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Curcumin pretreatment attenuates brain lesion size and improves neurological function following traumatic brain injury in the rat

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ABSTRACT

Turmeric has been in use since ancient times as a condiment and due to its medicinal properties. Curcumin, the yellow coloring principle in turmeric, is a polyphenolic and a major active constituent. Besides anti-inflammatory, thrombolytic and anti-carcinogenic activities, curcumin also possesses strong antioxidant property. The neuroprotective effects of curcumin were evaluated in a weight drop model of cortical contusion trauma in rat. Male Wistar rats (350–400 g, n = 9) were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and subjected to head injury. Five days before injury, animals randomly received an i.p. bolus of either curcumin (50 and 100 mg/kg/day, n = 9) or vehicle (n = 9). Two weeks after the injury and drug treatment, animals were sacrificed and a series of brain sections, stained with hematoxylin and eosin (H&E) were evaluated for quantitative brain lesion volume. Two weeks after the injury, oxidative stress parameter (malondialdehyde) was also measured in the brain. Curcumin (100 mg/kg) significantly reduced the size of brain injury-induced lesions ($P < 0.05$). Neurological examinations (rotarod and inclined-plane tests) were performed on days 1, 3, 7 and 14 post-brain injury. Control injured rats had a significant neurological deficit during 2 weeks ($P < 0.001$). The injury increased brain levels of the malondialdehyde by 35.6% and these increases were attenuated by curcumin (100 mg/kg). Curcumin treatment significantly improved the neurological status evaluated during 2 weeks after brain injury. The study demonstrates the protective efficacy of curcumin in rat traumatic brain injury model.

1. Introduction

Traumatic brain injury is a major cause of death and disability globally. Outcome for patients with brain injury has improved over the past decades due to improved pre-hospital and neurointensive care that has focused on avoiding/reducing secondary insults in these patients (Sharma and Vavilala, 2012) rather than through the use of new pharmacological treatments for brain injury. Despite promising results from experimental drug trials, none of the clinical trials of new drugs have been able to show a significant effect on outcome for patients with head injury (Zafonte et al., 2012). It appears that further improvements in care require continued focus on pathophysiological mechanisms responsible for the enhanced vulnerability of the brain to secondary insults after trauma. Understanding the effects of different secondary insults requires multimodality monitoring to elucidate each insult's effects on the tissue and at what time point the different insults are dangerous to the patient.

The cascade of delayed or secondary pathologic events that follow an injury is extremely complex, and the relative importance of each event appears to differ in each individual case. Therefore, a great need exists for a broad-spectrum neuroprotective agent that may target several pathophysiologic processes and thus confer widespread protection to the damaged brain.

Several mechanisms have been suggested to be involved in the etiology of brain injury including NMDA receptor activation leading to excitotoxicity, excessive nitric oxide (NO) generation and free radical mediated oxidative stress (Moojen et al., 2012; Ghorbani et al., 2012). Several studies have revealed that during brain injury there is excessive generation of oxidative stress parameters such as lipid peroxides and the antioxidant defense is impaired causing more vulnerability and damage to the brain (Slemmer et al., 2008; Sadeghnia et al., 2012). Of the many biological targets of oxidative stress, the lipids are the most involved class of biomolecules. A biomarker for lipid peroxidation is malondialdehyde (MDA), it is a highly toxic molecule and it has been implicated in a range of disease pathologies by producing oxidative damage in the tissues. A number of agents, both synthetic and natural, have been screened to evaluate their preventive and therapeutic efficacy against head injury. Dietary supplementation with blueberries, spinach and spirulina reduces ischemia/reperfusion induced apoptosis

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and cerebral infarction (Singleton et al., 2010). Turmeric (Curcuma longa rhizomes) has been extensively used as an effective therapeutic agent since ages. Turmeric as well as its constituent curcumin has been shown to exhibit anti-inflammatory, anti-carcinogenic and antioxidant activities, besides several other pharmacological properties (Khurana et al., 2012). Prophylactic/therapeutic effect of curcumin in cancer chemo-prevention, multiple sclerosis and myocardial infarction has been reported (Nagaraju et al., 2012). Curcumin has been found to be effective in the treatment of anterior uveitis and cystic fibrosis (Cartiera et al., 2010). More recently, the neuroprotective efficacy of curcumin in attenuating 3-nitropropionic acid (a fungal toxin) and lead induced neurotoxicity has been reported (Kumar et al., 2007). Pari and Murugan found that tetrahydrocurcumin prevented brain lipid peroxidation in streptozotocin induced diabetic rats (Pari and Murugan, 2007). The neuroprotective effect of curcumin was associated with its antioxidant potential in these studies (Merrell et al., 2009). Curcumin has been reported to cross the blood–brain barrier and based on the potential of curcumin to inhibit the formation of amyloid beta oligomers and fibrils in mice the use of curcumin has been recommended for the clinical trials to prevent or treat Alzheimer's disease (Wang et al., 2013). The effect of curcumin was studied in rats following intraperitoneal treatment, 30 min after MCAO, indicating its neuroprotective potential in ischemia. It has been suggested to be mediated through its antioxidant activity (Thiyagarajan and Sharma, 2004). Curcumin is recognized as a promising compound with multiple pharmacological properties and the present study was undertaken in rats treated with interaperitoneal curcumin before head injury to comprehensively evaluate the potential neuroprotective effects of curcumin with respect to the brain lesion, malondialdehyde (MDA), a marker of lipid peroxidation, and posttraumatic neurologic motor deficits following an experimental model of weight drop brain injury in the rat.

