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Neuroprotective effects of the polyphenolic antioxidant agent, Curcumin, against homocysteine-induced cognitive impairment and oxidative stress in the rat

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ABSTRACT

Aging is the major risk factor for neurodegenerative diseases and oxidative stress is involved in the pathophysiology of these diseases. In this study, the possible antioxidant and neuroprotective properties of the polyphenolic antioxidant compound, Curcumin against homocysteine (Hcy) neurotoxicity was investigated. Curcumin (5 and 50 mg/kg) was injected intraperitonealy once daily for a period of 10 days beginning 5 days prior to Hcy (0.2 μmol/μl) intrahippocampal injection in rats. Biochemical and behavioral studies, including passive avoidance learning and locomotor activity tests were studied 24 h after the last Curcumin or its vehicle injection. We detected Malondialdehyde (MDA) and Super oxide anion (SOA) in rats' hippocampi. Results indicated that Hcy could induce lipid peroxidation and increase MDA and SOA levels in rats' hippocampi. Additionally, Hcy impaired memory retention in passive avoidance learning test. However, Curcumin treatment decreased MDA and SOA levels significantly as well as improved learning and memory in rats. Histopathological analysis also indicated that Hcy could decrease hippocampus cell count and Curcumin inhibited this toxic effect. These results suggest that Hcy may induce lipid peroxidation in rats' hippocampi and polyphenol treatment (Curcumin) improved learning and memory deficits by protecting the nervous system against Hcy toxicity.

1. Introduction

The pathogenesis of neurodegenerative diseases such as Alzheimer or Parkinson is multifactorial with a complex combination of genetic components and environmental factors. The environmental or sporadic form represents the majority of these cases. Toxic reactions including inflammation, glutamatergic toxicity, the dysfunction of mitochondrial activity and ubiquitin/proteasome system, the activation of apoptosis pathways, the elevation of iron and nitric oxide, and the alteration of the homeostasis of antioxidants/oxidation are involved in the pathogenesis of neurodegenerative diseases (Wu, 2005).

Homocysteine (Hcy); a sulfur containing amino acid derived from the metabolism of methionine, is an independent risk factor for cardiovascular disease (Clarke et al., 1991). The thiol group of Hcy is readily oxidized in plasma and culture medium, resulting in the generation of reactive oxygen species (ROS). Moreover, Hcy has the ability to inhibit the expression of antioxidant enzymes such as glutathione peroxidase (GSH-Px), and super oxide dismutase (SOD) (Hankey and Eikelboom, 1999; Heinecke et al., 1988). Hcy is an excitatory amino acid, which markedly enhances the vulnerability of neuronal cells to excitotoxic and oxidative injury (Hankey and Eikelboom, 1999). An elevated plasma level of Hcy (more than 14 μM) is termed Hyperhomocysteinemia (HHCY) (Seshadri et al., 2002). Hcy is recognized as an independent risk factor for myocardial infarction, coronary artery disease, strokes, genetic disorders, Alzheimer's diseases (AD) and cognitive impairment (Schwartz et al., 1997). Recently, it has been suggested that chronic administration of Hcy to rats affected both long- and short-term memory in the Morris water maze task (Streck et al., 2004). Additionally, in another study it was found that in AD patients, high Hcy plasma levels favored neurodegeneration (Agnati et al., 2005). Wu (2005) showed that intrahippocampal Hcy (0.14 μmol/μl) injection could induce apoptosis in the hippocampal neurons of rats. Hcy is a glutamate agonist, which causes an increase in Ca^{2+} influx via the activation of the NMDA class of excitatory amino acid receptors, which in turn, results in neuronal cell death and apoptosis (Lipton et al., 1997). Kruman et al. (2000) showed that homocysteine administration via stereotaxic injection into the dorsal hippocampus of mice, using methods described previously (Bruce et al., 1996), could induce neuronal apoptosis and

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increase neuronal vulnerability to excitotoxicity by a mechanism involving DNA damage.

Antioxidants have been studied for their effectiveness in reducing deleterious effects and neuronal death by oxidative stress (Jara-Prado et al., 2003). Many but not all antioxidants have been shown to cross the blood-brain-barrier and to have a neuroprotective effect in human and animal models. The major limitations of these antioxidants appear to be their relatively short half-life, low bioavailability at the sites of free-radical production (Vitamins C and E) and the lack of potent protection (Blumenthal et al., 2000). Polyphenols are natural substances that are present in plants, fruits and vegetables including olive oil, red wine and tea (Ramassamy, 2006). The yellow pigment extracted from the rhizome of Curcuma longa, "Curcumin" a polyphenolic non-flavonon compound is the pharmacologically active substance of turmeric (Ganguli et al., 2000). Curcumin is nontoxic and has antioxidant, anti-inflammatory and anti-proliferative activities. Curcumin shows antioxidant activity equivalent to vitamins C and E (Blumenthal et al., 2000). Many studies have shown that Curcumin is a potent scavenger of reactive oxygen species, which cause oxidative damage; these studies have also shown improved learning and memory from treatment with Curcumin (Pan et al., 2008). Studies of animal models have shown that Curcumin inhibits lipid peroxidation and protects kidney cells, vascular endothelial cells, rat myocardium and collagen from oxidative damage (Blumenthal et al., 2000). Vajragupta et al. (2003) showed that three manganese complexes of Curcumin exhibited a great capacity to protect brain lipids against peroxidation, enhance SOD activity, and compared to other compound, showed highest inhibitory activity against H2O2-induced cell damage. Ahmed and Gilani (2009) showed the inhibitory effect of Curcuminoids (a mixture of Curcumin, Bisdemethoxycurcumin and Demethoxycurcumin) on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia. Therefore, the present study was investigated by the neuroprotective effect of curcumin against Hcy toxicity using behavioral studies, as well as biochemical and histological analysis to arrive at a conclusion.

2. Materials and methods

2.1. Drugs and chemicals

D-L-Homocysteine (Hcy), Curcumin, Butylhldroxytoluene (BHT), 2-Thiobarbituric acid (TBA), 1.1.3.3 -Tetramethoxypropan (99%), Nitro Blue Diformazan (NBD), Nitro Blue Tetrazolium (NBT), Trichloro acetic acid (TCA), Butanol and Ethyloleate, Hematoxylen, Eosin, Sodium pentobarbital (USP), Absolute Ethanol, Xylene, Formaldehyde were purchased from Sigma-Aldrich, Germany.

