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Protective effect of Curcumin, the active principle of turmeric (*Curcuma longa*) in haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes in rat brain

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Abstract

Tardive dyskinesia (TD) is a motor disorder of the orofacial region resulting from chronic neuroleptic treatment. A high incidence and irreversibility of this hyperkinetic disorder has been considered a major clinical issue in the treatment of schizophrenia. The molecular mechanism related to the pathophysiology of tardive dyskinesia is not completely known. Various animal studies have demonstrated an enhanced oxidative stress and increased glutamatergic transmission as well as inhibition in the glutamate uptake after the chronic administration of haloperidol. The present study investigated the effect of curcumin, an antioxidant, in haloperidol-induced tardive dyskinesia by using different behavioural (orofacial dyskinetic movements, stereotypy, locomotor activity, % retention), biochemical (lipid peroxidation, reduced glutathione levels, antioxidant enzyme levels (SOD and catalase) and neurochemical (neurotransmitter levels) parameters. Chronic administration of haloperidol (1 mg/kg i.p. for 21 days) significantly increased vacuous chewing movements (VCM's), tongue protrusions, facial jerking in rats which was dose-dependently inhibited by curcumin. Chronic administration of haloperidol also resulted in increased dopamine receptor sensitivity as evident by increased locomotor activity and stereotypy and also decreased % retention time on elevated plus maze paradigm. Pretreatment with curcumin reversed these behavioral changes. Besides, haloperidol also induced oxidative damage in all major regions of brain which was attenuated by curcumin, especially in the subcortical region containing striatum. On chronic administration of haloperidol, there was a decrease in turnover of dopamine, serotonin and norepinephrine in both cortical and subcortical regions which was again dose-dependently reversed by treatment with curcumin. The findings of the present study suggested for the involvement of free radicals in the development of neuroleptic-induced tardive dyskinesia and point to curcumin as a possible therapeutic option to treat this hyperkinetic movement disorder.

Keywords: Tardive dyskinesia; Curcumin; Neuroleptic-induced; Supersensitivity

1. Introduction

Tardive dyskinesia (TD) is a motor disorder of the orofacial region resulting from chronic neuroleptic treatment, that is characterized by repetitive involuntary movement in the orofacial regions and sometimes limb and trunk musculature. TD appears months or years after the initiation of antipsychotic treatment and it may persist even after drug withdrawal and may be irreversible in 20% of schizophrenic patients (Andreassen and Jorgensen, 1994). The occurrence and irreversibility of this hyperkinetic disorder has been considered a major clinical issue in the treatment of schizophrenia (Lohr et al., 2003).

The molecular mechanism related to the pathophysiology of tardive dyskinesia is not completely known. Increase in the density of striatal dopaminergic D2 receptors observed in humans and in experimental rodent models of tardive dyskinesia coincide with the appearance of extrapyramidal side effects. Role of the dopaminergic hypothesis as the main molecular mechanism of TD has been questioned on several

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grounds (Klawans and Rubovits, 1972). The participation of free radicals derived from the metabolism of dopamine and/or from an enhancement of the glutamatergic transmission, secondary to presynaptic dopamine receptors blockade has gained ample experimental support (Casey, 2000; Naidu and Kulkarni, 2001a,b; Tsai et al., 1998). Various animal studies have demonstrated an enhancement to glutamatergic participation as well as inhibition in the glutamate uptake after the chronic administration of haloperidol (Grimm et al., 1998; Burger et al., 2005a,b).

Lipid peroxidation levels in the blood and cerebrospinal fluid of the patients suffering from TD patient are significantly greater when compared to the normal patients as well as in case of different animal models of tardive dyskinesia such as haloperidol-induced tardive dyskinesia and reserpine induced orofacial hyperkinetic movements (Burger et al., 2003; Lohr et al., 2003). Different experimental paradigms have confirmed the protective action of different antioxidants (Burger et al., 2003; Naidu et al., 2003; Singh et al., 2003) whereas pro-oxidants, such as mitochondrial neurotoxin 3-nitropropionic acid, aggravate reserpine or haloperidol-induced orofacial dyskinesia (Calvente et al., 2002; Andreassen et al., 1998; Burger et al., 2003; Abilio et al., 2003).

The significance of *Curcuma longa* Linn (Turmeric) in health and nutrition has changed considerably since the discovery of the antioxidant property of naturally occurring phenolic compounds, curcuminoids. Curcumin is one of the main curcuminoids isolated from turmeric (Araujo and Leon, 2001). Curcumin has numerous pharmacological activities like anti-inflammatory activity, anti-protozoal activity, nematocidal activity, antibacterial activity, anti-mutagenic activity and hepatoprotective activity. Earlier studies have shown that curcumin inhibits reactive oxygen species (ROS) production (Quiles et al., 2002) as well as calcium entry (Balasubramanyam et al., 2003). It can also affect different other cellular processes such as activation of apoptosis, inhibition of platelet aggregation, inhibition of inflammatory cytokine production, inhibition of cyclooxygenase and lipoxygenase isoenzymes. It may also affect the activity of different key enzymes such as PKC, protein tyrosine kinases and calcium dependent endonuclease (Balasubramanyam et al., 2003).

In the present study, along with orofacial dyskinetic behaviours (vacuous chewing movements, tongue protrusions and facial jerkings) we also evaluated % retention (Elevated plus maze paradigm), stereotypic behaviour (Stereotypic rearing) and total locomotor activity (Actophotometer). Stereotypic behaviour and total locomotor activity were assessed to analyze the development of dopamine receptor supersensitivity with chronic administration of haloperidol (Bishnoi et al., 2006). Chronic administration of haloperidol is also associated with decreased retention ability. Hence, these behaviours were assessed to see the effect of co-administration of curcumin on the changes associated to retention ability and dopamine supersensitivity. There are several previous reports from our laboratory (Bishnoi et al., 2007a,b) as well as from other labs regarding the decrease in the levels of serotonin and norepinephrine by chronic neuroleptic treatment (Burnet et al., 1996; Ichikawa et al., 1998, Cahir et al., 2004; Lau et al., 2003). Alteration in catecholamine metabolism is one of the reasons of increased oxidative damage after haloperidol administration. Decreased levels of norepinephrine after the chronic administration of haloperidol reflect increased metabolism which can be correlated with the induction of oxidative damage. Similarly the involvement, if any of serotonin in haloperidol-induced neurotoxicity was assessed by estimating the brain levels of it.