2. Experimental procedure

2.1. Animals

Male Wistar rats (n = 40) weighing 350 to 400 g were housed individually in polycarbonate cages in a standard animal house maintained at 21 ± 2 °C and 50 ± 10% humidity with a 12-hour light:dark cycle. Rats were fed with standard laboratory chow and water. The experimental protocol was approved by an Institutional Review Committee of Mashhad Medical University for the use of Human or Animal Subjects and also the studies were carried out in accordance with the official regulations approved by the Animal Ethics Committee of Mashhad Medical University, Mashhad, Iran. The animals were also acclimatized to the laboratory conditions prior to experimentation. The rats were maintained in accordance with the National Institutes of Health guidelines for the care and use of laboratory rats.

2.2. Experimental groups

The rats were randomly allocated into four groups (n = 9) as follows; a control (sham-operated) group, a trauma group, and curcumin groups (50 and 100 mg/kg). Curcumin was administrated i.p. once daily for 5 days at the dose of 50 and 100 mg/kg/day. The dose of curcumin was chosen based on our previous experiments. The control group underwent craniotomy alone and received no medication. The trauma group underwent craniotomy followed by brain injury and received the vehicle. The curcumin groups underwent craniotomy followed by brain injury and received curcumin (50 and 100 mg/kg) 5 days before injury.

2.3. Drug treatment

Curcumin, (Sigma Chemicals, St, Louis, MO, USA), was suspended with dimethyl sulfoxide (DMSO) in double-distilled water. The vehicle given to the control animals had the same % of DMSO and had also similar osmolality and pH with the curcumin group. The solutions were sterilized through 0.22-mm filters and administered to animals by i.p. injection at the doses of 50 and 100 mg/kg once daily for 5 days. The final dose of curcumin was administered 30 min before the brain injury. The dose of curcumin was chosen based on previous experiments.

2.4. Surgical procedures and weight drop brain injury model

For the production of brain injury, we used the weight drop technique modified by Marklund et al. (2001) after Feeney et al. (1981). All rats were weighed before undergoing anesthesia. The animals were anesthetized by an intraperitoneal injection of 10 mg/kg xylazine (Sigma, St. Louis, USA,) and 50 mg/kg ketamine hydrochloride (Sigma, St. Louis, USA). Body temperature was monitored with a rectal probe and kept between 37.0 and 37.5 °C with a heating pad. After catheter preparation the animals were placed in a stereotaxic frame. All surgery was done under sterile conditions. A craniotomy (6×9 mm^2), centered over the right parietal cortex at bregma −3.5 and 3.5 mm lateral to the midline, was done using a dental drill. An 11 g weight was dropped from a height of 35 cm onto 4.5 mm diameter piston resting on the exposed dura. The device is constructed to prevent bouncing of the weight thus allowing only a single compression.

2.5. Locomotor activity

Rotarod, beam balance, and angel board tests were performed to evaluate the behavioral effect of curcumin on sensory-motor dysfunction after trauma. Neurological deficits in the vehicle- and drug-treated groups were determined after 1st, 3rd, 7th and 14th days after the brain injury.

2.5.1. Rotarod test

Rotarod testing took place over two consecutive days before injury. On day 1, the animals were trained until they were able to remain on the rod (speed = 16 RPM) for 120 s for two trials. The animals were given as many trials as required to reach this performance criterion, after which they were returned to their home cage until testing the following day. Animals that did not reach this criterion were eliminated from the present study. The rotarod test was conducted at 1st, 3rd, 7th and 14th days after brain injury. The latency time for each rat was determined from five separate trials; the lowest and highest outlier data were excluded and the remaining three data were averaged for the final result. On the test day, the animals were randomly assigned to a dose group (0, 50, 100 mg/kg/day; n = 9) and tested for three trials on the rod rotating at 16 rpm. The animals were allowed a minimum of 30 s rest period between trials. The dimensions of the rat rotarod were 8.5 cm in width and 7 cm in diameter. In order to assess the effect of curcumin on motor skill acquisition, the rats were transferred to the procedure room.

