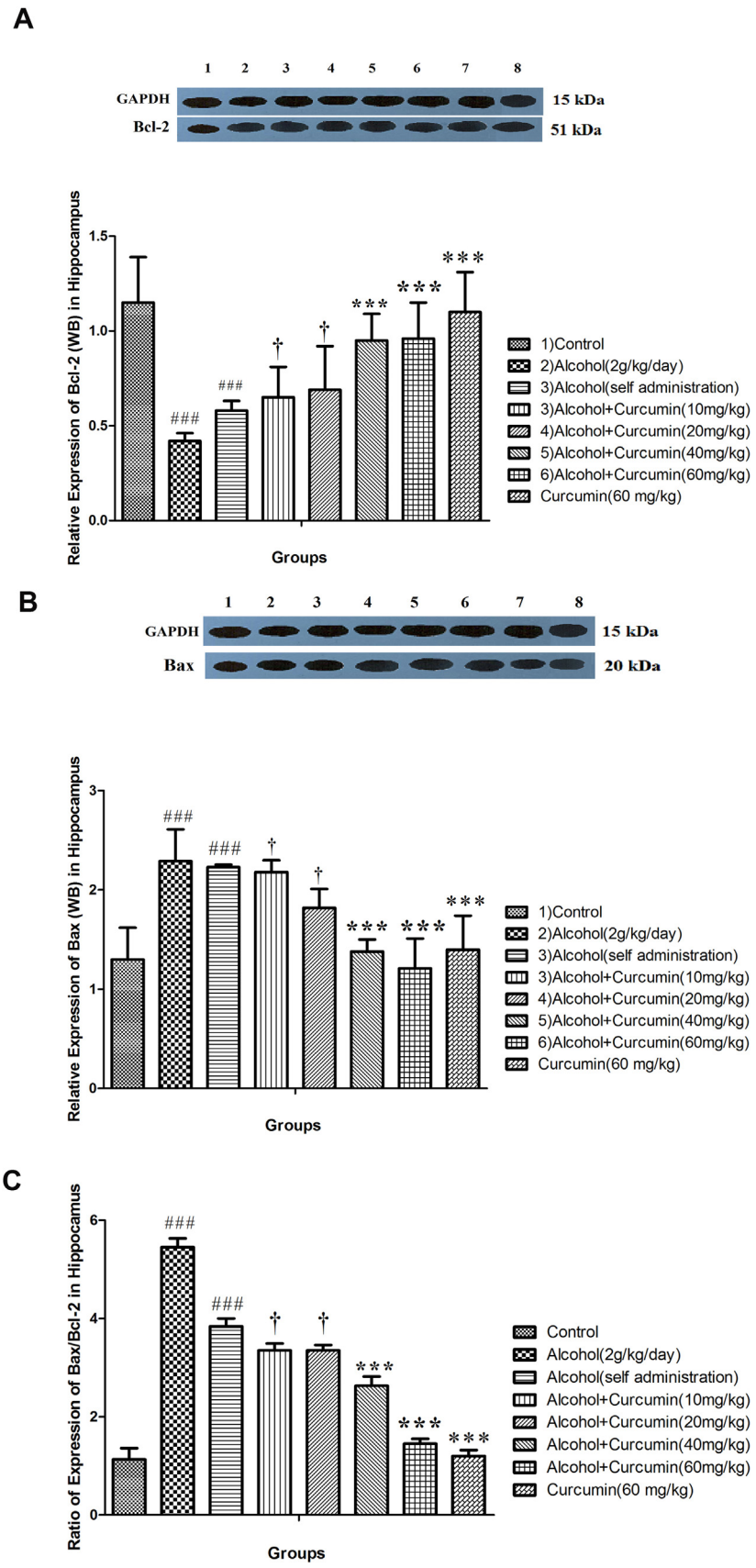

Fig. 9. The effects of various doses of curcumin (10, 20, 40 and 60 mg/kg) on alcohol-induced reduction in Bcl-2 (A) and alcohol induced increase in Bax (B) and ratio of Bax/Bcl-2 (C) protein expression in hippocampus. *** $p < 0.001$ vs. alcohol (2 g/kg/day) and alcohol self-administration groups. ### $p < 0.001$ vs. control group. † $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (60 mg/kg) group.

Table 4
The effects of various doses of curcumin on alcohol-induced reduction in hippocampal neuronal cell counting (number/mm).

Groups	CA1(number/mm)	Dentate Gyrus (number/mm)
Control	623.8 ± 8.1	688.11 ± 8.1
Alcohol (self administration)	500.6 ± 11.9[a]	546.2 ± 6.9[a]
Alcohol (2 g/kg/day)	472.3 ± 9.4[a]	529.10 ± 9.9[a]
Alcohol (2 mg/kg/day) + Curcumin (10 mg/kg)	502.3 ± 9.1	554.2 ± 9.8
Alcohol (2 mg/kg/day) + Curcumin (20 mg/kg)	545.6 ± 12	579.3 ± 7.2
Alcohol (2 mg/kg/day) + Curcumin (40 mg/kg)	577 ± 9.9[b,c]	617.3 ± 11.1[b,c]
Alcohol (2 mg/kg/day) + Curcumin (60 mg/kg)	600 ± 10.8[b,c]	626.3 ± 12.2[b,c]
Curcumin (60 mg/kg)	618.1 ± 9.1[b]	676.2 ± 10[b]

Data are represented as mean ± SEM (n = 8, each group).
[a] $p < 0.05$ vs. control group.
[b] $p < 0.05$ vs. alcohol (2 g/kg/day) and alcohol self-administered groups.
[c] $p < 0.05$ vs. alcohol (2 g/kg/day) in combination with curcumin (10 or 20 mg/kg) groups.