Considering the importance of searching for new compounds with potential usefulness in the treatment or prevention of TD, we examined the possible protective effect of curcumin in haloperidol-induced orofacial dyskinesia and related behavioural, biochemical and neurochemical alterations.

2. Methods and materials

2.1. Animals

Male Wistar rats (180–220 g; 10–12 rats/group) bred in the Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a normal light–dark cycle and free access of food and water. Animals were acclimatized to laboratory conditions before the test. Each animal was used only once in the experiment. All the experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Indian National Science Academy for the use and care of experimental animals.

2.2. Drugs and treatment schedule

The following drugs were used in the present study. Haloperidol (Serenace, Searle, India) was diluted with distilled water. Curcumin was dissolved in Carboxy Methyl Cellulose (CMC). Haloperidol and/or curcumin was/were administered intraperitoneally and per-orally respectively in a constant volume of 0.5 ml per 100 g of bodyweight of rat. Animals were divided in 5 groups. First group received vehicle(CMC), second group received haloperidol (1 mg/kg) plus vehicle (CMC), third group received haloperidol (1 mg/kg) plus curcumin (25 mg/kg), fourth group received haloperidol (1 mg/kg) plus curcumin (50 mg/kg), fifth group received only curcumin (50 mg/kg) Haloperidol and/or curcumin was/were administered simultaneously once daily (0900) in the morning for a period of 21 days and behavioural assessments were done every week (Day 7 and 14 (after 24 h of previous day's haloperidol injection) and last quantification was done 24 h after the last dose. Same set of animals was used for all the behavioural assessments.

2.3. Induction of orofacial dyskinesia

Haloperidol (1 mg/kg i.p.) was administered chronically to rats for a period of 21 days to induce oral dyskinesia. All the behavioural assessments were carried out every week and last

behavioural quantification was done after 24 h of last dose of haloperidol (Naidu et al., 2003).

2.4. Body weight change

The body weights of animals were recorded before the start of experiment and than before the behavioural assessment.

2.5. Behavioural assessment of orofacial dyskinesia

On the test day, rats were placed individually in a small (30×20×30 cm) Plexiglas cage for the assessment of oral dyskinesia. Animals were given 10 min to get acclimatized to the observation cage before behavioural assessments. To quantify the occurrence of oral dyskinesia, hand operated counters were employed to score tongue protrusion and vacuous chewing frequencies (VCMs). In the present study VCM are referred to as single mouth openings in the vertical plane not directed toward physical material. If tongue protrusion or VCM occurred during a period of grooming, they were not taken into account. Counting was stopped whenever the rat began grooming, and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. The behavioural parameters of oral dyskinesia were measured continuously for a period of 5 min. In all the experiments, the scorer was unaware of the treatment given to the animals (Naidu et al., 2003).

2.6. Elevated plus maze test

The elevated plus maze was used to evaluate spatial long term memory, following the procedure as described earlier (Reddy and Kulkarni, 1998). Briefly the apparatus consisted of two open arms and two closed arms. The arms extended from a central platform, and the maze was elevated to a height of 50 cm from the floor. On the first day, each animal was placed at the end of an open arm. Transfer latency (TL) is the time taken by the rat to move into one of the enclosed arm was recorded on the first day. If the animal did not enter an enclosed arm within 90 s it was gently pushed into one enclosed arm and the TL latency was assigned as 90 s. The rat was allowed to explore the maze for 20 s and then returned to the home cage. The rat was again placed on the maze on next day (24 h later) and TL was recorded (Itoh et al., 1994).

Percent retention was calculated by the formula —

$$\text{Transfer Latency (Day1)} - \text{Transfer Latency (Day2)}/\text{Transfer Latency (Day2)} \times 100$$

2.7. Locomotor activity

The locomotor activity was monitored using activity meter (IMCORP, India). Before subjecting the animal to cognitive task they were individually placed in activity meter and the total activity count was registered for 5 min. The locomotor activity was expressed in terms of total photo beams counts/5 min per animal (Reddy and Kulkarni, 1998).

2.8. Stereotypic behaviour assessment

Stereotypic rearing was measured in different groups of animals. Each animal was individually placed in a 1000 ml beaker and number of rearing was scored for the time period of 5 min. Number of rearing was scored as ++++ (between 12–16)=very high, +++ (between 8–12)=high, ++ (between 4–8)=moderate, +=low(between 2–4) (Kulkarni, 1999).

2.9. Dissection and homogenization

On day 22, behavioural assessments were done. Some of the animals were high VCM groups and some were in low VCM group. On the basis of behavioural changes animals were randomized in two groups, one each for biochemical studies and HPLC studies and sacrificed. Each of the group consists of equal number of high and low VCM animals. The brains were removed, forebrain was dissected out and cerebellum was discarded. Brains were put on ice and the cortex and subcortical regions (including striatum) were separated and weighed. A 10% (w v^{-1}) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). Whole brains (excluding cerebellum) from each group were separately stored at −80 °C for HPLC studies. Homogenate were centrifuged for 20 min at 12,000 ×*g* and supernatant was used for estimation of lipid peroxidation and reduced glutathione levels. The post nuclear fractions for catalase assay were obtained by centrifugation of the homogenate at 1000 ×*g* for 20 min, at 4 °C and for other enzyme assays centrifuged at 12,000 ×*g* for 60 min at 4 °C. The subcortical region of brain comprised of all the remaining parts of the forebrain except cortex.

2.10. Lipid peroxidation assay

The quantitative measurement of lipid peroxidation in forebrain was performed according to the method of Wills. The amount of malondialdehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm using Shimadzu UV/visible spectrophotometer. The results were expressed as nmol of malondialdehyde/mg protein using the molar extinction coefficient of chromophore (1.56×10^5 M^{-1} cm^{-1}) (Wills, 1966).