2.5.2. Inclined-plane test

We evaluated motor performance in rats, using a sliding apparatus two days before traumatic brain injury in rats and the following 2 weeks after trauma. The sliding apparatus had a 60 × 40-cm wood plane that could be inclined at an angle of 0° (horizontal) to 60°. Angel board testing took place over fourteen days. On day 1(before injury), each rat was placed on the 60°-angled inclined plane. The animals were trained until they were able to remain on the board for 120 s for two trials. The animals were given as many trials as required to reach this performance criterion, after which they were returned to their home cage until testing the following day. Animals that did not reach this criterion were eliminated from the study. The inclined-plane test was conducted at 1st, 3rd, 7th and 14th days after brain injury. The time for standing on an inclined angle board for each rat

was determined from five separate trials; the lowest and highest outlier data were excluded and the remaining three data were averaged for the final result. On the test day (1st, 3rd, 7th and 14th days after brain injury), the animals were tested for three trials on the board at 60°-angled inclined plane. The animals were allowed a minimum of 30 s rest period between trials.

2.6. Histopathological assessment

Immediately after the 2 weeks of neurologic motor testing, animals were over-anesthetized (sodium pentobarbital, 200 mg/kg, intraperitoneally) and transcardially perfused with 10% formalin. Brains were removed and postfixed in 10% formalin and then processed for paraffin embedding. In a subset of injured animals (vehicle, n = 9; 50 mg/kg/day, n = 9; 100 mg/kg/day, n = 9), a series of 6-μm coronal sections were cut on a rotary microtome at the levels of −3.3, −4.3, −5.3, and −6.3 mm bregma (Paxinos and Watson, 1990). One section at each level was stained with hematoxylin and eosin (H&E), and the area of the contralateral and ipsilateral cortices was then measured using a light microscope and image analysis system (MCID, Ontario, Canada), as previously described (Zhang et al., 1998). Following weight drop brain injury, the area of the damaged cortex was characterized by neuronal loss and neurons weakly stained with H&E. Under image analysis, this region was defined by low intensity of staining, thereby providing a clear distinction between healthy and lesioned tissues. The cortex of both hemispheres was outlined by hand, by an evaluator blinded to the injury and treatment status, and the total cortical area was calculated by the calibrated image analysis program. The lesion area then was calculated with the following formula: contralateral cortex − ipsilateral cortex.

2.7. Measurement of lipid peroxidation

For tissue lipid peroxidation measurements, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO). The formation of lipid peroxides was measured in the homogenates of the whole brain. The formation of MDA, an end product of fatty acid peroxidation was measured spectrophotometrically at 532 nm by using a thiobarbituric acid reactive substance (TBARS) and compared with values obtained from MDA standards, essentially by the method of Genet et al. (2002). The results were expressed as nmol of malondialdehyde (MDA)/mg tissue.

2.8. Mortality rate

The death numbers in the treated groups and non-treated groups were assessed during 2 weeks after brain injury (Fig. 4). We also recorded the day rats died during the experiment (Fig. 5). The number of animals for the low and high treatment groups respectively on test days was the following; 1st (n = 9 & 9), 3rd (n = 8 & 9), 7th (n = 7 & 8) and 14th days (n = 7 & 7).

2.9. Statistical analysis

Data were analyzed using a SPSS 13.0 for Windows program on a computer. The values were expressed as means ± SEM. The results were computed statistically using one-way analysis of variance for each of the test days (1, 3, 7 and 14). The Tukey–Kramer multiple comparison test post hoc testing was performed for intergroup comparisons using the least significant difference (LSD) test. A difference was considered significant at the $P < 0.05$ level.

3. Results

3.1. Behavioral evaluation

The motor function and cognitive tests were conducted by trained investigators who were blinded to the treatment and traumatic status of the animals.

3.1.1. Behavioral changes

3.1.1.1. Rotarod performance. The time of fall from the rod was significantly decreased at first at 1st, 3rd, 7th and 14th days after the brain injury as compared to the sham, suggesting an impairment in the rotarod performance. In the first day after the brain injury, the results showed that the high dose treated group (curcumin 100 mg/kg) stayed longer (40.9 s) on the rod compared to the low dose treated group (curcumin 50 mg/kg) (15.4 s) and vehicle group (20.1 s). However the statistical analysis showed no significant difference for this rise in time duration. On the third day the high dose treated group stayed longer (70.9 s) than the low dose treated (31 s) and vehicle group (16.6 s). However again, the statistical analysis showed no significant difference for this rise in time duration. On the seventh day all groups showed better motor performance, with the high dose treated group staying longer on the rod (78.5 s). The statistical analysis showed that this rise in time duration had significant difference in comparison to the vehicle group (32.4 s) ($^{**}p < 0.01$) but not to the low dose treated group (61.6 s). On the fourteenth day, the high dose treated group performed for a significantly longer duration (100.7 s) than the low dose treated (53 s) ($^{*}p < 0.05$) and the vehicle group (35.8 s) ($^{***}p < 0.001$). Even though the low dose treated group maintained longer time duration than the vehicle group (44.87 s), the statistical analysis showed that this difference was not significant. It is possible to surmise from the results of the rotarod test on the 1st, 3rd, 7th and 14th days, that the animals' ability to regain its motor functions was dose dependent on curcumin (Fig. 1).