2.2. Animals

Adult male Wister rats purchased from Pasteur institute (Tehran, Iran), weighing between 250–300 g were used in this study. The animals were housed at 22 °C in a controlled environment with a 12:12 hour light: dark cycle and were given access to standard laboratory food and water ad libitum. All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University, M.C.

2.3. Drugs-preparation and administration

Hcy powder was dissolved in hydrochloric acid (1 M) and diluted with Phosphate Buffered Saline (PBS) (Sigma-Aldrich, 2007). The PH of the solution was regulated at 7.4 by addition of 0.1 N NaOH. Solutions of Hcy were prepared freshly at concentration of 0.05, 0.1, 0.2 and 0.4 M. Hcy effective dose (0.2 μmol/μl) was calculated by drawing a dose–response diagram (Fig. 1). The yellow powder of Curcumin was dissolved in ethyl oleate as vehicle (Daniel et al., 2004). It was injected at low and high doses (5 and 50 mg/kg) intraperitonealy (i.p.) in rats. Curcumin dosages were selected on the basis of earlier reports which have demonstrated its antioxidant effects (Shen et al., 2007; Sumanont et al., 2007). Curcumin (5 and 50 mg/kg) was injected (i.p.) once daily for a period of 10 days beginning 5 days prior to Hcy (0.2 μmol/μl) injection in rats. This protocol was selected on the basis of earlier research, which demonstrated the Estrogen antioxidant effects against Hcy toxicity (Wu, 2005). Animals of the control group did not receive any injection .In the vehicle group, vehicle of Hcy (PBS) was injected into the hippocampus and vehicle of Curcumin (ethyl oleate) was injected (i.p.) for 10 days beginning 5 days prior to PBS injection. In the Hcy group, Hcy (0.2 μmol/μl) was injected intrahippocampaly and the vehicle of Curcumin (ethyl oleate) was injected (i.p.) 10 days beginning 5 days prior to Hcy injection. Biochemical, behavioral and histopathological

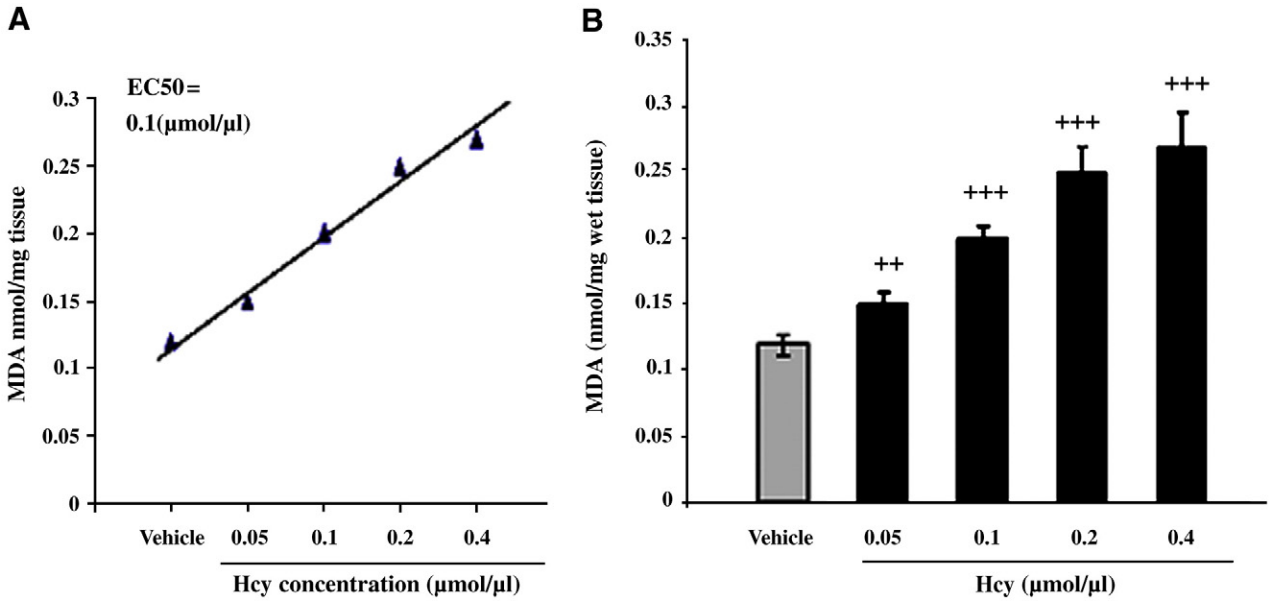

Fig. 1. (A) Regression line and EC50 of Hcy effect on MDA concentration (B) Hcy dose–response diagram on lipid peroxidation biomarker (MDA concentration) in rats' hippocampus. Each point is the mean ± SEM ($n=8$). ++ $p<0.01$, +++ $p<0.001$ difference from vehicle group.

analyses were done 24 h after the last Curcumin or its vehicle injection in the separate groups.

2.4. Intrahippocampal injection

The rats were anaesthetized with sodium pentobarbital (60 mg/kg i.p.) and placed in a stereotaxic frame. The rat skull was orientated according to Paxinos and Watson stereotaxic atlas (1986). After a sagittal incision, the bregma suture was located and holes were drilled with an electrical drill at the following co-ordinates; 3.3 mm posterior to bregma, 2.6 mm lateral to the sagittal suture and 3.6 mm ventral. Care was taken not to damage the meninges. A Hamilton syringe with a cannula of diameter of 0.3 mm was used to inject 1 μl of solutions of Hcy (0.05, 0.1, 0.2, 0.4 M) or its vehicle (PBS). The injection was carried out in the left and right dorsal hippocampus at a rate of 1 μl per 2 min. The cannula was left *in situ* for a further 5 min following Hcy injection to allow passive diffusion from the cannula tip and to minimize spread into the injection tract. The cannula was then slowly removed and the scalp, which was then closed with sutures. Animals were kept warm until recovery from the anesthesia.

2.5. Hippocampus removal

Five days after Hcy injection, rats were sacrificed by neck fracture and decapitated. The brain was exposed by making an incision through the bone on either side of the parietal suture, from the foramen magnum to near the orbit. The calvarium was removed, exposing the brain; the left and right hippocampus were removed carefully, immediately inserted in PBS solution (0.1 M) and stored at −70 ° C for the use in biochemical or histopathological analysis.