2.11. Estimation of non protein thiols (NPSH)

Non-protein thiols (NPSH) in the forebrain were estimated according to the method of Ellman. A 0.75 ml of homogenate was precipitated with 0.75 ml of 4% sulphosalicylic acid. The samples were centrifuged at 1200 ×*g* for 15 min at 4 °C. The assay mixture contained 0.5 ml of supernatant and 4.5 ml of 0.01 M (in 0.1 M phosphate buffer, pH 8.0) DTNB (5-5′-DithioBis-(2-Nitrobenzoic acid)). The yellow colour developed was read immediately at 412 nm using Shimadzu UV/visible spectrophotometer. The results were expressed as nmol of NPSH per mg protein (Ellman, 1959).

2.12. Enzyme assays

2.12.1. Superoxide dismutase activity

Superoxide dismutase activity was assayed according to the method of Kono et al., where in the reduction of nitazoblue tetrazolium (NBT) was inhibited by the superoxide dismutase is measured at 560 nm using Shimadzu UV/visible spectrophotometer. Briefly, the reaction was initiated by the addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post nuclear fraction of fore brain homogenate. The results were expressed as units/mg protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 50% (Kono, 1978).

2.12.2. Catalase activity

Catalase activity was assayed by the method of Luck et al, wherein the breakdown of H_2O_2 being measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H_2O_2 phosphate buffer (1.25×10^{-2} H_2O_2 m) and 0.05 ml of supernatant of forebrain homogenate (10%) and the changes in absorbance were recorded at 240 nm using Shimadzu UV/visible spectrophotometer. Enzyme activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.07). The results were expressed as μmol H_2O_2 decomposed/min/mg protein (Luck, 1963).

2.13. Protein estimation

The protein content was measured according to the method of Lowry et al. using bovine serum albumin as standard (Lowry et al., 1951).

2.14. Neurotransmitters estimation

Biogenic amines (dopamine, serotonin and norepinephrine) were estimated by HPLC with electrochemical detector. Waters standard system consisting of a high pressure isocratic pump, a 20 μl sample injector valve, C18 reverse phase column and electrochemical detector were used. Data was recorded and analyzed with the help of empower software. Mobile phase consisting of 2% citric acid, 2% $KHPO_4$, 1 mM EDTA, 1.2% MeOH, and 70 mg/ml of sodium octyl sulphate. pH of the mobile phase was adjusted to 3 with the help of HCl (6 N). Electrochemical conditions for the experiment were +0.800 V, sensitivity ranges from 5–50 nA. Separation was carried out at a flow rate of 1 ml/min. Samples (20 μl) were injected manually.

On the day of experiment frozen, forebrain samples were thawed and they were homogenized in homogenizing solution containing 0.1 M perchloric acid. After that samples were centrifuged at 12000 ×*g* for 5 min. The supernatant was further filtered through 0.25 μm nylon filters before injecting in the HPLC injection pump. Data was recorded and analyzed with the help of empower software (Church, 2005).

2.15. Statistical analysis

One specific group of rats was assigned to one specific drug treatment condition and each group comprised six rats ($n=6$). All the values are expressed as means±S.E.M. The data were analyzed by using analysis of variance (ANOVA) followed by Dunnett's test. In all tests, the criterion for statistical significance was $P<0.05$.

3. Results

3.1. Body weight change

Body weight was significantly decreased in haloperidol (1 mg/kg, i.p.) treated group as compared to control animals. Pretreatment with curcumin (25 and 50 mg/kg) prevented the decrease in body weight. Curcumin (50 mg/kg) *per se* did not cause any significant change in body weight as compared to control.

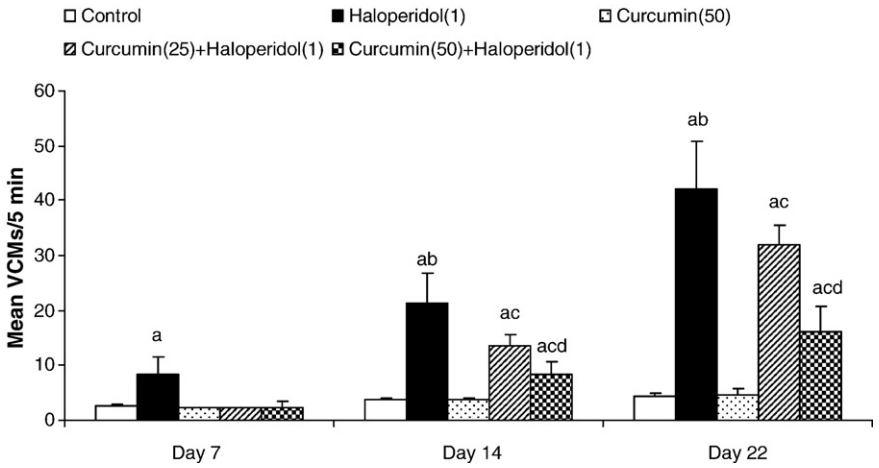

Fig. 1. Vacuous chewing movements (VCM's) recorded on day 7, 14, 22 (test day) in rats chronically treated with (a) vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1). Total number of animals in each group is 5–6 and data is expressed in Mean±SEM. [a]$p\leq0.05$ as compared to control group (on the day of behavioural assessment), [b]$p\leq0.05$ as compared to haloperidol-treated group of previous week, [c]$p\leq0.05$ as compared to haloperidol-treated group (on the day of behavioural assessment) [d]$p\leq0.05$ as compared to Curcumin (25)+haloperidol-treated group.