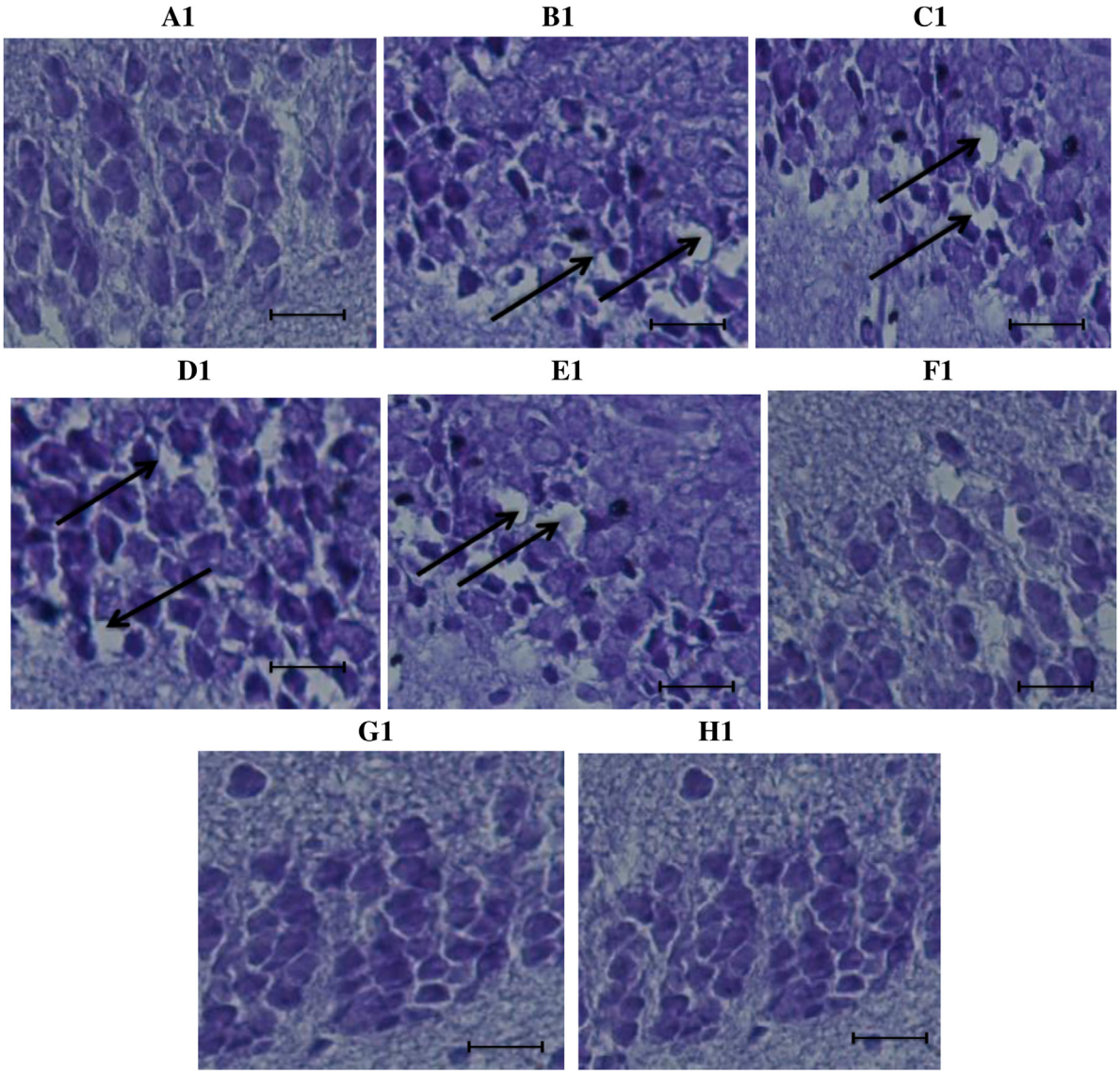

Fig. 10. Representative images of crystal violet-stained dentate gyrus. Control group (A1), alcohol (2 g/kg/day) group (B1), alcohol self-administration group (C1), alcohol (2 g/kg/day) in combination with curcumin (10, 20, 40 and 60 mg/kg, respectively) (D1, E1, F1 and G1) and curcumin only (60 mg/kg) group (H1). Arrows indicate the vacuolation and neuronal degeneration in dentate gyrus (Magnification ×400. Scale bar 100 μm).

4. Discussion

The current study demonstrates that various doses of curcumin can suppress alcohol-induced apoptosis, oxidative stress and inflammation in the hippocampus of adult rats. Furthermore, our study shows that these protective effects of curcumin are mediated via CREB-BDNF signaling pathway. We found that chronic alcohol administration alters behavioral parameters, such as motor

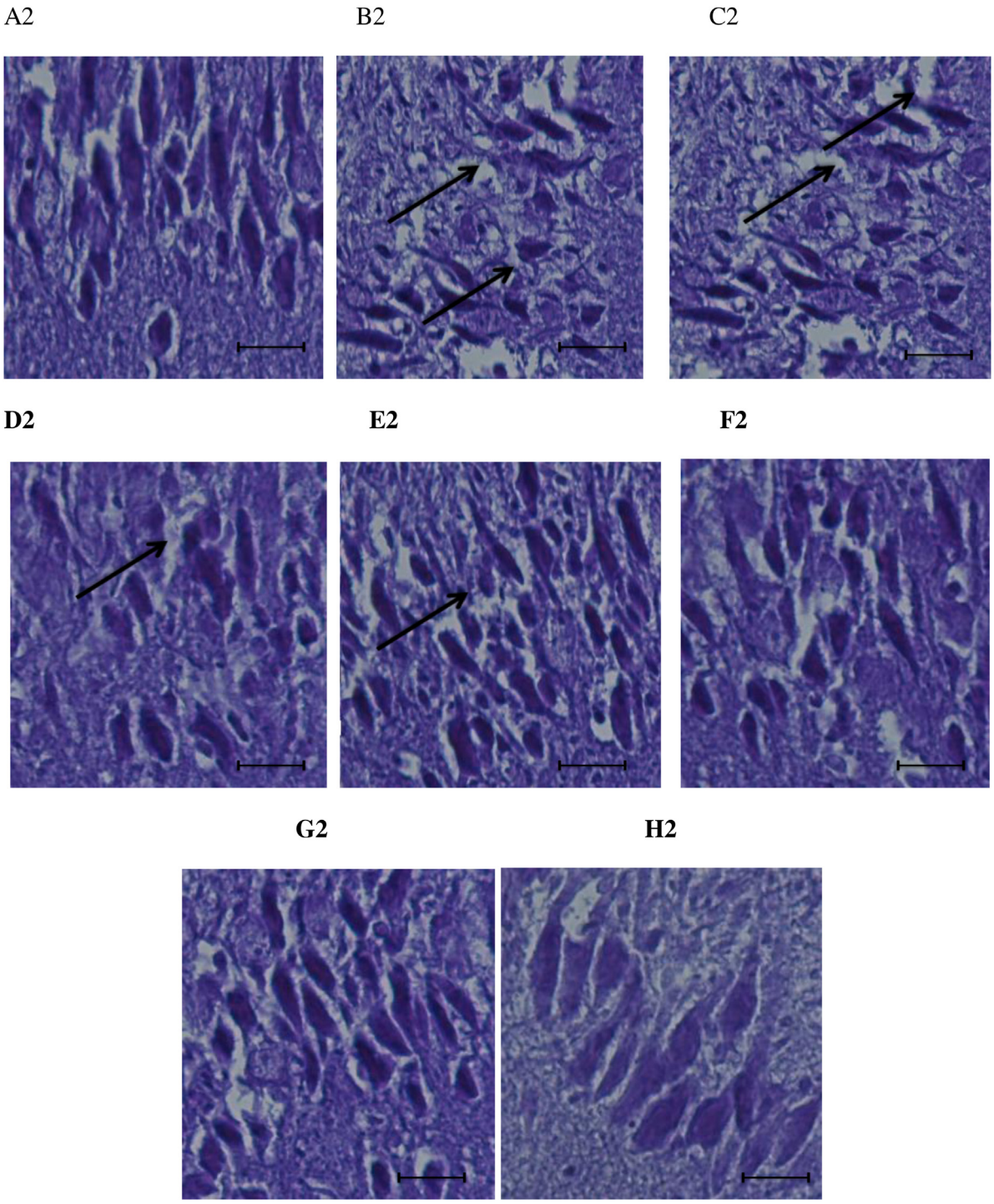

Fig. 11. Representative images of crystal violet-stained CA1 region of hippocampus. Control group(A2), alcohol (2 g/kg/day) group(B2), alcohol self-administration group(C2), alcohol (2 g/kg/day) in combination with curcumin (10, 20, 40 and 60 mg/kg, respectively) (D2, E2, F2 and G2) and curcumin only (60 mg/kg) group (H2). Arrows indicate the shrinkage and degeneration of neurons (Magnification ×400. Scale bar 100 μm).

activity and anxiety as assessed by OFT. Alcohol increases lipid peroxidation and oxidized form of glutathione (GSSG) while decreasing the reduced form of glutathione (GSH), GPx, GR and SOD activities in rat hippocampus. In contrast, curcumin (10, 20, 40 and 60 mg/kg) in a dose dependent manner attenuates lipid peroxidation and oxidative stress in both alcohol administrated groups. We found that alcohol treatment increases the level of pro-inflammatory cytokines like IL-β and TNF-α in the hippocampal tissues. Whereas, curcumin (10, 20, 40 and 60 mg/kg) has a strong potential for suppressing alcohol-induced neuroinflammation in a dose dependent manner. Moreover, both self-administered and forcefully-administered alcohol groups showed an elevated expression of an apoptotic protein Bax and Bax/Bcl-2 ratio, and a depressed expression of an anti-apoptotic protein Bcl-2. Interestingly, curcumin treatment antagonized alcohol-induced neuroapoptosis in a dose dependent manner. The key finding of

this study is that chronic alcohol consumption disrupts CREB-BDNF signaling, whereas, curcumin mediates neuroprotection against alcohol-induced toxicity by restoring CREB-BDNF signaling pathway.