2.6. Biochemical analysis of hippocampal homogenate

On the day of experiment, left and right rat's hippocampus were weighed (estimate 0.7 g), and homogenized (10% w/v) in 0.1 M PBS with Polytron homogenizer at pH 7.4. Homogenates were used immediately for detection of the biomarkers of lipid peroxidation: Malondialdehyde (MDA) and Super Oxide Anion (SOA).

Lipid peroxidation was determined according to the modified method (Placer et al., 1966). Hippocampal homogenates (1 ml) were incubated at 37 °C in an oscillating water bath for one hour. At the end of the incubation period, 0.5 ml of BHT (0.5 mg/ml in absolute ethanol) and 1 ml of TCA (25%) were added. The tubes were sealed and heated for 10 min in a boiling water bath to release MDA (the end product of lipid peroxidation) from proteins. To avoid adsorption of MDA to insoluble proteins, the samples were cooled to 4 °C and centrifuged at 2000×*g* for 20 min. Following centrifugation, 2 ml of the protein free supernatant was removed from each tube and 0.5 ml of TBA (0.33%) was added to this fraction. All tubes were heated for 1 h at 95 °C in a water bath. After cooling, the TBA-MDA complexes were extracted with 2 ml butanol. The light absorbance was red at 532 nm on UV/visible spectrophotometer and MDA level were determined from standard curve generated from 1,1,1,3 tetramethoxy propan. The results were represented as nmol/mg wet tissue.

For detection of SOA the assay procedure was a modification of the method described by Das et al. (1990). Hippocampal homogenate (1 ml) was incubated with 0.4 ml of NBT (0.1%) in an oscillating water bath for 1 hour at 37 °C. Termination of the assay and extraction of the reduced NBT was carried out by centrifuging the samples for 10 min at 2000×*g* followed by resuspension of the pellets with 2 ml of glacial acetic acid. The absorbance was measured at 560 nm on a spectrophotometer and converted to micromoles of Diformazan using a standard curve generated from NBD. The results were represented as μmol/mg wet tissue.

2.7. Hippocampus histopathological analysis

Removed hippocampal tissues were fixed in 10% neutral buffered formaldehyde for 24 h, embedded in paraffin and cut into at 3–4 μm thick sections by a microtome (Leica SM2000R, Germany). The tissue sections were deparaffinised in xylene. The slides were stained with Hematoxylin and Eosin (H&E) according to the procedure in Wilson and Gamble (2002), and viewed under a light microscope (Labomed, USA) for the structure and morphology of cells. Microscopic images obtained by a CCD camera and Digipro software. The cells also were counted in different regions of hippocampus (CA1, CA2, CA3 and DG). The results are represented as cell count per mm^2 tissue.

2.8. Behavioral study

The effects of Hcy alone or in combination with Curcumin on the rats' behavior were studied by passive avoidance learning task. Rats' locomotor activities were also studied by open field apparatus. All experiments were carried out 24 h after the last Curcumin (or its vehicle) injections which began in the fifth day following Hcy intrahippocampal injection in rats.

2.8.1. Passive avoidance learning apparatus

The apparatus (Shuttle box) consisted of equal sized light and dark compartments (30×20×30 cm), separated by a guillotine door (7×9 cm) that could be raised to 10 cm. A 40 W lamp was fixed 30 cm above the floor in the center of the light compartment. The floor was made from stainless steel (2.5 mm in diameter) and was connected to a shock stimulator. A single electrical shock (50 Hz, 1.5 s, 1.5–2 mA intensity) was delivered to the grid floor of the dark compartment by a stimulator.

2.8.1.1. Training. Five days after Hcy bilateral intrahippocampal injection, the rats were individually allowed to habituate to the apparatus 1 h prior to testing. All of the behavior tests were performed at 9:30–10:30 AM in a darkened, light and sound-attenuated and ventilated testing room; room temperature was 20–25 °C. Each animal was placed in the light compartment, and after 5 s, the door was opened, allowing the animal to enter a dark place. The habituation trial was repeated after 30 min and followed by the acquisition trial after an additional 30 min. Thus, every animal was placed in the light compartment and the latency time that elapsed before each animal had all four feet inside the dark compartment was measured in seconds and termed "Initial Latency" time (IL). Immediately after the rat entered the dark chamber with all the four feet inside, the door was closed and an electrical foot shock (1.5–2 mA, 50 Hz, 1.5 s) was delivered. After 20 s, the rats were removed from the dark chamber and returned to their own cages. Rats that had IL times of more than 100 s were excluded from further experiments. Any rat that was excluded in Initial latency test, replaced with a new rat so the numbers of rats in each group were kept fixed at 8. Two minutes later, the rats were retested in the same way. If the rats did not enter the dark compartment during 120 s, successful acquisition of passive avoidance response was recorded (Veerendra Kumar and Gupta, 2003).

2.8.1.2. Retention test. Twenty-four hours after training, the retention test was performed. Each rat was placed in the light compartment for 5 s, the door was opened and the step through latency time was measured and termed the "First Retention Latency" time (1st RL); however foot shock was not delivered. The test was concluded when the animal entered the dark compartment or remained in the light site for 600 s as an upper cut of time. Seven days after first retention test, latency times were measured again and termed "Second Retention Latency" time (2nd RL) (Veerendra Kumar and Gupta, 2003).

2.8.2. Open field test

The general stimulant or depression activity of a CNS-active drug may affect the response of an animal on behavioral paradigm. Therefore, the effect of Curcumin and Hcy were also studied on spontaneous locomotor activity in open field apparatus. Spontaneous locomotor activity was measured on days 5, 6 and 13 after Hcy intrahippocampal injection. In these days Initial, First and Second retention tests were also measured. Each animal was observed over a period of 5 min in a square open arena (30×30 cm) equipped with an infrared light-sensitive photocell using a digital photoactometer and was recorded by a video path analyzer. Measurements were expressed as locomotion, rest; distances traveled and speed of the animal. The apparatus was placed in a darkened, light and sound-attenuated and ventilated testing room with other behavioral testing apparatus (Yamamoto et al., 1988).

2.9. Data analysis

The data of behavioral and biochemical studies were expressed as Mean ± SEM (standard error of mean) and analyzed using one-way analysis of variance (ANOVA). If the F values were significant, the Student Newman–Keuls test was used to compare the experimental and control groups. In the histopathological study, cell morphology was analyzed by means of the Mann Whitney U test and cell density was analyzed using one-way ANOVA. p-value less than 0.05 ($p<0.05$) was considered to be statistically significant.