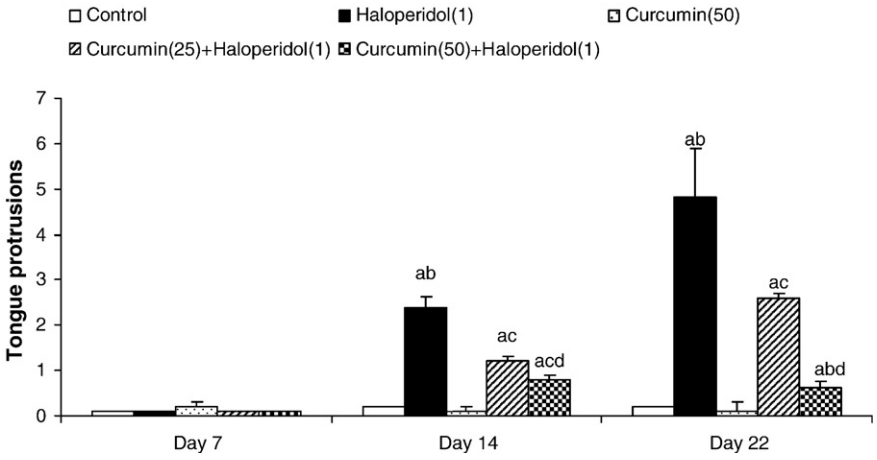

Fig. 2. Tongue protrusions recorded on day 7, 14, 22 (test day) in rats chronically treated with (a) vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1). Total number of animals in each group is 5–6 and data is expressed in Mean±SEM. [a]$p \leq 0.05$ as compared to control group (on the day of behavioural assessment), [b]$p \leq 0.05$ as compared to haloperidol-treated group of previous week, [c]$p \leq 0.05$ as compared to haloperidol-treated group (on the day of behavioural assessment) [d]$p \leq 0.05$ as compared to Curcumin (25)+haloperidol-treated group.

3.2. Behavioural assessment

3.2.1. Assessment of orofacial dyskinesia

Haloperidol (1 mg/kg, i.p.) treatment resulted into significant increase in VCM's, tongue protrusion and facial jerking. Pretreatment with curcumin (25 and 50 mg/kg) dose-dependently inhibited the increase of haloperidol-induced VCM, tongue protrusions and facial jerking. Curcumin (50 mg/kg) *per se* did not cause any significant change in VCM's, tongue protrusions and facial jerking as compared to control. (Figs. 1–3).

3.2.2. Stereotypic rearing behavior assessment

Haloperidol (1 mg/kg, i.p.) treatment resulted into decrease in stereotypic rearing behaviour up to 7th day which was thereafter increased up to last behavioural quantification. Pretreatment with curcumin (25 and 50 mg/kg) prevented this increase of stereotypic rearing behaviour. Curcumin (50 mg/kg) *per se* did not cause any significant change in stereotypic rearing behaviour as compared to control (Fig. 4).

3.2.3. Locomotor activity

Haloperidol (1 mg/kg, i.p.) treatment resulted into decrease in total locomotor activity (ambulatory and rearing) up to 14th day which was thereafter increased in last behavioural quantification. Pretreatment with curcumin (25 and 50 mg/kg) prevented this increase of locomotor activity. Curcumin (50 mg/kg) *per se* did not cause any significant change in total locomotor activity (ambulatory and rearing) as compared to control (Fig. 5).

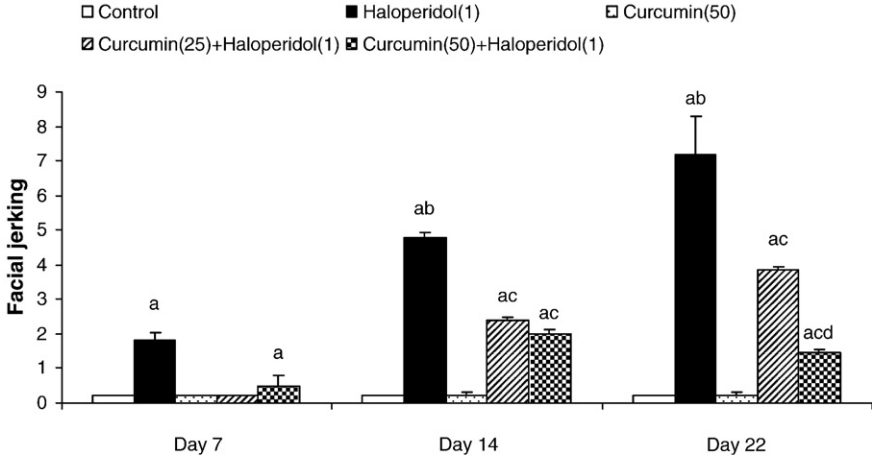

Fig. 3. Number of facial jerking recorded on day 7, 14, 22 (test day) in rats chronically treated with (a) vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1). Total number of animals in each group is 5–6 and data is expressed in Mean±SEM. [a]$p \leq 0.05$ as compared to control group (on the day of behavioural assessment), [b]$p \leq 0.05$ as compared to haloperidol-treated group of previous week, [c]$p \leq 0.05$ as compared to haloperidol-treated group (on the day of behavioural assessment) [d]$p \leq 0.05$ as compared to Curcumin (25)+haloperidol-treated group.

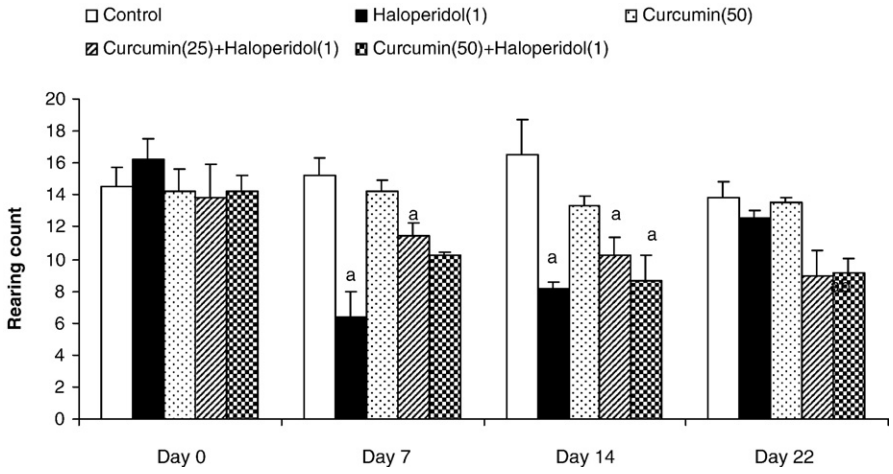

Fig. 4. Stereotypic rearing behaviour recorded on before drug administration (base line), day 7, 14, 22 (test day) in rats chronically treated with (a) vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1). Total number of animals in each group is 5–6 and data is expressed in Mean±SEM. $^{a}p \leq 0.05$ as compared to control group, $^{b}p \leq 0.05$ as compared to haloperidol-treated group.