3.1.1.2. Inclined-plane test. A significant impairment in duration stay on inclined-plane apparatus (40.) was observed following traumatic brain injury. The injured rats exhibited a decrease in total period of stay during the inclined-plane test as compared to the sham. On the first day after the brain injury, both the high dose treated group (59.9 s) and low dose group (66.9 s) showed an almost similar rise in time duration. The statistical analysis showed that this rise in time period was significant for both the high dose treated ($^{**}p < 0.01$) and low dose group ($^{*}p < 0.05$), in comparison to the vehicle group (25.1 s). However no significant difference was found between the two curcumin administered groups. On the third day the animals stayed longer on the angel board. The statistical analysis showed that the high dose treated group (122.4 s) and low dose treated (112 s) stayed for a significantly longer time duration compared to the vehicle group (39.2 s, $^{***}p < 0.001$). However no significant difference was found between the two curcumin administered groups. On the seventh day the animals stayed longer on the angel board. The statistical analysis showed that both the high dose and low dose treated group stayed for a significantly longer time duration compared to the vehicle group ($^{**}p < 0.01$). However no significant difference was found between the two curcumin administered groups. On the fourteenth day the results showed that even though the two curcumin administered groups stayed for a longer time duration (139.9 and 135.9 s respectively) compared to the vehicle group (97.9 s), the difference was not significant. The angle board test has shown that curcumin had a significant effect on promoting the recovery of balance during the first week after traumatic brain injury. However on the fourteenth day the *t*-test showed no difference between the curcumin-treated and vehicle groups, even though the treated groups displayed marginally higher time periods (Fig. 2).

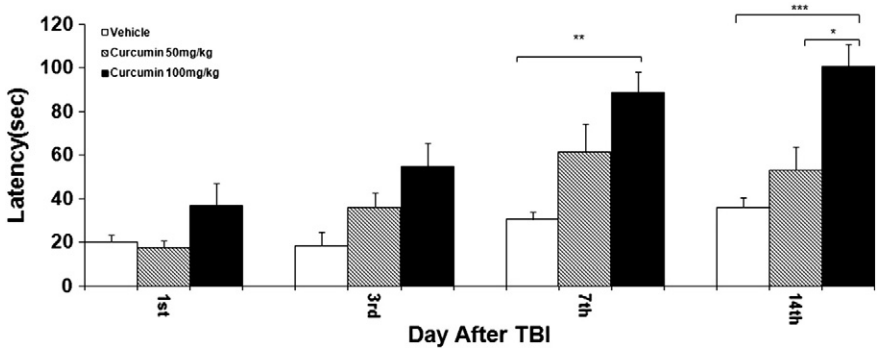

Fig. 1. Protective effect of curcumin on rotarod performance in rats subjected to traumatic brain injury. Curcumin was administered (i.p.) 5 days before brain injury. *$P < 0.05$, compared with the low dose of treated group. **$p < 0.01$, ***$p < 0.001$, compared with the vehicle group. Data are expressed as mean ± SE (n = 9).

3.2. Pathological results

In traumatic rats, hemispheric injury was well demarcated after 2 weeks of injury, as revealed by histological analysis of the brain. The contusion area in the vehicle group was found to be 30.1% compared to 26.1% in the low dose treated brain injury group. The statistical analysis confirmed that the difference between the two groups was not significant. Interestingly, pretreatment with high dose showed the smallest contusion area of 22.5%. By using ANOVA it was shown that the high dose pretreatment of curcumin showed a significant difference compare with the vehicle group (*$p < 0.05$) but not with the low dose treated group. No ischemic damage was noticed in brain sections of the sham-operated rats (Fig. 3).

3.3. Mortality rate

Of the 36 rats, 8 rats died in the course of the experiment (Fig. 3). No rats in the sham operation group died. Within the traumatic brain injury groups, 4 rats died (Fig. 4) at the 3rd, 4th, 7th and 11th days of injury (Fig. 5). These rats had not been allocated to a pretreatment prior to brain injury (Vehicle group). Whereas only 2 rats in the high dose treated groups (100 mg/kg/day) died at the 6th and 12th days and also 2 rats in the low dose treated group (50 mg/kg/day) died at the 3rd and 7th days after the brain injury respectively (Figs. 4 and 5).