3. Results

3.1. Hcy dose–response diagram

Fig. 1 shows Hcy dose–response diagram on rats' hippocampal lipid peroxidation. It was found that 0.2 μmol/μl is the safe effective dose of Hcy to induce lipid peroxidation. Also EC50 was 0.1 μmol/μl. A higher concentration of Hcy (0.4 μmol/μl) caused side effects as well as some morbidity and mortality.

3.2. Estimation of oxidative stress parameters

Fig. 2 shows the effects of drugs on lipid peroxidation biomarker (MDA). One-way ANOVA indicated that hippocampal MDA levels in Hcy group was increased significantly as compared to the vehicle group [F (4, 35) = 46.25, $p<0.001$]. Curcumin treatment (5 and 50 mg/kg; i.p.) significantly decreased the MDA levels at 5 mg/kg ($p<0.01$) and 50 mg/kg ($p<0.001$).

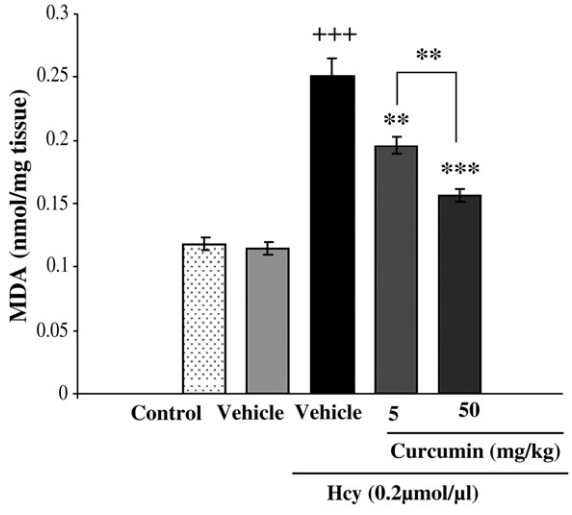

Fig. 2. Effects of Curcumin (5 and 50 mg/kg) on MDA concentration in Hcy (0.2 μmol/μl) treated rats. Curcumin at the dose of 50 mg/kg decreased MDA concentration less than 5 mg/kg ($p<0.01$). Each point is the mean ± SEM ($n=8$). $^{**}p<0.01$, $^{***}p<0.001$ difference from Hcy group. +++ $p<0.001$ difference from vehicle group.

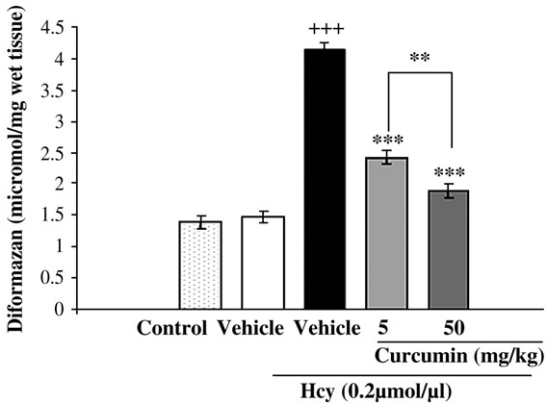

Fig. 3. Effects of Curcumin (5 and 50 mg/kg) on Super oxide anion (Diformazan) concentration in Hcy (0.2 μmol/μl) treated rats. Curcumin at the dose of 50 mg/kg decreased SOA (Diformazan) concentration less than 5 mg/kg ($p<0.01$). Each point is the mean ± SEM ($n=8$).*** $p<0.001$ difference from Hcy group. +++ $p<0.001$ difference from vehicle group.

Fig. 3 shows the effects of drugs on SOA levels (Diformazan) in the rats' hippocampi. One-way ANOVA indicated that hippocampal SOA levels in Hcy group were significantly greater than that of vehicle group [F (4, 35) = 116.13, $p<0.001$] Curcumin treatment led to a significant decrease in SOA levels with 5 and 50 mg/kg dosages ($p<0.001$).

Fig. 4 shows the effect of Curcumin alone on lipidperoxidation biomarker (MDA). There were not any significant differences between the effects of Control, Vehicle and Curcumin alone (5, 50 mg/kg) groups on MDA concentrations.

3.3. Estimation of histopathological parameters

Mann Whitney U test indicated that there were not any significant differences in the morphology and structure of hippocampus neurons between experimental groups. On the other hand, one-way ANOVA indicated that the cell density of Dentate Gyrus (cell count per mm^2) in Hcy group (Fig. 5A-a) was significantly ($p<0.001$) lower than that of vehicle group (Fig. 5A-b). However, after Curcumin treatment the cell density was increased significantly in comparison with Hcy group at 5 and 50 mg/kg dosage ($p<0.01$). Also there were not any significant differences between the effects of Curcumin 5 and 50 mg/kg on cell density in Dentate Gyrus (Fig. 5A-e, A-f, 5B).

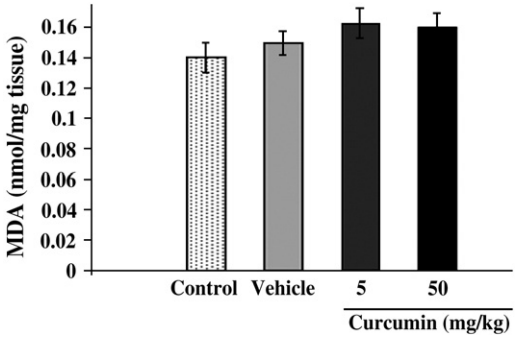

Fig. 4. Effect of Curcumin alone (5–50 mg/kg) on lipid peroxidation biomarker (MDA) and comparison with vehicle and Control groups.