3.2.4. Elevated plus maze test

Haloperidol (1 mg/kg, i.p.) treatment resulted into decrease in % retention as compared to control group. Pretreatment with curcumin (25 and 50 mg/kg), prevented this decrease in % retention at the day of last behavioural quantification. Curcumin (50 mg/kg) *per se* did not cause any significant change in % retention as compared to control (Fig. 6).

3.3. Biochemical assessment

3.3.1. Lipid peroxidation assay

Chronic haloperidol treatment (1 mg/kg, i.p.) resulted in significant increase in lipid peroxidation in both the regions (cortex, subcortical (including striatum) of the brain as compared to control animals. Pretreatment with curcumin (25 and 50 mg/kg) prevented the increase in lipid peroxidation in both the regions. Curcumin (50 mg/kg) *per se* did not cause any significant change in lipid peroxidation as compared to control (Fig. 7).

3.3.2. Estimation of reduced glutathione

Chronic haloperidol treatment (1 mg/kg, i.p.) resulted in significant decrease in reduced glutathione in both the regions (cortex, subcortical (including striatum) of the brain as compared to control animals. Pretreatment with curcumin (25 and 50 mg/kg) prevented the decrease in reduced glutathione in both the regions. Curcumin (50 mg/kg) *per se* did not cause any significant change in reduced glutathione as compared to control (Fig. 8).

3.3.3. Catalase activity

Chronic haloperidol treatment (1 mg/kg, i.p.) resulted in significant decrease in catalase in both the regions (cortex, subcortical (including striatum) of the brain as compared to

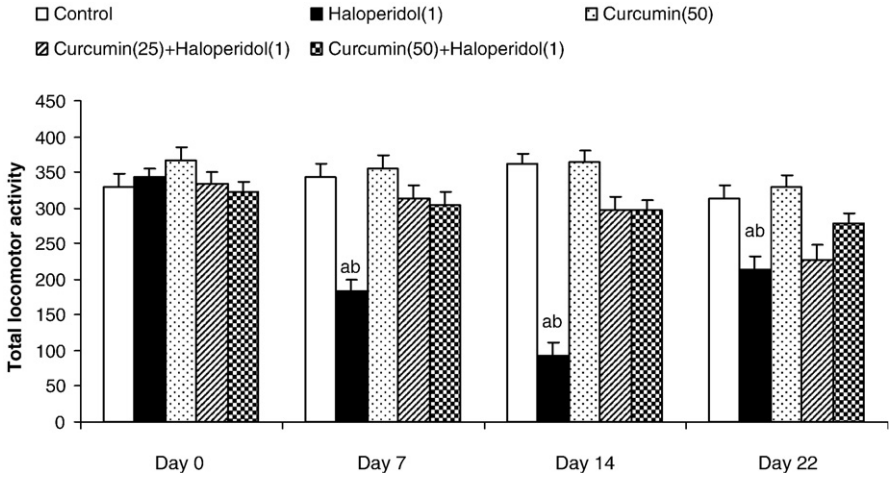

Fig. 5. Total locomotor activity recorded on before drug administration (base line), day 7, 14, 22 (test day) in rats chronically treated with(a) vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1). Total number of animals in each group is 5–6 and data is expressed in Mean±SEM. $^{a}p \leq 0.05$ as compared to control group, $^{b}p \leq 0.05$ as compared to haloperidol-treated group.

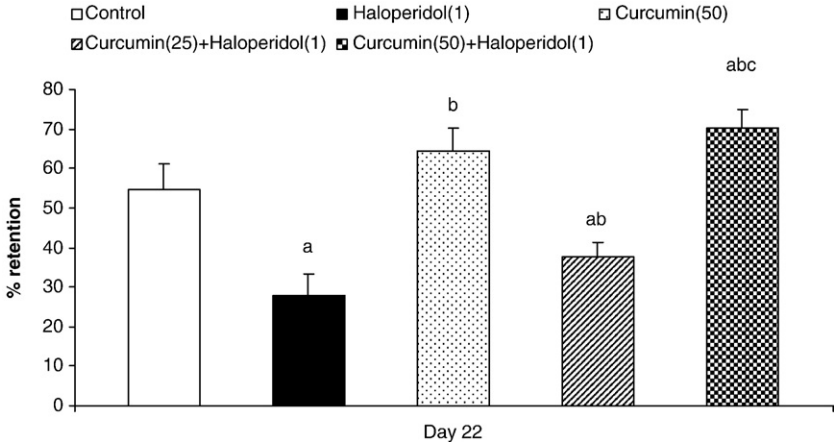

Fig. 6. Percentage retention recorded on day 22 (test day) in rats chronically treated with (a) vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1). Total number of animals in each group is 5–6 and data is expressed in Mean±SEM. [a]$p \leq 0.05$ as compared to control group, [b]$p \leq 0.05$ as compared to haloperidol-treated group, [c]$p \leq 0.05$ as compared to curcumin(25)+Haloperidol(1).

control animals. Pretreatment with curcumin (25 and 50 mg/kg) prevented the decrease in catalase in both the regions. Curcumin (50 mg/kg) *per se* did not cause any significant change in catalase as compared to control (Fig. 9).

3.3.4. Superoxide dismutase activity

Chronic haloperidol treatment (1 mg/kg, i.p.) resulted in significant decrease in superoxide dismutase in both the regions (cortex, subcortical (including striatum) of the brain as compared to control animals. Pretreatment with curcumin (25 and 50 mg/kg) prevented the decrease in superoxide dismutase in both the regions. Curcumin (50 mg/kg) *per se* did not cause any significant change in superoxide dismutase as compared to control (Fig. 10).

3.4. Neurochemical assessment

3.4.1. Neurotransmitter estimation

Chronic administration of haloperidol resulted into decreased levels of dopamine, norepinephrine and serotonin in homogenates of cortical and subcortical regions (including striatum) which was prevented by pretreatment with curcumin (25 and 50 mg/kg). Curcumin (50 mg/kg) *per se* did not cause any significant change in dopamine, norepinephrine and serotonin concentration as compared to control (Table 1).