Alcohol, due to its sedative effect, carries a high potential for being abused [5,40,56]. Studies have reported numerous alcohol-induced behavioral disturbances. Cippitelli et al., demonstrated that rats with chronic ethanol abuse have impaired spatial navigation skills in morris water maze test and poor normal behavior in elevated plus maze task, which were related to altered molecular functions in brain [6]. Our results indicated that 2 g/kg/day alcohol as well as voluntary consumption of alcohol in rats decrease the ambulation number and distance in OFT. In contrast to this, curcumin at 40 and 60 mg/kg doses increases ambulation number and distance in alcohol exposed rats. This curcumin-induced behavioral improvement is in accordance with previous studies [37,57]. In addition, some previous works have shown that chronic alcohol administration can alter motor activity in animal models and human subjects [37,58–60]. Similar to previous reports [61–63], the current study demonstrates that alcohol abuse decreases the number of central square entries and time spent in central square in OFT, which is indicative of alcohol-induced anxiety-like behavior in rats. Furthermore, curcumin at doses of 40 and 60 mg/kg was found to be effective in attenuating anxiety levels in rats. However, this study showed that curcumin (60 mg/kg) alone did not change ambulation number and distance and also did not affect the number of central square entries and time spent in central square in OFT. By these findings, it can be discussed that the mentioned changes in OFT is due to alcohol consumption and curcumin can prevent these effects of alcohol in OFT, while the curcumin alone did not cause an anxiety like behavior or motor activity disturbances in OFT. Previous study from our laboratory and other studies have reported such anxiolytic and anti-depressant effects of curcumin on drug induced disturbances [1,2,24,64,65]. In accordance to previous studies, the current study shows that curcumin at highest dose studied (60 mg/kg) is more efficient in evoking neuroprotective effects on brain [2]. Some reports claim that in traditional medicine this herbal compound was used as a sedative and tranquilizer [66,67], and other reports showed that curcumin can modulate drug-induced motor activity disturbances in abusers and can prevent anxiety and depression in animal and human subjects [68–70].

Previous studies have demonstrated that chronic abuse of alcohol in adult and juvenile rats leads to mitochondrial dysfunction and respiratory enzyme imbalance, which eventually induces oxidative stress in brain cells [11,71–73]. Nevertheless, the exact mechanism of action of alcohol in this regard still remains unclear. Our results indicated that voluntary and involuntary administration of alcohol increases hippocampal MDA level, whereas, curcumin treatment (10, 20, 40 and 60 mg/kg) reduces alcohol-induced rise in lipid peroxidation in the brain. The inhibitory effect of curcumin on MDA level was more pronounced at high doses (60 mg/kg) compared to low doses used in this study (10 and 20 mg/kg). Also our data indicated that curcumin alone did not affect significantly the MDA level in hippocampus.

These results are similar to previous findings which indicated alcohol-induced lipid peroxidation in the brain [12,71]. According to these findings, it seems that part of destructive effects of alcohol is mediated through mitochondrial dysfunction and probably curcumin is somehow modulating this process.

Furthermore, it has been confirmed by previous studies that curcumin exerts neuroprotective effects by inhibiting the formation of free radicals [74,75]. The role of curcumin as a scavenger for free radicals is well-evident in literature [45]. Our results indicated that alcohol decreases mitochondrial GSH content, while increasing GSSG level in the hippocampal tissues. According to previous studies, the neurotoxic effects of alcohol can be at least partly attributed to its ability to convert the protective reduced glutathione form (GSH) to the toxic oxidized form (GSSG) [12,71]. The reduced form of glutathione inhibits lipid peroxidation and acts as an antioxidant mediator by scavenging free radicals [45,76]. Many previous studies clarified that alcohol abuse can affect glutathione pathway and by this key change can start and activate neurodegenerative signals in brain[76,77], these reports that alcohol inhibit the enzymes which convert glutathione destructive form (GSSG) to protective form(GSH) and by this mechanism cause harmful effect on glutathione cycle and consequently causes neural cell death [76,77]. Moreover, we found that various doses of curcumin, specially 40 and 60 mg/kg, improve GSH content, while reducing GSSG level in animals with alcohol consumption. These findings have also been reported already by previous studies indicating that curcumin can be therapeutically beneficial against neurodegenerative diseases as it promotes GSH formation [76,78]. According to these findings and already reported, an important parts of curcumin neuroprotection properties could be attributed to the positive role of curcumin on glutathione circle [76].

In our study, forceful and self administration of alcohol decreased GPx, GR and SOD activities in isolated hippocampal tissues. Previous studies have reported that alcohol abuse diminishes antioxidant defenses which may result in neuro-degeneration [79,80]. It has been shown that GR is the main enzyme responsible for converting the oxidized glutathione (GSH) to the reduced form (GSSG) [1,76]. Thus, alcohol-induced decrease in GR activity results in elevated GSSG and reduced GSH levels as observed in our results (Table 3). Some novel reports showed that alcohol consumption causes inhibition of antioxidant activity in multiple cells, and these effects cause alcohol induced degenerative effects on body cells such as brain, liver, heart and other cells [81–83], These data confirms the destructive properties of alcohol on inhibition of antioxidant enzymes such as GPx, GR, CAT and SOD [81–83].

We observed that curcumin treatment dose-dependently improves the activity of antioxidant enzymes. Curcumin by activating GR, increases the conversion of GSSG to GSH and thus, protects the brain against alcohol-induced oxidative stress. Previous in vivo and in vitro studies have also confirmed such antioxidative properties of curcumin [24,45,84]. It has been proposed that herbal drugs such as curcumin actively inhibit glutathione-redox cycle and inhibit GSSG formation by increasing enzymes like GR and GPx [24,85]. In addition, our results confirmed the previous findings regarding the decrease in SOD activity following alcohol abuse [86]. In accordance with previous studies, treatment by curcumin was found to be effective in reversing this alcohol-induced reduction in SOD activity in the hippocampal tissues [12,85,87]. The role of curcumin in improvement of antioxidant defenses and increase of activities of antioxidant enzymes were clarified by multiple studies [88,89]. All of these reports believed that curcumin and other similar herbal compounds can activate antioxidant enzymes and by this type of activation could be involved in neuroprotection against some neurotoxic agent such as alcohol, morphine and amphetamine type stimulant properties [1,88–90]. Improvement of antioxidant defenses by curcumin can prohibit formation of free radicals and oxidative molecules and thus can inhibit cell death [1,89]. In addition, our data showed that curcumin alone did not significantly affect the GPx, GR and SOD activities in hippocampus which confirmed that in group under treatment by alcohol in combination with curcumin, alcohol consumption decreases the GPx, GR and SOD activities and this was inhibited by curcumin.

We demonstrated that chronic alcohol administration increases the inflammatory biomarkers in the hippocampus. Our results are

in agreement with previous findings which have reported the rise of pro-inflammatory cytokines following alcohol abuse [6,74,91,92]. The role of activation of neuroinflammtion by drug abuse especially alcohol consumption, in animal and human subjects, were reported already [6,93].These studies suggested that neuroinflammation and production of inflammatory biomarkers attribute to alcohol induced cell death [6,93].

We found that curcumin treatment in a dose dependent manner suppresses alcohol-induced neuroinflammation, as observed by reduced IL-1β and TNF-α levels. Similarly, a study on alzheimer's disease reveals the potential of this herbal compound in depressing the inflammation caused by activated astrocytes and microglia[94]. Moreover, curcumin has shown to block multiple sites of TNF-α and TGF-β signaling cascades, thereby protecting the tissue against inflammation and injury [38,95–97]. Anti inflammatory role of curcumin was confirmed in previous studies [76,98], and this property is the reason for its effectiveness in autoimmune disorders [76,98], and neuroprotective effects against neurotoxic agent induced inflammation [68,76,98].