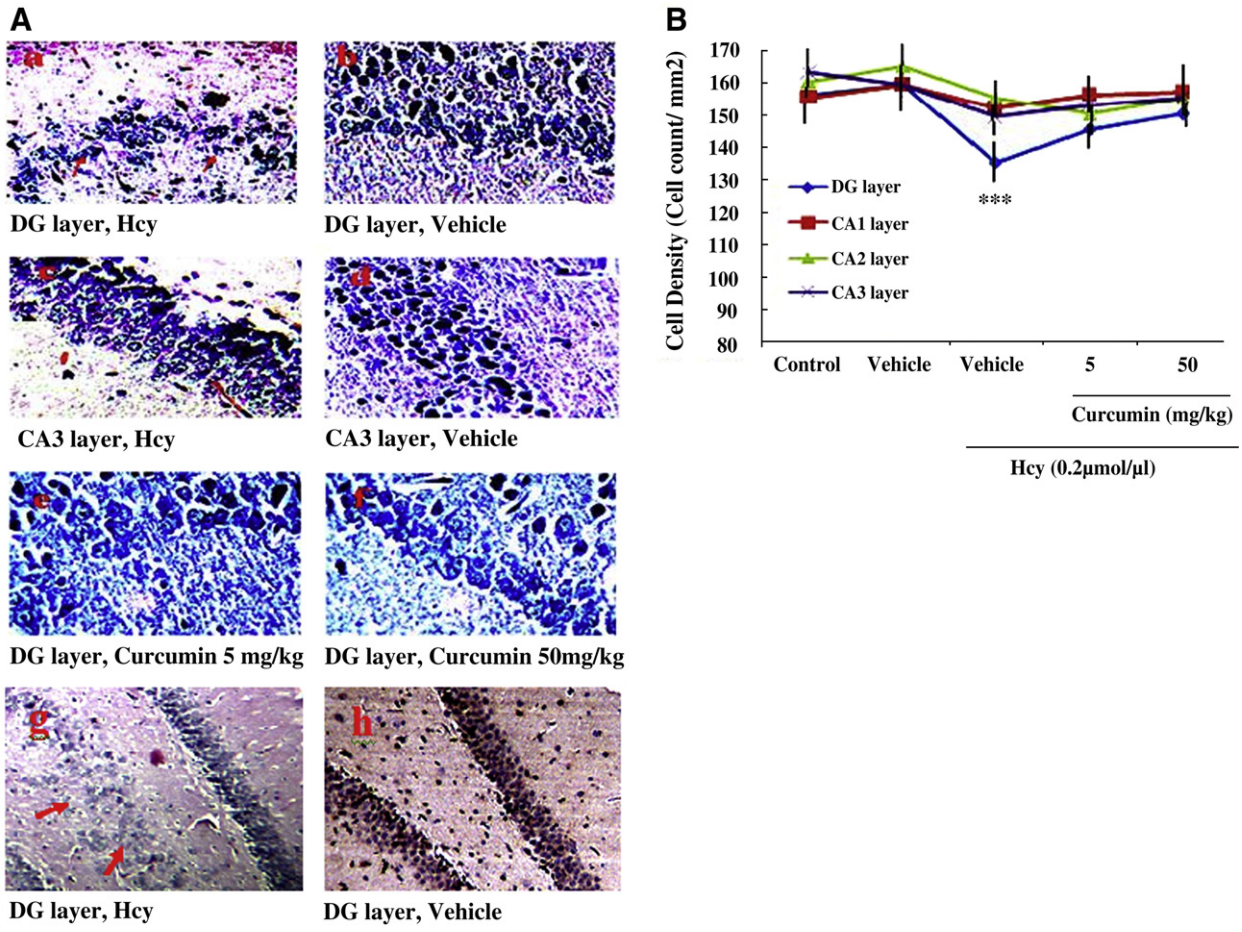

Fig. 5. A: Photographs were taken from rats' hippocampal sections stained with Hematoxylin and Eosin x40. (a) DG (dentate gyrus cells) of Hcy group, (b) DG of vehicle group, (c) CA3 of Hcy group, (d) CA3 of vehicle group, (e) DG of Curcumin (5 mg/kg) group (f) DG of Curcumin (50 mg/kg) group, (g) DG of Hcy group × 10, (h) DG of vehicle group × 10 . Significant changes in morphology (cell structure) were not observed between groups, but the cell count per mm^2 (cell density of DG layer) in Hcy group was decreased significantly in comparison with the vehicle group ($p<0.001$). Curcumin (5 and 50 mg/kg) increased cell density in comparison with Hcy group ($p<0.01$). B: Cell density curve from different layers of rats' hippocampal sections.Each point is the mean ± SEM ($n=5$). *** $p<0.001$ difference from Hcy group.

3.4. Estimation of learning and memory parameters

3.4.1. Passive avoidance task

In this set of experiments, Panel A in Fig. 6 represents initial latency times that were recorded in the training process before electrical shock and these times were naturally less than the first retention latency times (1st RL) (panel B, Fig. 6) that were recorded in retention test, 24 h after the electrical shock and represents Short term memory. Panel C represents Second retention latency times (2nd RL) that were recorded 7 days after the first retention test and indicates long term memory (Veerendra Kumar and Gupta, 2003). One-way ANOVA indicated that initial latency time (IL) did not differ between control and experimental groups (Fig. 6A) while the mean 1st RL (Fig. 6B) in Hcy group were significantly [F (4, 35) = 17.99, $p<0.001$] less than that in vehicle group ($p<0.001$). However, Curcumin at the doses of 5 and 50 mg/kg significantly reversed the Hcy-mediated decrease in step through latency time and increased mean 1st RL times ($p<0.001$). Furthermore, although one-way ANOVA indicated that Hcy (0.2 μmol/μl) significantly decreases the 2nd RL times in comparison with the vehicle group [F (4, 35) = 14.49, $p<0.001$], Curcumin at the doses of 5 and 50 mg/kg significantly reversed the Hcy-mediated decrease in 2nd RL and increased 2nd RL ($p<0.001$) (Fig. 6C). Thus, the Curcumin-treated rats had higher step through latency, mean 1st RL, and 2nd RL times than those in the Hcy-treated rats (Fig. 6C).

Fig. 7 shows the effect of Curcumin alone on passive avoidance learning (1st RL). There were not any significant differences between the effects of Control, Vehicle and Curcumin alone (5, 50 mg/kg) groups on rats' memory.

3.4.2. Locomotor activity

One-way ANOVA indicated that there were not any significant differences in locomotor activities between control and experimental groups on the days of 5, 6 and 13 after Hcy injection.

4. Discussion

The aim of this study was to investigate and clarify the neuroprotective effects of Curcumin, a polyphenolic non-enzymatic antioxidant agent, against Hcy neurotoxicity. The goal was to establish an animal model of oxidative stress in hippocampus. Previously Kim et al. (2007) showed the neuroprotective effects of Ginsenoside (Rg_3) against homocysteine-induced excitotoxicity in rat hippocampus. They showed that Hcy induced selective neuronal loss in the dorsal blade of dentate gyrus and the CA3 of rats' hippocampi.