4. Discussion

In the present study, haloperidol-treated animals developed orofacial dyskinesia, which was determined by an increase in VCM's, FT and TP. The administration of curcumin dose-dependently showed a protective effect against haloperidol-induced orofacial dyskinesia. Literature data indicates that an imbalance in production and detoxification of free radicals may be associated with chronic neuroleptic use and it contributes to the initiation of hyperkinetic movements in the orofacial regions (Cadet et al., 1986). Typical neuroleptics block dopamine D2 receptors (Creese et al., 1976; Seeman et al., 1976) and this blockade is associated with particularly increased dopamine turnover in catecholamine rich region such as basal ganglia. These regions of the brain are highly vulnerable to the free radical overproduction caused by increased dopamine turnover,

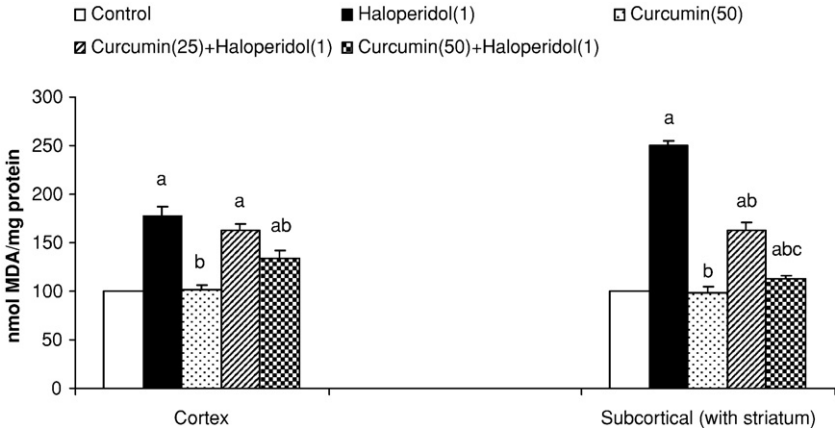

Fig. 7. Lipid peroxidation in different brain regions (cortex, subcortical regions(including striatum)) in rats chronically treated with vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1). Total number of animals in each group is 5–6 and data is expressed in Mean±SEM. [a]$p \leq 0.05$ as compared to control group, [b]$p \leq 0.05$ as compared to haloperidol-treated group, [c]$p \leq 0.05$ as compared to curcumin(25)+Haloperidol(1).

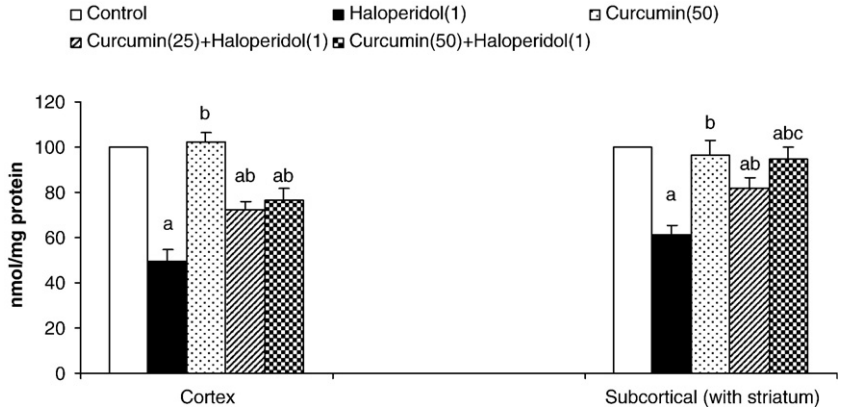

Fig. 8. Reduced glutathione levels in different brain regions (cortex, subcortical regions (including striatum)) in rats chronically treated with vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1). Total number of animals in each group is 5–6 and data is expressed in Mean±SEM. [a]$p \leq 0.05$ as compared to control group, [b]$p \leq 0.05$ as compared to haloperidol-treated group, [c]$p \leq 0.05$ as compared to curcumin(25)+Haloperidol(1).

because they use elevated amounts of energy and contain considerable amounts of polyunsaturated fatty acids (Lohr et al., 2003). The increased oxidative metabolism in these regions after chronic haloperidol administration may be associated with decrease in antioxidant brain defense, as evidenced by increased lipid peroxidation, reduced glutathione levels and markedly reduced antioxidant enzyme (catalase and superoxide dismutase) levels. These results are in accordance to with data from the literature showing increased oxidative damage parameters in different regions of brain after the chronic administration of haloperidol (Naidu et al., 2003; Singh et al., 2003). However, there are several points of evidence in the literature that an increase in lipid peroxidation and decrease in reduced glutathione can be correlated to an increase in other more direct measure of oxidative stress such as protein carbonyls and 8-oxa-2-deoxyguanosine formation (Campos et al., 2005; Kumarguruparam et al., 2005; Rodrigo et al., 2005).

Curcumin dose-dependently protected haloperidol-treated rats against the increase in lipid peroxidation, decrease in reduced glutathione, decrease in catalase and superoxide dismutase levels in the cortical as well as subcortical (including striatum) regions of the brain. Recently it has been demonstrated that chronic administration of haloperidol to rats results into significant reduction in glutamate uptake in synaptosomes obtained from subcortical regions not from cortical and striatal regions, which is further correlated with increase in orofacial dyskinetic movements (Burger et al., 2005a,b). Curcumin might have prevented the oxidative damage and excitotoxicity induced by decreased uptake of glutamate.

Using haloperidol-induced animal model of tardive dyskinesia, several groups have demonstrated that haloperidol treatment and oral dyskinesia are closely associated with the oxidative stress process (Naidu et al., 2003; Singh et al., 2003; Abilio et al., 2004; Perry et al., 2004), as well as neuropathological alteration within the basal ganglia (Andreassen and Jorgensen, 2000). Further support for the involvement of oxidative stress as a causative agent of orofacial dyskinesia was recently obtained, where they showed that a decreased striatal catalase activity is involved in the development of orofacial dyskinesia in rats (Abilio et al., 2004).