Current study showed that high doses of curcumin (40 and 60 mg/kg) markedly decrease IL-1β and TNF-α level as compared to low doses of curcumin (10 or 20 mg/kg), which confirms its dose dependent effects on anti-inflammatory parameters. Also our data showed that curcumin alone did not affect on IL-1β and TNF-α levels in hippocampus which confirmed that in group under treatment by alcohol in combination with curcumin, alcohol consumption can cause inflammation and this can be inhibited by curcumin

In addition to oxidative stress and inflammation, the current study confirms alcohol-induced apoptosis in rat hippocampus. Voluntary and involuntary administration of alcohol increased Bax and decreased Bcl-2 protein expressions. These results are in accordance with the previous studies which have reported that the alcohol and tobacco abuse trigger apoptosis, and eventually lead to brain damage [6,99]. One of the proposed pathways for alcohol induced neurodegeneartion was activation of apoptosis in brain cells which caused cell death [100]. Infact these type of activation of apoptosis was suggested as the main and key pathway for alcohol induced cell damage and breakdown [100].

The results of present study demonstrated the anti-apoptotic effect of curcumin (especially in 40 and 60 mg/kg) against alcohol toxicity, as indicated by reduced Bax and improved Bcl-2 expressions in the hippocampus. Wang et al., demonstrated that curcumin treatment markedly attenuates cleaved caspase-3 and nuclear condensation resulting from brain ischemia-reperfusion injury[38]. Curcumin properties in management and inhibition of cell death were suggested by various reports [1,30]. Herbal compounds such as curcumin can inhibit pro-apoptotic and can inhibit drug-induced cell death by inhibition of apoptotic and pro-apoptotic factors in various brain areas, which this property somehow take a part in neuroprotective role of curcumin [1,101]. Our data also indicates that curcumin at high doses (40 and 60 mg/kg) significantly decreases alcohol-induced apoptosis as compared to lower doses (10 or 20 mg/kg) which confirms its dose dependent effects on alcohol-induced toxicity. Also our data showed that curcumin alone did not affect the Bcl-2 and Bax protein expressions in hippocampus which confirmed that in group under treatment by alcohol in combination with curcumin, alcohol consumption can cause apoptosis which this effect was inhibited by high doses of curcumin.

Brain-derived neurotrophic factor (BDNF), an important nerve growth factor, plays a vital role in synaptic plasticity, neurogenesis and neuronal survival [35]. BDNF and its receptor, tropomyosin-related kinase B, are regulated by cAMP and CREB. Furthermore, CREB-BDNF signaling has been implicated in regulating several neural functions like learning, memory, mood balances and reward mechanisms [102]. Previous works have reported the reduced expression of CREB, CREB-P and BDNF in alcohol abuse animal models. Perturbation in CREB-BDNF signaling pathway during alcohol-abuse has been speculated in cell death and neurodegeneration [103]. Bison and Crews demonstrated that a 4-day binge ethanol treatment reduces CREB-P immunoreactivity in the hilus of DG and in CA3 and CA2 fields of hippocampus [104]. Furthermore, ethanol-induced reduction in cAMP, CREB and CREB-P expression was associated with impairment of mitochondrial function and cellular toxicity in human neuroblastoma cells [105]. Lovinger et al., demonstrated ethanol-induced inhibition of glutamate receptor activation in hippocampal neurons [106], which may in turn reduce BDNF production by disturbing calcium-mediated signaling pathways [107]. In addition, ethanol inhibits cell proliferation by interfering with mitogenic growth factors like bFGF and IGF-1 (basic fibroblast growth factor and insulin-like growth factor 1, respectively) [108]. The growth factors via PI3K-Akt (Phosphatidylinositol-3-Kinase-Protein Kinase B) pathway activate CREB and may influence BDNF production [109,110].

The key focus of the current study was to investigate the molecular mechanisms involved in curcumin-induced neuroprotection against alcohol abuse and in this regards, we studied CREB-BDNF signaling pathway. Similar to previous reports, we found that voluntary and involuntary administration of alcohol suppresses expression of CREB and CREB-P in the DG and CA1 regions of hippocampus, and consequently reduces BDNF expression at gene and protein levels. Interestingly, curcumin in a dose dependent manner (specially at 40 and 60 mg/kg) increases CREB, CREB-P and BDNF expression in the hippocampal tissues. Since, ethanol consumption has shown to perturb cAMP–protein kinase A signaling within mesolimbic reward pathway [111], it can be suggested that curcumin treatment restores the cAMP-CREB cascade and improves BDNF levels in alcohol-addicted rats. Kumar et al., demonstrated that curcumin treatment improves diabetes-induced alternations in dopaminergic signaling by improving expression of phospholipase C and CREB [36]. In addition, curcumin promotes neuronal survival by evoking a dose- and time- dependent increase in BDNF protein [38]. Fig. 12 vdescribes the alcohol-induced perturbation of possible signaling pathways which can influence BDNF production, and the inhibitory effect of curcumin on these signaling perturbations. Moreover, similar to previous studies[112], our data showed that curcumin alone did not significantly affect on cAMP-CREB cascade protein expression in hippocampus which confirmed that in group with alcohol consumption in combination with curcumin, alcohol abuse can cause decrease of cAMP-CREB cascade protein expression which could inhibited by high doses of curcumin.

The histological findings of present study indicated a significant neurodegeneration caused by alcohol consumption in the DG and CA1 areas of hippocampus. However, curcumin treatment reduced alcohol-induced cell degeneration and improved neuronal survival by reducing apoptosis, oxidative stress, neuroinflammation and perturbation in CREB-BDNF signaling. We found that curcumin alone did not significantly affect the histology of DG and CA1 areas of hippocampus which confirmed that in group received alcohol in combination with curcumin, alcohol abuse can cause histological changes in DG and CA1 areas of hippocampus which could be inhibited by high doses of curcumin. Ying et al., demonstrated the potential of curcumin in reversing impaired hippocampal neurogenesis in chronically stressed rats via up-regulation of BDNF and serotonin receptors [113]. Furthermore, Dong and his group also reported curcumin-induced diverse effects on genes related to growth and plasticity [101]. Studies have shown that oxidative stress, inflammation and apoptosis are suitable indicators representing mitochondrial dysfunction[114]. A number of diseases such as parkinson's disease, gliomas and sclerosis are associated