3.4. Lipid peroxidation assay

Fig. 6 shows the level of lipid peroxidation marker, MDA, in the normal and experimental rats. Lipid peroxidation was measured as the formation of MDA in whole homogenates of the rat brain from the control and curcumin-treated injured animals. There was a significant elevation in MDA concentration in the traumatic group when compared with corresponding control group ($P < 0.001$). Pretreatment of rat in the higher dose of curcumin significantly decreased lipid peroxidation end product, MDA (by 19%) ($P < 0.0\,5$) (Fig. 6).

4. Discussion

Head injury and trauma remain serious complications which contribute to increased patient mortality and long-term disability (Sharma and Vavilala, 2012). The present study suggests, for the first time, that the doses of curcumin attenuate cerebral damage and improve neurological outcome following traumatic brain injury. An impairment in the sensorimotor functions is well known following head injury. In the present study, the neurological disorders were found to be increased due to brain damage. Pretreatment with curcumin resulted in significant decrease in the neurological disorders. Rotarod and inclined-plane tests are two of the sensitive tests to monitor the spontaneous locomotor activity and muscle co-ordination. Any deficit in the placement of forelimbs, foot-fault error and co-ordination is easily detected through these tests. Traumatic rats with low to high injury have been reported to show impairment in rotarod and inclined-plane tests (Lekic et al., 2011). Errors in the placement of foot and impaired co-ordination following brain injury for 2 weeks were also observed in the present study. Rotarod performance and inclined-plane tests were affected following head injury in the rats, which provided another evidence of impaired balance and muscle co-ordination due to traumatic brain injury. It has been reported that rotarod performance in the rats is

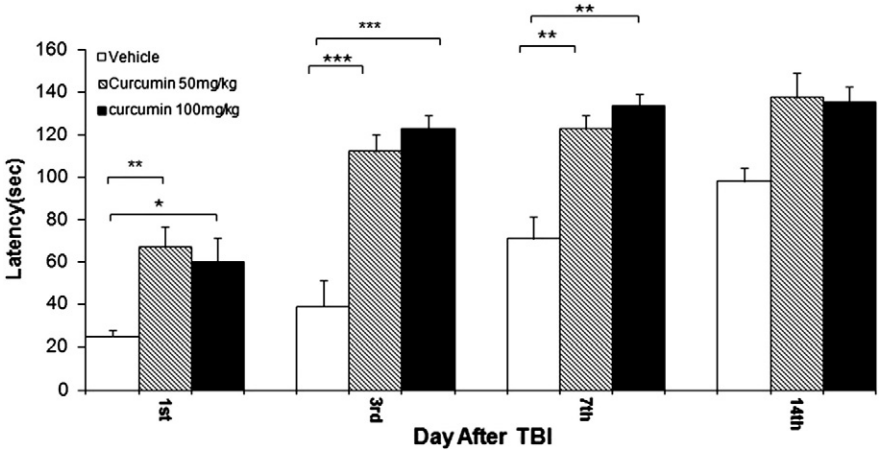

Fig. 2. Protective effect of curcumin on inclined-plane test in rats subjected to traumatic brain injury. Curcumin was administered (i.p.) 5 days before brain injury. *$P < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with the vehicle group. Data are expressed as mean ± SE (n = 9).

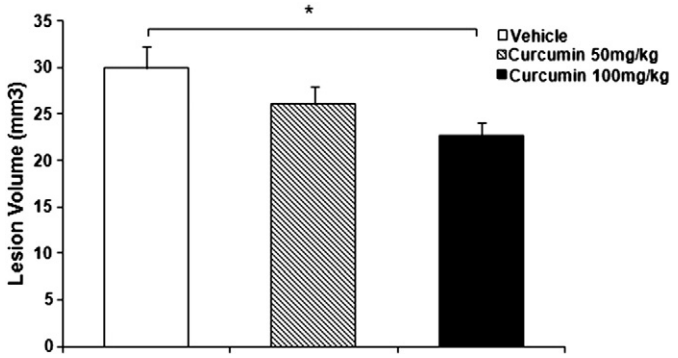

Fig. 3. Protective effect of curcumin on lesion volume (mm3) in rats subjected to traumatic brain injury. Curcumin was administered (i.p.) 5 days before brain injury. The section at each level was stained with hematoxylin and eosin, and the area of the contralateral and ipsilateral cortices was then measured using a light microscope. $^{*}P < 0.05$compared with the vehicle group.