Results of the present study indicated that Hcy was neurotoxic for rats. Biochemical results revealed that five days after Hcy intrahippocampal injection, MDA and SOA levels were significantly increased in the hippocampi in comparison with the vehicle and control groups (Figs. 2 and 3). These results are in accord with those of earlier report that also demonstrated the oxidative stress induced after acute

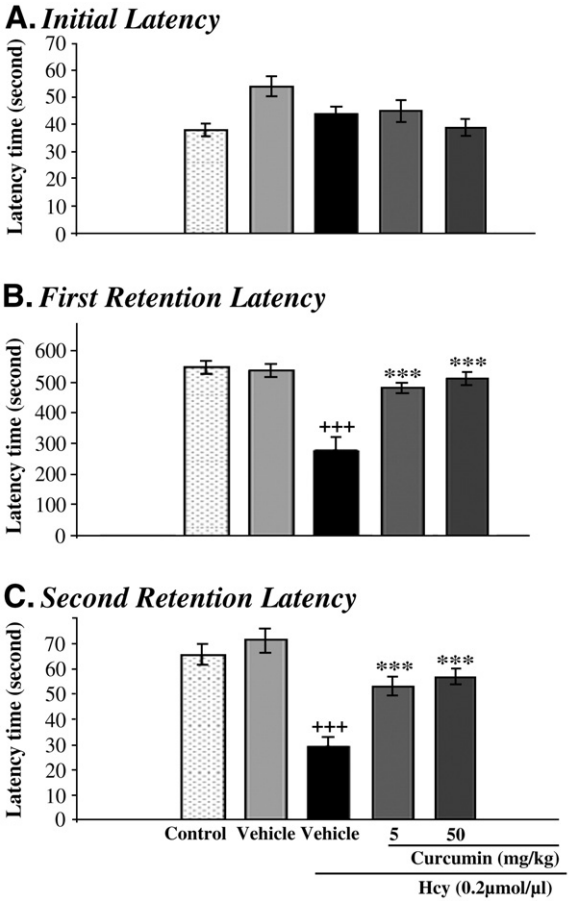

Fig. 6. Effects of Curcumin (5 and 50 mg/kg) on passive avoidance behavior after Hcy (0.2 µmol/µl) Intrahippocampal injection in rats. The effects of drugs on (A) Initial latency times (IL) before electrical shock in the training process ($n=8$), (B) First Retention Latency times (1st RL), 24 h after electrical shock in the first retention test ($n=8$), (C) Second Retention Latency times (2nd RL) 7 days after electrical shock in second retention test ($n=8$). Each point is the mean ± SEM ($n=8$). $^{***}p<0.001$ difference from Hcy group. $+++p<0.001$ difference from vehicle group.

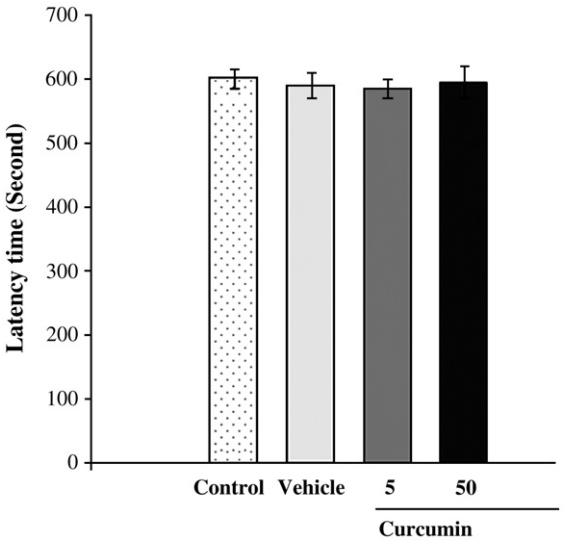

Fig. 7. Effect of Curcumin alone (5–50 mg/kg) on passive avoidance learning (1st RL) and comparison with Vehicle and Control groups.

Hyperhomocysteinemia in the rat brain (Matté et al., 2009). They initially investigated the effect of chronic Hyperhomocysteinemia on some parameters of oxidative damage, namely total radical-trapping antioxidant potential and antioxidant enzymes activities (super oxide dismutase, catalase and glutathione peroxidase), as well as on DNA damage in parietal cortex and blood of rats. Wu (2005) also showed that Hcy could induce lipid peroxidation and apoptosis in the rat's brain.

In the present study, it was observed that Hcy over dose can be lethal for rats and some morbidity and mortality and Parkinson like behaviors (tremor, rotation) were observed after administration of 0.4 µmol/µl or higher concentrations. Furthermore, our results indicated that Hcy (0.2 µmol/µl) could diminish passive avoidance learning behavior significantly, and decreased latency time in the retention test. In retention tests no shock was delivered and only latency time in passing through was recorded. The Hcy group had significantly lower latency time than other groups, indicating that Hcy could diminish long term as well as short term memory in rats. The effects of Hcy on the memory were investigated in some of the earlier studies. It was found that Long-term exposure of Hcy induced alterations in spatial learning, hippocampal signaling and synaptic plasticity (Algaidi et al., 2005). Streck et al. (2004) revealed that chronic Hyperhomocysteinemia impaired memory in the rat. It was suggested that Hcy might generate reactive oxygen species (ROS), which attack the poly unsaturated fatty acids (PUFA) of neuronal cell membranes and induce lipid peroxidation in the hippocampus. Hcy may also modulate intracellular signaling, ultimately leading to neuronal death via apoptosis or necrosis (Agnati et al., 2005). ROS are highly reactive with all biological macromolecules (e.g. proteins, DNA and lipids). PUFAs are extremely sensitive to free radicals due to their double bonds. ROS attack PUFAs, which initiates a chain reaction called "lipid peroxidation" (Wu, 2005). In the course of this process, many detrimental metabolites, such as peroxides, aldehydes, ketones and alcoholes are generated. Peroxides and aldehydes, especially 4-hydroxy-nonenal (HNE) and MDA, are the key-factors for the initiation and the propagation of atherosclerosis, inflammation, cancer and neurodegenerative diseases (Pratico and Delanty, 2000).

In the present study the loss of dentate gyrus cells of hippocampus by Hcy was observed. The role of dentate gyrus of hippocampus on the memory was investigated in previous studies. Kim et al. (2010) investigated the effect of treadmill exercise on memory in relation to neurogenesis and apoptosis in the hippocampal dentate gyrus of old-aged rats. They showed that loss of memory by aging was associated with a decrease in neurogenesis and an increase in apoptosis in the hippocampal dentate gyrus. McHugh et al. (2007) showed dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. They tested this hypothesis by generating and analyzing a mouse strain that lacks the gene encoding the essential subunit of the N-methyl-D-aspartate (NMDA) receptor NR1, specifically in dentate gyrus granule cells. The mutant mice performed normally in contextual fear conditioning, but were impaired in the ability to distinguish two similar contexts.