Curcumin, one of the curcuminoids obtained from *Curcuma longa* can affect number of cellular processes as well as certain key enzymes. Curcumin inhibits ROS production (Quiles et al., 2002; Ramirez-Tortosa, 2002) and calcium entry (Balasubra-

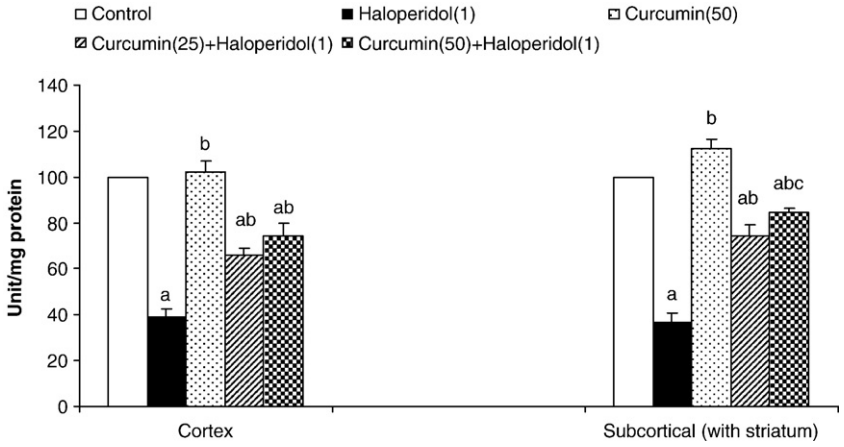

Fig. 9. Catalase activity in different brain regions (cortex, subcortical regions(including striatum)) in rats chronically treated with vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1). Total number of animals in each group is 5–6 and data is expressed in Mean±SEM. [a]$p \leq 0.05$ as compared to control group, [b]$p \leq 0.05$ as compared to haloperidol-treated group, [c]$p \leq 0.05$ as compared to curcumin(25)+Haloperidol(1).

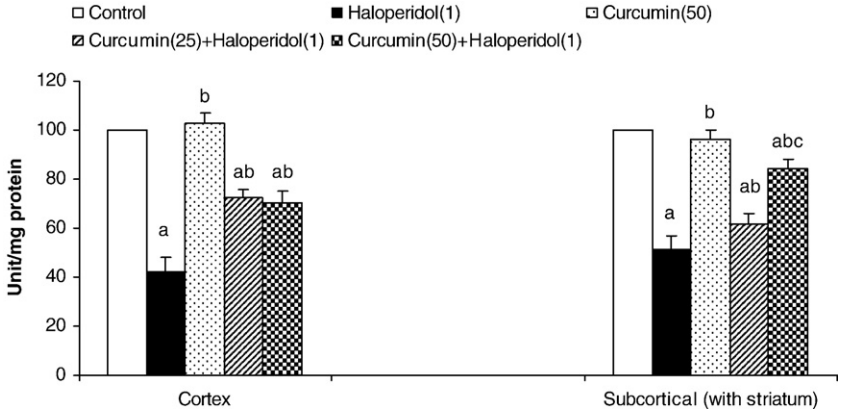

Fig. 10. Superoxide dismutase activity in different brain regions (cortex, subcortical regions(including striatum)) in rats chronically treated with vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1). Total number of animals in each group is 5–6 and data is expressed in Mean±SEM. [a]$p \leq 0.05$ as compared to control group, [b]$p \leq 0.05$ as compared to haloperidol-treated group, [c]$p \leq 0.05$ as compared to curcumin(25)+Haloperidol(1).

manyam et al., 2003). Curcumin is a good antioxidant and it inhibits lipid peroxidation in liver microsomes, erythrocyte membranes and brain homogenates (Araujo and Leon, 2001). It provides a protection of hemoglobin from oxidation at the concentration as low as 0.08 μmol (Araujo and Leon, 2001). Curcumin lowers the lipid peroxidation by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase at higher levels. It is also capable of scavenging oxygen free radicals such as superoxide anions, singlet oxygen, NO and hydroxyl radicals which are important to the initiation of lipid peroxidation and causing oxidative damage (Quiles et al., 2002; Ramirez- Tortosa, 2002). This is even more effective than alpha-tocopherol. Other than different mechanisms described earlier antioxidant effects of curcumin involve inhibition of calcium influx (Balasubramanyam et al., 2003). Since it is believed that rise in glutamate dependent intracellular calcium levels represents the final common pathway of several injurious stimuli and it is the attainment of a critical calcium concentration that is responsible for triggering the pro-oxidants and protease system that ultimately cause neuronal death, hence lowering of intracellular calcium and resultant decrease in the glutamate release would be the key mechanism to explain the neuroprotective effects of curcumin in different neurodegenerative disorders such as tardive dyskinesia. The likely mechanisms of inhibition of calcium entry by curcumin are (a) inhibition of SERCA (sarco/cendoplasmic reticulum calcium ATPase) calcium pumps (all isoforms) (Bilmen et al., 2001) (b) inhibition of inositol-triphosphate sensitive calcium channels (Dyer et al., 2002) (c) inhibition of cytosolic signals that are responsible for calcium entry into the cells (Balasubramanyam et al., 2003). Curcumin (turmeric) is a potent inhibitor of the SERCA Ca2+ pumps (all isoforms), prevent the entry of calcium within the cells and thus inhibiting Ca2+-dependent ATPase activity with IC50 values of between 7 and 15 μm. It also inhibits ATP-dependent Ca2+-uptake in a variety of microsomal membranes, although for cerebellar and platelet microsomes, a stimulation in Ca2+ uptake is observed at low curcumin concentrations (<10 μm). Hence it will prevent the increase in the intracellular free Ca2+, suggesting the neuroprotective action of curcumin (Bilmen et al., 2001). Increased expression of cyclooxygenase and lipoxygenase gene and PGE2 and LTB4 production has been implicated in neurodegeneration in several pathological setting (Wang et al., 2005). Recently it was reported that cyclooxygenase enzyme inhibitors attenuates the development of tardive dyskinesia in rats (Naidu and Kulkarni, 2001a,b). Besides this COX inhibition prevents the formation of oxidant species dopamine–quinine which has been implicated in the

Table 1
Levels of dopamine, serotonin and norepinephrine in cortical and subcortical regions of the animals treated with vehicle, haloperidol (1 mg/kg, i.p. 21 days), curcumin (50), curcumin (25)+haloperidol(1), curcumin (50)+haloperidol(1)