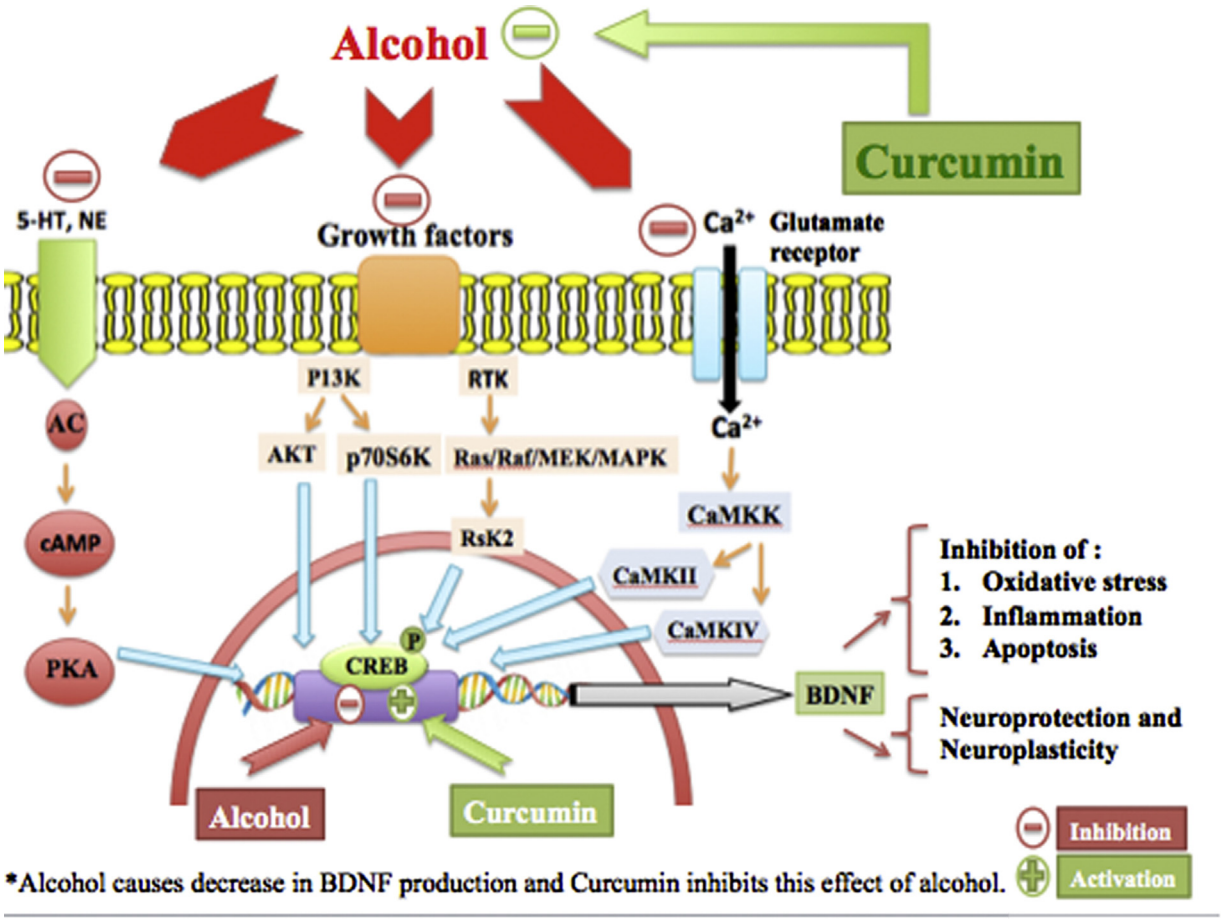

Fig. 12. Schematic presentation of neuroprotective effects of curcumin on alcohol-induced neurotoxicity..

with dysfunction of mitochondrial complexes. Inhibition of mitochondrial respiratory chain complexes can induce cell death by enhancing the production of reactive oxygen species[109,114]. Studies have shown that CREB-BDNF signaling pathway protects neuronal mitochondria against various malicious agents [109].

5. Conclusion

For the first time, the current study shows that curcumin treatment reduces the alcohol-induced apoptosis, oxidative stress and inflammation probably via activation of CREB-BDNF pathway. Thus, curcumin may be useful as a therapeutic agent for reducing deficits caused by alcohol abuse and other neurodegenerative diseases. However, further studies regarding human dosage and toxicity are warranted.

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References

[1] M. Motaghinejad, M. Karimian, O. Motaghinejad, B. Shabab, I. Yazdani, S. Fatima, Protective effects of various dosage of Curcumin against morphine induced apoptosis and oxidative stress in rat isolated hippocampus, Pharmacol. Rep. 67 (2) (2015) 230–235.
[2] M. Motaghinejad, M.Y. Bangash, P. Hosseini, S.M. Karimian, O. Motaghinejad, Attenuation of morphine withdrawal syndrome by various dosages of curcumin in comparison with clonidine in mouse: possible mechanism, Iran. J. Med. Sci. 40 (2) (2015) 125–132.
[3] S. Bent, Herbal medicine in the United States: review of efficacy, safety, and regulation, J. Gen. Intern. Med. 23 (6) (2008) 854–859.
[4] J. Sarris, A. Panossian, I. Schweitzer, C. Stough, A. Scholey, Herbal medicine for depression, anxiety and insomnia: a review of psychopharmacology and clinical evidence, Eur. Neuropsychopharmacol. 21 (12) (2011) 841–860.
[5] M. Motaghinejad, M.A. Ghaleni, O. Motaghinejad, Preventive effects of forced exercise against alcohol-induced physical dependency and reduction of pain perception threshold, Int. J. Prev. Med. 5 (10) (2014) 1299–1307.
[6] A. Cippitelli, R. Damadzic, K. Frankola, A. Goldstein, A. Thorsell, E. Singley, R.L. Eskay, M. Heilig, Alcohol-Induced neurodegeneration, suppression of transforming growth factor-β, and cognitive impairment in rats: prevention by group II metabotropic glutamate receptor activation, Biol. Psychiatry 67 (9) (2010) 823–830.
[7] F.T. Crews, K. Nixon, Mechanisms of neurodegeneration and regeneration in alcoholism, Alcohol Alcohol. 44 (2) (2009) 115–127.
[8] D.F. Hermens, J. Lagopoulos, J. Tobias-Webb, T. De Regt, G. Dore, L. Juckes, N. Latt, I.B. Hickie, Pathways to alcohol-induced brain impairment in young people: a review, Cortex 49 (1) (2013) 3–17.
[9] A.J. Heinz, A. Beck, A. Meyer-Lindenberg, P. Sterzer, A. Heinz, Cognitive and neurobiological mechanisms of alcohol-related aggression, Nat. Rev. Neurosci. 12 (7) (2011) 400–413.
[10] F.T. Crews, Alcohol-related neurodegeneration and recovery: mechanisms from animal models, Alcohol Res. Health 31 (4) (2008) 377–388.
[11] S.K. Das, D. Vasudevan, Alcohol-induced oxidative stress, Life Sci. 81 (3) (2007) 177–187.
[12] J. Haorah, S.H. Ramirez, N. Floreani, S. Gorantla, B. Morsey, Y. Persidsky, Mechanism of alcohol-induced oxidative stress and neuronal injury, Free Radic. Biol. Med. 45 (11) (2008) 1542–1550.
[13] C.A. Casey, S.M. Lee, R. Aziz-Seible, B.L. McVicker, Impaired receptor-mediated endocytosis: its role in alcohol-induced apoptosis, J. Gastroenterol. Hepatol. 23 (s1) (2008) S46–S49.
[14] Y. Chen, F. Tassone, R.F. Berman, P.J. Hagerman, R.J. Hagerman, R. Willemsen, I. N. Pessah, Murine hippocampal neurons expressing Fmr1 gene premutations show early developmental deficits and late degeneration, Hum. Mol. Genet. 19 (1) (2010) 196–208.