Results of the present study indicated that Curcumin treatment in both low and high doses (5 and 50 mg/kg) inhibited lipid peroxidation significantly and decreased MDA and SOA levels in the Hcy-treated hippocampi (Figs. 2 and 3). On the other hand, the high dose of Curcumin (50 mg/kg) decreased lipid peroxidation less than the dose of 5 mg/kg ($p<0.01$). Nonetheless, Curcumin also improved Hcy-mediated memory deficits and increased latency times in the first and second passive avoidance tests (Fig. 6). Also histopathological analysis showed that Hcy reduced cell density in some parts of hippocampus. Cell density in dentate gyrus of the dorsal hippocampus was decreased in the Hcy group in comparison with the vehicle group. However, this deficit was significantly improved by treatment with Curcumin (5 and 50 mg/kg; Fig. 5).

In our research sodium pentobarbital was used as an anesthetic agent in animal surgery at the dose of 60 mg/kg according to Dairam research (2006). Pentobarbital was the principal agent used for general anesthesia in small animals. It is still commonly used as an

anesthetic in laboratory situations for rodents. Because of its deep anesthetic effect, animal uncomfortatability during surgery decreased. Additionally, behavioral studies began 5 days after animal surgery so anesthetic drug was eliminated after this duration from animals' body. Also all of experimental groups received the same anesthetic agent. Therefore its behavioral effect was very low.

According to results, Curcumin has a dose-related antioxidant effects on the hippocampus. Curcumin alone did not affect on learning and memory (Fig. 7), but improved learning and memory process that was impaired by Hcy. In a previous study, Pan et al. (2008) indicated that Curcumin improved learning and memory in mice and investigated the neuroprotective effect of Curcumin on the memory of AD mice in the step-through test. Curcumin reduced the number of step-through errors and prolonged step-through latency in AD mice (Pan et al., 2008). Curcumin also attenuated the neuropathological changes in the hippocampus and inhibited apoptosis accompanied by an increase in Bcl-2 but not Bax level (Pan et al., 2008). According to present knowledge, apoptosis is mainly involved in the first two conditions, (cancer and neurodegeneration), while aging is likely more affected by inflammation (Salvioli et al., 2007). Lim et al. (2001) showed that Curcumin reduced oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. They also showed that low and high doses of Curcumin significantly lowered oxidized proteins and interleukin-1beta, a proinflammatory cytokine, elevated in the brains of these mice (Lim et al., 2001). Kumar et al. (2007a,b) also showed that chronic treatment with Curcumin (10, 20 and 50 mg/kg; per os) once daily for a period of 8 days beginning 4 days prior to 3-Nitropropionic acid (3-NP) administration dose-dependently improved the 3-NP-induced motor and cognitive impairment. Biochemical analysis revealed that Curcumin administration significantly attenuated 3-NP-induced oxidative stress (lipid peroxidation estimation, reduced glutathione and nitrite activity) in the brains of rats (Kumar et al., 2007a,b). Another study showed that chronic treatment with Curcumin (5–50 mg/kg; per os) twice daily for a period of 25 days beginning 4 days prior to Colchicines injection significantly improved the Colchicines-induced cognitive impairment. Also, chronic administration of Curcumin significantly reduced the Colchicines-induced elevated lipid peroxidation (Kumar et al., 2007a,b). There are multiple biological activities of Curcumin (Maheshwari et al., 2006), such as its anti-inflammatory activity via down regulation of cyclo-oxygenase 2 and nitric oxide synthase through suppression of NF-kappa B activation (Bengmark, 2006). Results of this study suggest that the antioxidant property of Curcumin may be responsible for protection against Hcy oxidative stress, possibly by increasing the endogenous defenses against oxidative stress. Our results suggest that Curcumin may scavenge SOA from hippocampus tissue. Therefore, Curcumin has protective effects against lipid peroxidation and has decreased MDA and SOA formation. In conclusion, Curcumin at low or high doses (5 and 50 mg/kg) could prevent neurotoxicity of Hcy in the rat hippocampus. If Hyperhomocysteinemia is one of the pathological reasons for neurodegenerative disorders such as sporadic AD or Parkinson's disease, Curcumin may be an effective prophylactic agent in the prevention of oxidative stress by Hcy. Furthermore, our data suggest that Curcumin's mechanism for protecting the hippocampus against the toxicity of Hcy may be to inhibit the generation of ROS in rats' brain. Further studies are needed to reveal the exact mechanism of Hcy in cell death process (apoptosis or necrosis) and the neuroprotective properties of Curcumin must be studied in more detail.