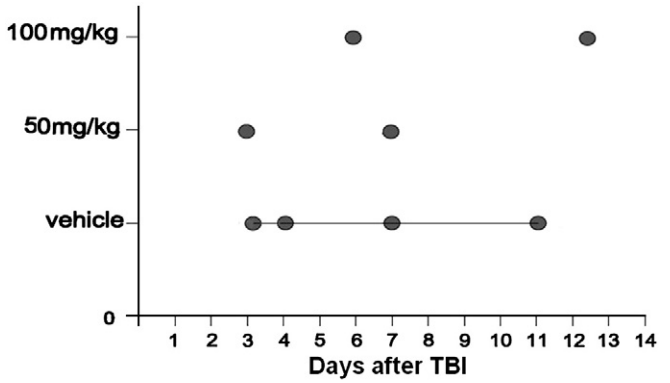

Fig. 5. The distribution of the dead rats during the experiment (2 weeks) in each group. Curcumin was administered (i.p.) 5 days before brain injury.

significantly affected following brain injury. A linear relationship between the severity of injury and time during which rats stay on the accelerating rod has been reported (Yang et al., 2010). Significantly, pretreatment with curcumin in the rats with brain injury caused an improvement in the co-ordination as evident by the evaluation of duration to stay on beam. The time of fall from the rotating rod and inclined-plane tests were also significantly increased in these animals suggesting that curcumin pretreatment could significantly prevent impairment of sensorimotor functions. Further, a significant improvement in neurological disorders following curcumin pretreatment, observed in the present study, is quite interesting and is consistent with the above findings. Curcumin does have muscle relaxant properties that persist for several hours post administration (Tamaddonfard et al., 2012), which may explain why curcumin-treated animals did not show significant improvement on neurologic motor scores in the acute posttraumatic period. Sham (uninjured) animals treated with curcumin do indeed show some sedation and muscle relaxation up to several hours posttreatment (unpublished data), which may lower motor function scores. Brain trauma and cerebral ischemia appear to share certain pathophysiological cellular events that lead to neuronal death (McIntosh et al., 1996). Similar to our observations in brain injury, curcumin has been reported to protect against postischemic cell death in hippocampal CA1 regions, prevent memory loss, and reduce hemispheric infarct volume in rodent models of both global and focal cerebral ischemia (Sun et al., 2011). On the other hand, the delayed effect of curcumin on cortical lesion in the present study, along with a functional improvement two or three weeks after curcumin treatment, suggests that curcumin's effect on early oxidant events may become more evident as cell death progresses over several weeks postinjury.

We have studied gross neuroprotective effects of curcumin without going into details of the mechanisms at the cellular or molecular levels. Enhanced oxidative stress due to increased generation of free radicals has been reported during brain injury (Slemmer et al., 2008). An increase in the levels of oxygen and hydroxyl radicals following traumatic brain injury has also been shown (Nishio et al., 1997). Kuroda et al. (1996) suggested that generation of free radical species is an important contributor to brain damage (Kuroda et al., 1996). The protective effect of curcumin against cerebral ischemia in rats has been studied by Thiyagarajan and Sharma (2004). They attributed the neuroprotective effect of curcumin to its antioxidant property. One of the most popular secondary damage theories is the free radical theory of trauma which proposes that ROS leads to oxidative damage over the time of the injury. Thus, trauma is usually associated with increasing level of oxidation and an imbalance between the formation and removal of ROS and the development of oxidative stress plays an important role in secondary damage after injury (Aronowski and Zhao, 2011). ROS alters proteins, carbohydrates, and lipids, and inactivates enzymes and transporters, damages DNA and the transcriptional machinery, and initiates the chain reactions that peroxidize polyunsaturated fatty acids in membrane phospholipids (Gill and Tuteja, 2010). As a general rule, tissue levels of free radicals, lipid peroxidation products, and antioxidants show important changes after injury (Farahmand et al., 2013). In our study, lipid peroxidation level was evaluated by measuring endogenous MDA level in brain hemogenate. Increased endogenous MDA level in injured rats indicated

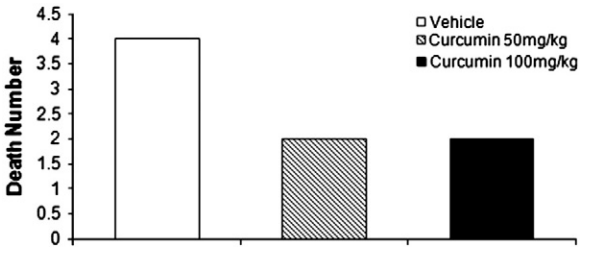

Fig. 4. The death numbers in the treated and non-treated groups were assessed during 2 weeks after brain injury. Curcumin was administered (i.p.) 5 days before brain injury.