[15] L. Jing, C.-m. Jin, S.-s. Li, F.-m. Zhang, L. Yuan, W.-m. Li, Y. Sang, S. Li, L.-j. Zhou, Chronic alcohol intake-induced oxidative stress and apoptosis: role of CYP2E1 and calpain-1 in alcoholic cardiomyopathy, Mol. Cell. Biochem. 359 (1–2) (2012) 283–292.
[16] Y. Sari, J.M. Weedman, M. Nkrumah-Abrokwah, Neurotrophic peptides, ADNF-9 and NAP, prevent alcohol-induced apoptosis at midgestation in fetal brains of C57BL/6 mouse, J. Mol. Neurosci. 49 (1) (2013) 150–156.
[17] M. Comporti, C. Signorini, S. Leoncini, C. Gardi, L. Ciccoli, A. Giardini, D. Vecchio, B. Arezzini, Ethanol-induced oxidative stress: basic knowledge, Genes Nutr. 5 (2) (2010) 101–109.
[18] S.A. Morris, D.W. Eaves, A.R. Smith, K. Nixon, Alcohol inhibition of neurogenesis: a mechanism of hippocampal neurodegeneration in an adolescent alcohol abuse model, Hippocampus 20 (5) (2010) 596–607.
[19] M.A. Taffe, R.W. Kotzebue, R.D. Crean, E.F. Crawford, S. Edwards, C.D. Mandyam, Long-lasting reduction in hippocampal neurogenesis by alcohol consumption in adolescent nonhuman primates, Proc. Natl. Acad. Sci. U. S. A. 107 (24) (2010) 11104–11109.
[20] Y. Zhong, G. Dong, H. Luo, J. Cao, C. Wang, J. Wu, Y.-Q. Feng, J. Yue, Induction of brain CYP2E1 by chronic ethanol treatment and related oxidative stress in hippocampus, cerebellum, and brainstem, Toxicology 302 (2) (2012) 275–284.
[21] I. Mohanty, D.S. Arya, A. Dinda, S. Joshi, K.K. Talwar, S.K. Gupta, Protective effects of Curcuma longa on ischemia-reperfusion induced myocardial injuries and their mechanisms, Life Sci. 75 (14) (2004) 1701–1711.
[22] H. Awasthi, S. Tota, K. Hanif, C. Nath, R. Shukla, Protective effect of curcumin against intracerebral streptozotocin induced impairment in memory and cerebral blood flow, Life Sci. 86 (3) (2010) 87–94.
[23] J. Mehla, K. Reeta, P. Gupta, Y.K. Gupta, Protective effect of curcumin against seizures and cognitive impairment in a pentylenetetrazole-kindled epileptic rat model, Life Sci. 87 (19) (2010) 596–603.
[24] G.M. Cole, B. Teter, S.A. Frautschy, Neuroprotective Effects of Curcumin, The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease, Springer, 2007, pp. 197–212.
[25] U. Subudhi, K. Das, B. Paital, S. Bhanja, G.B. Chainy, Supplementation of curcumin and vitamin E enhances oxidative stress, but restores hepatic histoarchitecture in hypothyroid rats, Life Sci. 84 (11) (2009) 372–379.
[26] Y. Sumanont, Y. Murakami, M. Tohda, O. Vajragupta, H. Watanabe, K. Matsumoto, Prevention of kainic acid-induced changes in nitric oxide level and neuronal cell damage in the rat hippocampus by manganese complexes of curcumin and diacetylcurcumin, Life Sci. 78 (16) (2006) 1884–1891.
[27] H.-C. Huang, K. Xu, Z.-F. Jiang, Curcumin-mediated neuroprotection against amyloid-(-induced mitochondrial dysfunction involves the inhibition of GSK-3β, J. Alzheimer's Dis. JAD 32 (4) (2011) 981–996.
[28] S.J. Kim, T.G. Son, H.R. Park, M. Park, M.-S. Kim, H.S. Kim, H.Y. Chung, M.P. Mattson, J. Lee, Curcumin stimulates proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus, J. Biol. Chem. 283 (21) (2008) 14497–14505.
[29] R. Bhandari, A. Kuhad, Neuropsychopharmacotherapeutic efficacy of curcumin in experimental paradigm of autism spectrum disorders, Life Sci. 141 (2015) 156–169.
[30] A. Jagota, M. Reddy, The effect of curcumin on ethanol induced changes in suprachiasmatic nucleus (SCN) and pineal, Cell. Mol. Neurobiol. 27 (8) (2007) 997–1006.
[31] T. Ak, İ. Gülçin, Antioxidant and radical scavenging properties of curcumin, Chem. Biol. Interact. 174 (1) (2008) 27–37.
[32] V.P. Menon, A.R. Sudheer, Antioxidant and Anti-inflammatory Properties of Curcumin, The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease, Springer, 2007, pp. 105–125.
[33] D. Tardito, J. Perez, E. Tiraboschi, L. Musazzi, G. Racagni, M. Popoli, Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview, Pharmacol. Rev. 58 (1) (2006) 115–134.
[34] A.Y. Wen, K.M. Sakamoto, L.S. Miller, The role of the transcription factor CREB in immune function, J. Immunol. 185 (11) (2010) 6413–6419.
[35] S. Dworkin, T. Mantamadiotis, Targeting CREB signalling in neurogenesis, Expert Opin. Ther. Targets 14 (8) (2010) 869–879.
[36] R.J. Donati, M.M. Rasenick, G protein signaling and the molecular basis of antidepressant action, Life Sci. 73 (1) (2003) 1–17.
[37] T.P. Kumar, S. Antony, G. Gireesh, N. George, C. Paulose, Research Curcumin modulates dopaminergic receptor, CREB and phospholipase c gene expression in the cerebral cortex and cerebellum of streptozotocin induced diabetic rats, J. Biomed. Sci. 31 (17) (2010) 17–43.
[38] R. Wang, Y.-H. Li, Y. Xu, Y.-B. Li, H.-L. Wu, H. Guo, J.-Z. Zhang, J.-J. Zhang, X.-Y. Pan, X.-J. Li, Curcumin produces neuroprotective effects via activating brain-derived neurotrophic factor/TrkB-dependent MAPK and PI-3 K cascades in rodent cortical neurons, Prog. Neuropsychopharmacol. Biol. Psychiatry 34 (1) (2010) 147–153.
[39] M. Thiyagarajan, S.S. Sharma, Neuroprotective effect of curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats, Life Sci. 74 (8) (2004) 969–985.
[40] M. Motaghinejad, M.Y. Bangash, O. Motaghinejad, Attenuation of alcohol withdrawal syndrome and blood cortisol level with forced exercise in comparison with diazepam, Acta Med. Iran. 53 (5) (2015) 311–316.