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References

Agnati LF, Genadani S, Rasio G. Studies on homocysteine plasma levels in Alzheimer's patients for neurodegeneration. Neural Transm 2005;112:163–9.
Ahmed T, Gilani AH. Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease. Pharmacol Biochem Behav 2009;91:554–9.
Algaidi SA, Christie LA, Jenkinson AM, Whaley L, Riedel G, Platt B. Long-term homocysteine exposure induces alterations in spatial learning, hippocampal signaling and synaptic plasticity. Exp Neurol 2005;197:8-12.
Bengmark S. Curcumin, a toxic antioxidant and natural NFkappaB, cyclooxygenase-2, lipooxygenase, and inducible nitric oxide synthase inhibitor: a shield against acute and chronic diseases. JPEN J Parenter Enteral Nutr 2006;30:45–51.
Blumenthal M, Goldberg A, Brinkman J. Herbal Medicine: the expanded commission E monographs. Newton, MA: Integrative Medicine Communications; 2000. p. 330–4.
Bruce A, Boling W, Kindy S, Peschon J, Kraemer J. Altered neuronal and microglial responses to brain injury in mice. Nat Med 1996;2:788–94.
Clarke L, Daly K, Robinson E, Naughten E, Cahalane S, Fowler B, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 1991;324: 1149–55.
Dairam A, Chetty P, Daya S. Non-steroidal anti-inflammatory agents, tolmetin and sulindac, attenuate oxidative stress in rat brain homogenate and reduce quinolinic acid-induced neurodegeneration in rat hippocampal neurons. Metab Brain Dis 2006;21:221–33.
Daniel S, Limson JL, Dairam A, Watkins GM, Daya S. Through metal binding, curcumin protects against lead- and cadmium-induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain. J Inorg Biochem 2004;98:266–75.
Das UN, Padma M, Saga PS. Stimulation of free radical generation in human leukocytes by various agents including tumor necrosis is a calmodulin process. Biochem Biophys Res 1990;167:1030–6.
Ganguli M, Chandra V, Kamboh MI. Apolipoprotein E polymorphism and Alzheimer's disease: the Indo-US Cross-National Dementia Study. Neurology 2000;57:824–30.
Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. Lancet 1999;354: 407–13.
Heinecke JW, Cerruti PA, Fridovich I, McCord JM. Super oxide-mediated oxidation of low density lipoprotein by thiols. Oxy-Radicals in Molecular Biology and Pathology. 1st ed. New York: Alan R Liss; 1988. p. 443–57.
Jara-Prado A, Ortega-Vazquez A, Martinez-Ruano L, Rios C, Santamaria A. Homocysteine-induced brain lipid peroxidation, effects of NMDA receptor blockade, antioxidant treatment, and nitric oxide synthase inhibition. Neurotoxicology 2003;5:237–43.
Kim JH, Cho SY, Lee JH, Jeong SM, Lee BH, Lee JH, et al. Neuroprotective effects of ginsenoside Rg_3 against homocysteine-induced excitotoxicity in rat hippocampus. Brain Res 2007;1136:190–9.
Kim SE, Ko IG, Kim BK, Shin MS, Cho S, Kim CJ, et al. Treadmill exercise prevents aging-induced failure of memory through an increase in neurogenesis and suppression of apoptosis in rat hippocampus. Exp Gerontol 2010;45(5):357–65.
Kruman I, Culmsee C, Chan SL, Kruman Y, Guo Z, Penix L, et al. Homocysteine elicits a DNA damage response in neurons that promostes apoptosis and hypersensitivity to excitotoxicity. J Neurosci 2000;20:6920–6.
Kumar A, Naidu PS, Seghal N, Padi SS. Effect of curcumin on intracerebroventricular colchicine-induced cognitive impairment and oxidative stress in rats. J Med Food 2007a;10:486–94.
Kumar P, Padi SS, Nadiu PS, Kumar A. Possible neuroprotective mechanisms of curcumin in attenuating 3-nitropropionic acid-induced neurotoxicity. Methods Find Exp Clin Pharmacol 2007b;29:19–25.
Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM. The curry spice curcumin reduces oxidative damage and Amyloid pathology in an Alzheimer transgenic mouse. Neuroscience 2001;21:8370–7.
Lipton SA, Kim WK, Choi YB, Kumar S, Emilia DM, Rayudu PV. Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. Proc Natl Acad Sci 1997;94:5923–8.
Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin, a short review. Life Sci 2006;78:2081–7.
Matté C, Mackedanz V, Stefanello FM, Scherer EB, Andreazza AC, Zanotto C, et al. Chronic hyperhomocysteinemia alters antioxidant defenses and increases DNA damage in brain and blood of rats: protective effect of folic acid. Neurochem Int 2009;54:7-13.
McHugh T, Jennifer M, Quinn J, Balthazar N, Coppery R. Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. Science 2007;317:94–9.
Pan R, Qiu S, DX LU, Dong J. Curcumin improves learning and memory ability and its neuroprotective mechanism in mice. Chin Med J 2008;121:832–9.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. San Diego: Academic Press; 1986.
Placer ZA, Cushman LL, Johnson AC. Estimation of Product lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem 1966;16:359–64.
Pratico D, Delanty N. Oxidative injury in disease of the central nervous system. Focus on Alzheimer's disease. Am J Med 2000;109:577–85.
Ramassamy C. Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets. Eur J Pharmacol 2006;545:51–64.

Salvioli S, Sikora E, Cooper E. Curcumin in cell death process: a challenge for CAM of age-related pathologies. E CAM 2007;4:181–90.
Schwartz S, Siscovick D, Malinow M. Myocardial infarction in young women in relation to plasma total homocysteine, folate, and a common variant in the methylenetetrahydrofolate reductase gene. Circulation 1997;96:412–7.
Seshadri S, Beiser A, Selhub J, Jacques F, Rosenberg IH, D'agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476–83.
Shen SQ, Zhang Y, Xiang JJ, Xiong CL. Protective effect of curcumin against liver warm ischemia/reperfusion injury in rat model is associated with regulation of heat shock protein and antioxidant enzymes. World J Gastroenterol 2007;13:1953–61.
Sigma Aldrich Co. Catalog, Specification Sheet. 2007; H4628. P: 1.
Streck EL, Bavaresco CS, Netto CA, Wyse AT. Chronic hyperhomocysteinemia provokes a memory deficit in rats in the Morris water maze task. Behav Brain Res 2004;153: 377–81.
Sumanont Y, Murakami Y, Tohda M, Vajragupta O, Watanabe H, Matsumoto K. Effects of manganese complexes of curcumin and diacetylcurcumin on kainic acid-induced neurotoxic responses in the rat hippocampus. Biol Pharm Bull 2007;30:1732–9.
Vajragupta O, Boonchoong P, Watanabe H, Tohda M, Kummasud N, Sumanont Y. Manganese complexes of curcumin and its derivatives: evaluation for the radical scavenging ability and neuroprotective activity. Free Radic Biol Med 2003;35: 1632–44.
Veerendra Kumar MH, Gupta YK. Effect of *Centella asiatica* on cognition and oxidative stress in an intracerebroventricular streptozotocin model of Alzheimer's disease in rat. Clin Exp Pharmacol Physiol 2003;30:336–42.
Wilson I, Gamble M. The hematoxylin and eosin. In: Bancroft JD, Gamble, editors. Theory and Practice of Histological Techniques. 5th ed. London: Churchill Livingston; 2002. p. 125–38.
Wu WM. An investigation into the neuroprotective effects of Estrogen and Progesterone in a model of Homocysteine – induced neurodegeneration. Thesis of Master in Pharmacy. South Africa: Rhodes University E-Publishing; 2005. p. 65-180.
Yamamoto M, Tamura A, Kirino T. Behavioral changes after focal cerebral ischemia by left middle cerebral ischemia by left middle cerebral artery occlusion in rats. Brain Res 1988;452:323–8.