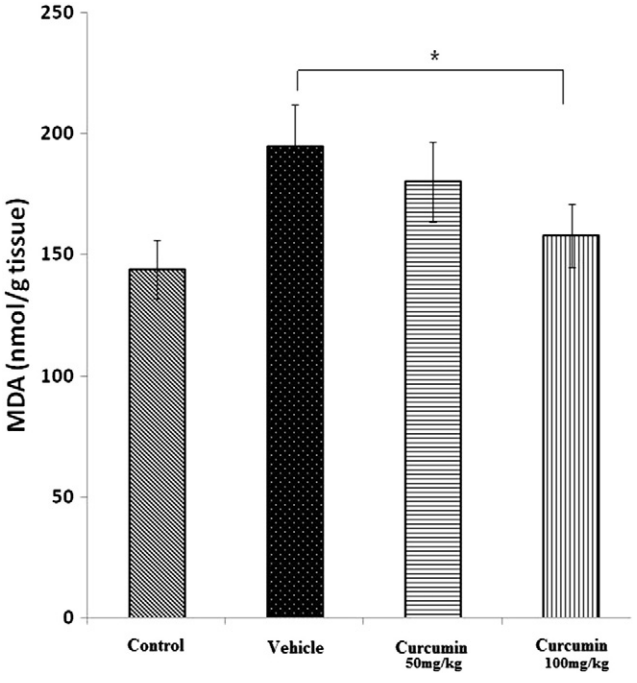

Fig. 6. The effect of curcumin (50 and 100 mg/kg) on brain MDA level in traumatic brain injury in rat. Curcumin was administered (i.p.) 5 days before brain injury.

that the balance between oxidant and antioxidant agents changed on behalf of pro-oxidation in the brain homogenates of traumatic rats. Therefore, decrease MDA levels after curcumin pretreatment may play an additional role in decreasing oxidative stress.

A decrease in body weight following brain injury, observed in the present study (data was not shown), is consistent with the earlier reports of traumatic brain injury loss in body weight (Wei et al., 2012). Moon et al. (2009) reported that the body weight decrease was probably due to injury affecting feeding behavior and injury to the anterior hypothalamus (Moon et al., 2009). The decrease in body weight in traumatic rats in the present study could be due to decreased food intake of these rats. Since multi-factorial mechanisms are involved in brain injury, a number of synthetic and natural agents have been used to investigate their protective and therapeutic efficacy (Pierre et al., 1999). Vitamin E, Vitamin B3, NMDA receptor antagonists, Na^+ channel blockers and nitric oxide synthase (NOS) inhibitors have been used and found effective in the experimental model of brain injury (Hall et al., 2012). Use of herbal products in the management of outcome of head injury has also been advocated because of their high antioxidant activity (He et al., 2012). Dietary supplementation with spinach, blueberries and spirulina has been reported to reduce brain injury-induced apoptosis and cerebral trauma. Modulation of oxidative stress has been one of the most extensively pursued therapeutic strategies in experimental traumatic brain injury. It has been observed that agents that inhibit lipid peroxidation or have strong antioxidant activity are useful in the treatment of this disease (Nishio et al., 1997). Curcumin, an important ingredient of turmeric, is a cyclooxygenase inhibitor and possesses multiple pharmacological properties including anti-inflammatory, anti-carcinogenic and anti-thrombotic (Merrell et al., 2009). These properties of curcumin might have also contributed to its anti-injury efficacy but it is difficult to confirm them in the present study. The free radical scavenging activity of curcumin and its protective effect against reactive species are well documented. Curcumin is unique over other natural antioxidants since it possesses both the phenolic and diketonic groups which help in the scavenging of free radicals. In contrast, other natural antioxidants possess either phenolic or diketonic groups (Priyadarsini, 1997). The efficacy of compounds modulating oxidative stress has been demonstrated through evaluation of posttraumatic neuromotor function, neurochemical outcom (Motterlini et al., 2000). In the present study, we observed that one of the antioxidants, curcumin, significantly reduced tissue loss in the cortex to injury at 2 weeks following brain injury in the rat. This neuroprotective effect may, in part, explain the improvement of neurologic motor function. These results suggest that use of antioxidant may be useful in treating several aspects of the posttraumatic pathologic cascade.