[41] V. Jevtovic-Todorovic, R.E. Hartman, Y. Izumi, N.D. Benshoff, K. Dikranian, C.F. Zorumski, J.W. Olney, D.F. Wozniak, Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits, J. Neurosci. 23 (3) (2003) 876–882.
[42] K. Nixon, F.T. Crews, Binge ethanol exposure decreases neurogenesis in adult rat hippocampus, J. Neurochem. 83 (5) (2002) 1087–1093.
[43] M. Motaghinejad, M. Motevalian, S.F. Larijani, Z. Khajehamedi, Protective effects of forced exercise against methylphenidate-induced anxiety, depression and cognition impairment in rat, Adv. Biomed. Res. 27 (4) (2015) 134–137.
[44] M. Motaghinejad, M. Motevalian, A. Ebrahimzadeh, Reduction of methylphenidate induced anxiety, depression and cognition impairment by various doses of venlafaxine in rat, Int. J. Prev. Med. 6 (52) (2015) 25–28.
[45] M. Motaghinejad, S.M. Karimian, O. Motaghinejad, B. Shabab, M. Asadighaleni, S. Fatima, The effect of various morphine weaning regimens on the sequelae of opioid tolerance involving physical dependency, anxiety and hippocampus cell neurodegeneration in rats, Fundam. Clin. Pharmacol. 29 (3) (2015) 299–309.
[46] M. Motaghinejad, M. Motevalian, B. Shabab, Neuroprotective effects of various doses of topiramate against methylphenidate induced oxidative stress and inflammation in rat isolated hippocampus, Clin. Exp. Pharmacol. Physiol. 43 (3) (2016) 360–371.
[47] M. Motaghinejad, M. Motevalian, B. Shabab, Effects of chronic treatment with methylphenidate on oxidative stress and inflammation in hippocampus of adult rats, Neurosci. Lett. 619 (2016) 106–113.
[48] M. Motaghinejad, Z. Seyedjavadein, M. Motevalian, M. Asadi, The neuroprotective effect of lithium against high dose methylphenidate: possible role of BDNF, Neurotoxicology 56 (2016) 40–54.
[49] M. Motaghinejad, M. Motevalian, Involvement of AMPA/kainate and GABA A receptors in topiramate neuroprotective effects against methylphenidate abuse sequels involving oxidative stress and inflammation in rat isolated hippocampus, Eur. J. Pharmacol. 784 (2016) 181–191.
[50] M. Motaghinejad, M. Motevalian, B. Shabab, Possible involvements of glutamate and adrenergic receptors on acute toxicity of methylphenidate in isolated hippocampus and cerebral cortex of adult rats, Fundam. Clin. Pharmacol. (2016) (Epub ahead of print).
[51] M. Motaghinejad, M. Motevalian, B. Shabab, S. Fatima, Effects of acute doses of methylphenidate on inflammation and oxidative stress in isolated hippocampus and cerebral cortex of adult rats, J. Neural. Transm. (Vienna) 1–11 (2016) (Epub ahead of print).
[52] M.W. Pfaffl, G.W. Horgan, L. Dempfle, Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR, Nucleic Acids Res. 30 (9) (2002) e36.
[53] S. Gingerich, G. Kim, J. Chalmers, M. Koletar, X. Wang, Y. Wang, D. Belsham, Estrogen receptor alpha and G-protein coupled receptor 30 mediate the neuroprotective effects of 17β-estradiol in novel murine hippocampal cell models, Neuroscience 170 (1) (2010) 54–66.
[54] D.L. Champagne, R.C. Bagot, F. van Hasselt, G. Ramakers, M.J. Meaney, E.R. de Kloet, M. Joëls, H. Krugers, Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress, J. Neurosci. 28 (23) (2008) 6037–6045.
[55] A. Sahay, R. Hen, Adult hippocampal neurogenesis in depression, Nat. Neurosci. 10 (9) (2007) 1110–1115.
[56] R. Cramb, Drug and alcohol abuse reviews. liver pathology and alcohol, J. Med. Microbiol. 38 (3) (1993) 235.
[57] L. Helson, S. Chiu, Intravenous Curcumin and Derivatives for Treatment of Neurodegenerative and Stress Disorders, Google Patents, 2011.
[58] H.J. Hanchar, P.D. Dodson, R.W. Olsen, T.S. Otis, M. Wallner, Alcohol-induced motor impairment caused by increased extrasynaptic GABAA receptor activity, Nat. Neurosci. 8 (3) (2005) 339–345.
[59] M. Motaghinejad, O. Motaghinejad, Preventive effects of forced exercise against alcohol induced physical dependency and reduction of pain perception threshold, Int. J. Prev. Med. 5 (10) (2014) 307–311.
[60] S. Bulle, V.D. Reddy, A.V. Hebbani, P. Padmavathi, C. Challa, P.K. Puvvada, E. Repalle, D. Nayakanti, C.A. Narasimhulu, V. Nallanchakravarthula, Nephro-protective action of P. santalinus against alcohol-induced biochemical alterations and oxidative damage in rats, Biomed. Pharmacother. 84 (2016) 740–746.
[61] M.A. Schuckit, V. Hesselbrock, Alcohol dependence and anxiety disorders, FOCUS (2014) 25–30.
[62] R. Spanagel, Alcoholism: a systems approach from molecular physiology to addictive behavior, Physiol. Rev. 89 (2) (2009) 649–705.
[63] L. Letenneur, S. Larrieu, P. Barberger-Gateau, Alcohol and tobacco consumption as risk factors of dementia: a review of epidemiological studies, Biomed. Pharmacother. 58 (2) (2004) 95–99.
[64] G.M. Abu-Taweel, J.S. Ajarem, M. Ahmad, Protective effect of curcumin on anxiety, learning behavior, neuromuscular activities, brain neurotransmitters and oxidative stress enzymes in cadmium intoxicated mice, J. Behav. Brain Sci. 3 (01) (2013) 74–80.
[65] M. Motaghinejad, M.Y. Bangash, P. Hosseini, S.M. Karimian, O. Motaghinejad, Attenuation of morphine withdrawal syndrome by various dosages of curcumin in comparison with clonidine in mouse: possible mechanism, Iran J. Med. Sci. 40 (2) (2015) 125–132.
[66] M. Akaberi, Z. Sobhani, B. Javadi, A. Sahebkar, S.A. Emami, Therapeutic effects of Aloe spp. in traditional and modern medicine: a review, Biomed. Pharmacother. 84 (2016) 759–772.