Treatment (mg/kg i.p.)	Dopamine (pg/mg tissue)	Serotonin (pg/mg tissue)	Norepinephrine (pg/mg tissue)	Dopamine (pg/mg tissue)	Serotonin (pg/mg tissue)	Norepinephrine (pg/mg tissue)
	Cortical			Subcortical		
Control	514±45.2	352±25.2	1348±105.4	753±45.2	607±45.2	1642±125.4
Haloperidol (1)	196±56.2[a]	175±16.2[a]	889±85.5[a]	375±56.2[a]	394±56.2[a]	886±85.5[a]
Curcumin(50)	748±84.2[b]	248±74.2[b]	1124±205.1[b]	648±76.2[b]	548±44.2[b]	1924±205.1[b]
Curcumin(25)+Haloperidol(1))	248±75.0[a]	250±35.0	1024±102.5[a,b]	405±15.0	403±15.0[a]	382±102.5[a,b]
Curcumin(50)+Haloperidol(1)	326±32.2[a,b]	311±22.2[a,b]	1301±96.5[b,c]	472±22.2[a,b]	453±22.2[a,b]	1017±96.5[a,b,c]

Total number of animals in each group is 5–6 and data is expressed in Mean±SEM.
[a] $p \leq 0.05$ as compared to control group.
[b] $p \leq 0.05$ as compared to haloperidol-treated group.
[c] $p \leq 0.05$ as compared to curcumin(25)+Haloperidol(1).

pathogenesis of tardive Dyskinesia (Teismann et al., 2003). Curcumin is a potent inhibitor of both COX (IC50 = 5–10 μM) and LOX and its protective action may be because of its COX/LOX inhibitory activity (Zhang et al., 1999). NFKappaB is stimulated by oxidative damage and contributed to neurodegeneration. As curcumin is also strong inhibitor of activation of NFKappaB this could be another possible molecular mechanism of its protective effect in tardive dyskinesia, a neurodegenerative disease (Panet et al., 2004). Besides this, curcumin has also been reported to prevent the activation of AP-1 activity, vascular endothelial growth factor mRNA up-regulation and the resultant increase in the DNA synthesis in microvascular endothelial cells (Okamoto et al., 2002).

In HPLC studies, chronic haloperidol administration resulted in a decrease in dopamine, norepinephrine and serotonin turnover in cortex as well as subcortical regions of the brain (Cahir et al., 2004; Bishnoi et al., 2007a,b). Chronic administration of haloperidol may increase the number of dormant receptors, hence resulting in decrease in dopamine turnover in extracellular spaces in the brain (Bishnoi et al., 2007a,b; Cara et al., 2001). Curcumin attenuates the decrease in dopamine release. As a consequence, the increase in the number of receptors would be less intense leading to a delayed development of supersensitivity. Curcumin dose-dependently prevented the decrease of dopamine turnover in extracellular spaces. Depletion in serotonin can be correlated with increase in glutamate neurotransmission, resulting in increased orofacial dyskinesia and oxidative damage. Curcumin dose-dependently prevented this depletion and hence limited the excitotoxicity and oxidative damage induced by depletion of serotonin in the brain. We might predict role of norepinephrine in development of tardive dyskinesia as its turnover had been also decreased significantly, which was prevented by curcumin. Increase in dopamine and norepinephrine receptor density after chronic haloperidol administration is well reported (Lau et al., 2003). Other than its antioxidant action curcumin has also a direct action on the central monoaminergic system. In several animal models such as forced swim and olfactory bulbectomy, decrease in the levels serotonin (5-HT), noradrenaline (NA), high 5-hydroxyindoleacetic acid (5-HIAA) and 4-dihydroxyphenylacetic acid (DOPAC) in the hippocampus were observed, and were completely reversed by curcumin administration. A slight decrease in 5-HT, NA and dopamine (DA) levels was found in the frontal cortex of OB rats which was also reversed by curcumin treatment (Xu et al., 2005a). In addition, the neurochemical assays showed that curcumin produced a marked increase of serotonin and noradrenaline levels at 10 mg/kg in both the frontal cortex and hippocampus. Dopamine levels were also increased in the frontal cortex and the striatum. Moreover, curcumin was found to inhibit monoamine oxidase activity in the mouse brain. These findings suggest that the neuroprotective effects of curcumin may involve the central monoaminergic neurotransmitter systems (Xu et al., 2005b).

Curcumin dose-dependently prevented dopamine supersensitivity development as suggested by behavioural experiments of stereotypy rearing, locomotor activity (rearing and ambulatory activity). Haloperidol showed initial decrease in both stereotypic as well as total locomotor activity. After first week of haloperidol treatment both the behaviors were time dependently increased showing development of dopaminergic supersensitivity. There are several research articles suggesting that development of orofacial dyskinesia (after chronic administration of haloperidol and other agents) is associated with memory and cognitive deficit in both genders (Silva et al., 2002). In several other reports it is very well established that schizophrenic patients with TD demonstrate impaired cognitive functioning. The neurochemical and structural changes underlying TD may produce specific deficits in memory for visual materials (Sorokin et al., 1988) as well as spatial working memory (Pantelis et al., 2001). Based on this we also tried to find out if there is any effect of curcumin on decrease in % retention induced by haloperidol. Curcumin dose-dependently prevented the decrease in % memory retention, induced by chronic haloperidol administration. It is quite possible that it may be because of the decrease in the locomotion (dopamine receptor blockade) but there is plethora of direct literature (Naidu et al., 2006; Naidu et al., 2004) suggesting that decrease in retention after chronic administration of haloperidol is not related to any locomotor related effects.

In present study, curcumin was able to reverse the behavioural, biochemical and neurochemical changes caused by exposure to haloperidol possibly by virtue of its antioxidant effect, inhibition of calcium entry, inhibition of COX/LOX enzymes effect. Pharmacologically, curcumin is reported as safe. A phase I human trial with 25 subjects using up to 8000 mg curcumin per day for 3 months found no toxicity. 5 other human trials using 1125–2500 mg of curcumin per day also showed no toxicity (Chainani-Wu, 2003). Based on this, curcumin should be considered for continuation of animal and possibly clinical studies as a potential therapeutic agent to prevent development of neuroleptic induced tardive dyskinesia.

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