Curcumin has been found to be neuroprotective against different neurotoxicants (Sood et al., 2011). One of the characteristic properties of curcumin is that it does not affect the normal cells. Thiyagrajan and Sharma also observed inhibition in lipid peroxidation following intraperitoneal treatment with curcumin (Thiyagarajan and Sharma, 2004). The effect was more marked at a higher dose (300 mg/kg) of curcumin. In our present study, we also showed the higher dose of curcumin had more potency in inhibiting neurological disorders, the brain lesion volume and MDA level after brain injury. Absorption of curcumin in the body may occur both by gavage and intraperitoneal routes although bioavailability and pharmacokinetic properties are different (Sood et al., 2011). In the present study, pretreatment with intraperitoneal administration of curcumin beginning 5 days before traumatic brain injury was able to confer a significant degree of neuroprotection with respect to both behavioral and, neurologic motor function which lasted for 2 weeks following brain injury and drug treatment. As curcumin has xanthine oxidase inhibitory activity (Shen and Ji, 2009), it may prevent production of superoxide during brain injury. Curcumin has been reported to cross the blood–brain barrier in aged mice and its use is recommended in the treatment/prevention of Alzheimer's disease (Wang et al., 2013). Sharma et al. in an interesting study reported that curcumin inhibited the formation of lipid peroxides even in the presence of agents that induced lipid peroxidation suggesting its strong antioxidant property (Sharma et al., 2010). In the study on the ischemic model in rats, treatment with curcumin increased the superoxide dismutase activity in ischemic rats, which further provides an evidence of its antioxidant potential. The modulation of oxidative stress function has been shown previously to attenuate posttraumatic regional cerebral edema in a variety of experimental models of brain injury (Shapira et al., 1990), the administration of curcumin in the present study was also observed to have an effect in reducing the extent of cortical lesion volume. The evidence exists to suggest that improvements in neurologic motor are dependent on the reduction of cortical cell loss. In an interesting study, curcumin has been shown to improve neurobehavioral outcome without having significant effects on posttraumatic lesion volume assessed at 48 h postinjury using 2,3,5-triphenyltetrazolium chloride staining (McIntosh et al., 1996). The lack of demonstrable neuroprotective effect in this earlier study may have been due to the fact that secondary injury leading to formation of a well-defined lesion develops over a period of days (Bramlett et al., 1997). In the present study, attenuation of cortical lesion volume was observed in high dose curcumin-pretreated animals up to 2 weeks postinjury.

The two substantially different mechanisms determine the damage after traumatic brain injury. Firstly, the primary insult occurs at the moment of impact. Next, secondary insult represents consecutive pathological processes such as oxidative stress, which is initiated at the moment of injury (Seifman et al., 2008). Imbalance between cellular production of free radicals and the ability of cells to defend against them is referred to as oxidative stress. Oxidative stress begins immediately after brain injury and initiates the events resulting in neuronal dysfunction and death. It may have a significant role in secondary damage and is responsible for morbidity and mortality following brain injury. However, their underlying mechanisms are complex and remain unclarified (Ferguson et al., 2010). Oxidative stress due to excessive generation of reactive oxygen species with consequent impairment of endogenous antioxidant defense mechanisms plays a significant role in the secondary events leading to neuronal death and then sensorymotor dysfunction (Eghwrudjakpor and Allison, 2010). On the other hand, there is a strong relationship between severity of brain injury and oxidative stress.

The delayed effect of curcumin as an antioxidant agent and also, in order to consider the effect of administration of curcumin on the early oxidant events in traumatic brain injury, in this study, therefore, we hypothesized that the pretreatment effect of curcumin on functional outcome, neuronal damage and on the early oxidative events, may become more effective on the improvement of cell death progress and secondary injuries over two weeks after the traumatic brain injury of rats. The present findings on 5 days pretreatment of curcumin provided evidences that pretreatment of curcumin is involved in early oxidative changes in traumatic rats that can influence the clinical outcomes of traumatic brain injury of rats after 2 weeks versus the untreated traumatic rats.

The knowledge of the pathophysiology after brain injury, is necessary for adequate and successful treatment. Thus, in this study, we investigated pretreatment of curcumin on neurological outcome and oxidative stress marker in rats with traumatic brain injury.

The role of intracellular calcium in brain damage and the protective effect of calcium channel blockers in head injury have been suggested. As a result of brain trauma, the ionic gradients across the membranes are affected. Efflux of K^+ from cells producing cellular depolarization and the movement of extracellular calcium into cells through calcium channels are physiological consequences. An increase in intracellular calcium enhances breakdown of phospholipids, proteins and nucleic acids and thus is linked with calcium toxicity in cerebral trauma (Barone et al., 1997). Recently, Matteucci et al. found that curcumin treatment protected rat retinal neurons against excitotoxicity by decreasing the intracellular calcium levels modulated by NMDA receptors

(Matteucci et al., 2005). An increase in intracellular calcium level following traumatic brain injury and its inhibition following curcumin treatment in traumatic rats is interesting. It is, however, difficult to comment whether such an effect is due to formation of any complex or blocking of calcium channels. Low mortality in traumatic rats treated with curcumin as compared to traumatic rats treated with vehicle suggests that curcumin may affect the mortality by brain trauma by providing protection. It is possible that pretreatment with curcumin for more time and/or at higher dose might be more effective on the outcome of brain injury but that needs further study. The present dose and duration were based on literature reports. The precise mechanisms underlying the neuroprotective effects of curcumin in models of brain trauma are currently under investigation.

Finally, the present study demonstrates that pre-injury administration of curcumin produced significant attenuation of cortical lesion volume associated with traumatic brain injury in rats. These observations suggest that inhibition of oxidative stress may be a beneficial therapeutic approach toward reducing posttraumatic neurobehavioral and motor function deficits and improving neuronal survival following traumatic brain injury.

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