[67] A. Shojaii, M. Motaghinejad, S. Norouzi, M. Motevalian, Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of astragalus hamosus in animal models, Iran J. Pharm. Res. 14 (1) (2015) 263–270.
[68] A.L. Lopresti, M. Maes, G.L. Maker, S.D. Hood, P.D. Drummond, Curcumin for the treatment of major depression: a randomised double-blind, placebo controlled study, J. Affect. Disord. 167 (2014) 368–375.
[69] S. Kulkarni, A. Dhir, K.K. Akula, Potentials of curcumin as an antidepressant, Sci.World J. 9 (2009) 1233–1241.
[70] M. Gazal, M.R. Valente, B.A. Acosta, F.N. Kaufmann, E. Braganhol, C.L. Lencina, F.M. Stefanello, G. Ghisleni, M.P. Kaster, Neuroprotective and antioxidant effects of curcumin in a ketamine-induced model of mania in rats, Eur. J. Pharmacol. 724 (2014) 132–139.
[71] E. Agar, S. Demir, R. Amanvermez, M. Bosnak, M. Ayyildiz, C. Celik, The effects of ethanol consumption on the lipid peroxidation and glutathione levels in the right and left brains of rats, Int. J. Neurosci. 13 (12) (2009) 1643–1652.
[72] O.L. Erukainure, J.A. Ajiboye, R.O. Adejobi, O.Y. Okafor, S.O. Adenekan, Protective effect of pineapple (Ananas cosmosus) peel extract on alcohol? induced oxidative stress in brain tissues of male albino rats, Asian Pac. J. Trop. Dis. 1 (1) (2011) 5–9.
[73] S. Bulle, V.D. Reddy, P. Padmavathi, P. Maturu, V.N. Ch, Modulatory role of Pterocarpus santalinus against alcohol-induced liver oxidative/nitrosative damage in rats, Biomed. Pharmacother. 83 (2016) 1057–1063.
[74] E. Albano, Alcohol, oxidative stress and free radical damage, Proc. Nutr. Soc. 65 (03) (2006) 278–290.
[75] J. Haorah, B. Knipe, J. Leibhart, A. Ghorpade, Y. Persidsky, Alcohol-induced oxidative stress in brain endothelial cells causes blood-brain barrier dysfunction, J. Leukoc. Biol. 78 (6) (2005) 1223–1232.
[76] B. Jagatha, R.B. Mythri, S. Vali, M.S. Bharath, Curcumin treatment alleviates the effects of glutathione depletion in vitro and in vivo: therapeutic implications for Parkinson's disease explained via in silico studies, Free Radic. Biol. Med. 44 (5) (2008) 907–917.
[77] K. Bala, B. Tripathy, D. Sharma, Neuroprotective and anti-ageing effects of curcumin in aged rat brain regions, Biogerontology 7 (2) (2006) 81–89.
[78] M.H. Stridh, F. Correa, C. Nodin, S.G. Weber, F. Blomstrand, M. Nilsson, M. Sandberg, Enhanced glutathione efflux from astrocytes in culture by low extracellular Ca2+ and curcumin, Neurochem. Res. 35 (8) (2010) 1231–1238.
[79] P.S. Brocardo, J. Gil-Mohapel, B.R. Christie, The role of oxidative stress in fetal alcohol spectrum disorders, Brain Res. Rev. 67 (1) (2011) 209–225.
[80] P.S. Brocardo, F. Boehme, A. Patten, A. Cox, J. Gil-Mohapel, B.R. Christie, Anxiety-and depression-like behaviors are accompanied by an increase in oxidative stress in a rat model of fetal alcohol spectrum disorders: protective effects of voluntary physical exercise, Neuropharmacology 62 (4) (2012) 1607–1618.
[81] L. Zhao, Y. Wang, J. Liu, K. Wang, X. Guo, B. Ji, W. Wu, F. Zhou, Protective effects of genistein and puerarin against chronic alcohol-Induced liver injury in mice via antioxidant, anti-inflammatory, and anti-apoptotic mechanisms, J. Agric. Food Chem. 64 (38) (2016) 7291–7297.
[82] D.V.N. Kumar, K. Shanmugam, C. Ramakrishna, G.V. Subbaiah, K.S. Reddy, Bacopa monniera modulates antioxidant enzymes responses against alcohol-induced oxidative stress in rat brain, Indo Am. J. Pharm. Res. 6 (2) (2016) 4569–4575.
[83] X. Joya, O. Garcia-Algar, J. Salat-Batlle, C. Pujades, O. Vall, Advances in the development of novel antioxidant therapies as an approach for fetal alcohol syndrome prevention, Birth Defects Res. A Clin. Mol. Teratol. 103 (3) (2015) 163–177.
[84] A. Dairam, R. Fogel, S. Daya, J.L. Limson, Antioxidant and iron-binding properties of curcumin, capsaicin, and S-allylcysteine reduce oxidative stress in rat brain homogenate, J. Agric. Food Chem. 56 (9) (2008) 3350–3356.
[85] T. Nishinaka, Y. Ichijo, M. Ito, M. Kimura, M. Katsuyama, K. Iwata, T. Miura, T. Terada, C. Yabe-Nishimura, Curcumin activates human glutathione S-transferase P1 expression through antioxidant response element, Toxicol. Lett. 170 (3) (2007) 238–247.
[86] X. Cai, L. Bao, N. Wang, M. Xu, R. Mao, Y. Li, Dietary nucleotides supplementation and liver injury in alcohol-Treated rats: a metabolomics investigation, Molecules 21 (4) (2016) 435.
[87] P.K. Shukla, V.K. Khanna, M.M. Ali, M.Y. Khan, R.C. Srimal, Anti-ischemic effect of curcumin in rat brain, Neuroch. Res. 3 (6) (2008) 1036–1043.
[88] W. Zhong, K. Qian, J. Xiong, K. Ma, A. Wang, Y. Zou, Curcumin alleviates lipopolysaccharide induced sepsis and liver failure by suppression of oxidative stress-related inflammation via PI3 K/AKT and NF-κB related signaling, Biomed. Pharmacother. 83 (2016) 302–313.
[89] S. Hu, P. Maiti, Q. Ma, X. Zuo, M.R. Jones, G.M. Cole, S.A. Frautschy, Clinical development of curcumin in neurodegenerative disease, Expert Rev. Neurother. 15 (6) (2015) 629–637.
[90] H. Luo, R. Cao, L. Wang, L. Zhu, Protective effect of Cistanchis A on ethanol-induced damage in primary cultured mouse hepatocytes, Biomed. Pharmacother. 83 (2016) 1071–1079.
[91] S. Alfonso-Loeches, M. Pascual, U. Gómez-Pinedo, M. Pascual-Lucas, J. Renau-Piqueras, C. Guerri, Toll-like receptor 4 participates in the myelin disruptions associated with chronic alcohol abuse, Glia 60 (6) (2012) 948–964.
[92] K.W. Kelley, R. Dantzer, Alcoholism and inflammation: neuroimmunology of behavioral and mood disorders, Brain Behav. Immun. 25 (2011) S13–S20.
[93] M. Pascual, P. Baliño, C.M. Aragón, C. Guerri, Cytokines and chemokines as biomarkers of ethanol-induced neuroinflammation and anxiety-related behavior: role of TLR4 and TLR2, Neuropharmacology 89 (2015) 352–359.
[94] H.-M. Wang, Y.-X. Zhao, S. Zhang, G.-D. Liu, W.-Y. Kang, H.-D. Tang, J.-Q. Ding, S.-D. Chen, PPARγ agonist curcumin reduces the amyloid-β-stimulated inflammatory responses in primary astrocytes, J. Alzheimers Dis. 20 (4) (2010) 1189–1199.
[95] J.-J. Kuo, H.-H. Chang, T.-H. Tsai, T.-Y. Lee, Positive effect of curcumin on inflammation and mitochondrial dysfunction in obese mice with liver steatosis, Int. J. Mol. Med. 30 (3) (2012) 673–679.
[96] B. Ray, D.K. Lahiri, Neuroinflammation in Alzheimer's disease: different molecular targets and potential therapeutic agents including curcumin, Curr. Opin. Pharmacol. 9 (4) (2009) 434–444.
[97] Y. Panahi, M.S. Hosseini, N. Khalili, E. Naimi, L.E. Simental-Mendía, M. Majeed, A. Sahebkar, Effects of curcumin on serum cytokine concentrations in subjects with metabolic syndrome: a post-hoc analysis of a randomized controlled trial, Biomed. Pharmacother. 82 (2016) 578–582.
[98] K.M. Kim, H.-O. Pae, M. Zhung, H.-Y. Ha, Y.A. Ha, K.-Y. Chai, Y.-K. Cheong, J.-M. Kim, H.-T. Chung, Involvement of anti-inflammatory heme oxygenase-1 in the inhibitory effect of curcumin on the expression of pro-inflammatory inducible nitric oxide synthase in RAW264. 7 macrophages, Biomed. Pharmacother. 62 (9) (2008) 630–636.
[99] N.B. Farber, C.E. Creeley, J.W. Olney, Alcohol-induced neuroapoptosis in the fetal macaque brain, Neurobiol. Dis. 40 (1) (2010) 200–206.
[100] B.L. McVicker, D.J. Tuma, A.A. Naji, C.A. Casey, Alcohol and Apoptosis, Elsevier Inc., 2004, pp. 105–112.
[101] S. Dong, Q. Zeng, E.S. Mitchell, J. Xiu, Y. Duan, C. Li, J.K. Tiwari, Y. Hu, X. Cao, Z. Zhao, Curcumin enhances neurogenesis and cognition in aged rats: implications for transcriptional interactions related to growth and synaptic plasticity, PLoS One 7 (2) (2012) e31211.
[102] S.C. Pandey, H. Zhang, A. Roy, K. Misra, Central and medial amygdaloid brain-derived neurotrophic factor signaling plays a critical role in alcohol-drinking and anxiety-like behaviors, J. Neurosci. 26 (32) (2006) 8320–8331.
[103] W. Guo, E.L. Crossey, L. Zhang, S. Zucca, O.L. George, C.F. Valenzuela, X. Zhao, Alcohol exposure decreases CREB binding protein expression and histone acetylation in the developing cerebellum, PLoS One 6 (5) (2011) e19351.
[104] S. Bison, F. Crews, Alcohol withdrawal increases neuropeptide Y immunoreactivity in rat brain, Alcohol. Clin. Exp. Res. 27 (7) (2003) 1173–1183.
[105] Z. Liu, Y. Liu, R. Gao, H. Li, T. Dunn, P. Wu, R.G. Smith, P.S. Sarkar, X. Fang, Ethanol suppresses PGC-1α expression by interfering with the cAMP-CREB pathway in neuronal cells, PLoS One 9 (8) (2014) e104247.
[106] D.M. Lovinger, G. White, F.F. Weight, Ethanol inhibits NMDA-activated ion current in hippocampal neurons, Science 243 (4899) (1989) 1721–1724.
[107] D.A. Fortin, T. Srivastava, D. Dwarakanath, P. Pierre, S. Nygaard, V.A. Derkach, T.R. Soderling, Brain-derived neurotrophic factor activation of CaM-kinase via transient receptor potential canonical channels induces the translation and synaptic incorporation of GluA1-containing calcium-permeable AMPA receptors, J. Neurosci. 32 (24) (2012) 8127–8137.
[108] M.W. Miller, Effects of alcohol on the generation and migration of cerebral cortical neurons, Science 233 (4770) (1986) 1308–1311.
[109] S. Impey, R.H. Goodman, CREB signaling-timing is everything, Sci. STKE 2001 (82) (2001) pe1.
[110] Z.-H. Zhang, X.-B. Fang, G.-M. Xi, W.-C. Li, H.-Y. Ling, P. Qu, Calcitonin gene-related peptide enhances CREB phosphorylation and attenuates tau protein phosphorylation in rat brain during focal cerebral ischemia/reperfusion, Biomed. Pharmacother. 64 (6) (2010) 430–436.
[111] M. Wand, L. Zweifel, W. Schwindinger, T. Abel, The cAMP–protein kinase A signal transduction pathway modulates ethanol consumption and sedative effects of ethanol, J. Neurosci. 21 (14) (2001) 5297–5303.
[112] J. Gil-Mohapel, F. Boehme, L. Kainer, B.R. Christie, Hippocampal cell loss and neurogenesis after fetal alcohol exposure: insights from different rodent models, Brain Res. Rev. 64 (2) (2010) 283–303.
[113] Y. Xu, B. Ku, L. Cui, X. Li, P.A. Barish, T.C. Foster, W.O. Ogle, Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats, Brain Res. 1162 (2007) 9–18.
[114] Z. Wu, P. Puigserver, U. Andersson, C. Zhang, G. Adelmant, V. Mootha, A. Troy, S. Cinti, B. Lowell, R.C. Scarpulla, Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1, Cell 98 (1) (1999) 115–124.