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Attenuation of arsenic neurotoxicity by curcumin in rats

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ABSTRACT

In view of continued exposure to arsenic and associated human health risk including neurotoxicity, neuroprotective efficacy of curcumin, a polyphenolic antioxidant, has been investigated in rats. A significant decrease in locomotor activity, grip strength (26%) and rota-rod performance (82%) was observed in rats treated with arsenic (sodium arsenite, 20 mg/kg body weight, p.o., 28 days) as compared to controls. The arsenic treated rats also exhibited a decrease in the binding of striatal dopamine receptors (32%) and tyrosine hydroxylase (TH) immunoreactivity (19%) in striatum. Increased arsenic levels in corpus striatum (6.5 fold), frontal cortex (6.3 fold) and hippocampus (7.0 fold) associated with enhanced oxidative stress in these brain regions, as evident by an increase in lipid perioxidation, protein carbonyl and a decrease in the levels of glutathione and activity of superoxide dismutase, catalase and glutathione peroxidase with differential effects were observed in arsenic treated rats compared to controls. Simultaneous treatment with arsenic (sodium arsenite, 20 mg/kg body weight, p.o., 28 days) and curcumin (100 mg/kg body weight, p.o., 28 days) caused an increase in locomotor activity and grip strength and improved the rota-rod performance in comparison to arsenic treated rats. Binding of striatal dopamine receptors and TH expression increased while arsenic levels and oxidative stress decreased in these brain regions in co-treated rats as compared to those treated with arsenic alone. No significant effect on any of these parameters was observed in rats treated with curcumin (100 mg/kg body weight, p.o., 28 days) alone compared to controls. A significant protection in behavioral, neurochemical and immunohistochemical parameters in rats simultaneously treated with arsenic and curcumin suggest the neuroprotective efficacy of curcumin.

Introduction

Extensive applications of arsenic in mining, smelting and refining of certain ores have distributed it into the environment (Goyer, 1991; Polissar et al., 1990; Klaassen, 2001). Burning of coal has also contributed to disperse arsenic in the environment. High levels of arsenic in ground water have been detected in some regions in India and several other countries and thus pose health risk to humans (Das et al., 1995; NRC, 2001; Hassan et al., 2003). Exposure to arsenic in humans has also been reported through folk medicines and by consuming contaminated food material particularly sea food (ATSDR, 2005; WHO, 1992; Francesconi and Edmonds, 1987; Foa et al., 1984; Vahidnia et al., 2007). Although both organic and inorganic forms of arsenic exist in nature, humans are mainly exposed from inorganic arsenic through drinking water and occupational sources. Arsenic and its inorganic compounds have long been known to be neurotoxic (Vahidnia et al., 2007). Peripheral neuropathy following arsenic exposure is well documented (Chhuttani and Chopra, 1979; Schoolmeester and White, 1980; Brouwer et al., 1992; Heaven et al., 1994). A decrease in peripheral nerve conduction velocity has been reported following chronic exposure to arsenic dust (Blom et al., 1985; Vahidnia et al., 2007). An association between arsenic ingestion and increased risk of microvascular diseases including neurological disorders has been reported (Chiou et al., 2005). Gharibzadeh and Hoseini (2008) suggested that arsenic exposure may be a risk factor for Alzheimer's disease by inducing apoptosis in cortical neurons.

Arsenic easily crosses the blood brain barrier (Tripathi et al., 1997) and accumulates in the brain leading to neurobehavioral abnormalities (Itoh et al., 1990). Although not much information about the precise target of arsenic in brain is known, basal ganglia has been shown to be quite vulnerable (Ghafgazi et al., 1980; Rodriguez et al., 2001). Studies have been carried out in whole brain (Flora et al., 2005; Gupta and Flora, 2006) and brain regions to understand the mechanism of arsenic induced neurotoxicity (Shila et al., 2005a, 2005b, 2005c). It was observed that arsenic has marked effect on corpus striatum, cortex and hippocampus (Shila et al., 2005c). Delayed maturation of Purkinje cells and their defective migration have been reported in rats exposed to sodium arsenite during rapid

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brain growth period from postnatal days 4 to 10 (Dhar et al., 2007). Impaired learning and memory in arsenic exposed individuals and children have been reported (Danan et al., 1984; Calderon et al., 2001). Alteration in motor behavior has also been reported in arsenic exposed rats (Rodriguez et al., 2001, 2003).

A number of studies have been carried out to understand the biochemical mechanisms involved in arsenic induced neurotoxicity. Levels of dopamine, norepinephrine and serotonin have been found to be altered following arsenic exposure in experimental studies suggesting the role of biogenic amines in the neurotoxicity of arsenic (Tripathi et al., 1997; Kannan et al., 2001). Besides effect on the catacholaminergic system, enhanced oxidative stress associated with decreased antioxidant defense in the brain has been reported in arsenic neurotoxicity (Gupta et al., 2005; Shila et al., 2005a, 2005b; Flora and Gupta, 2007; Sinha et al., 2008). Arsenic enhances generation of free radicals leading to increased lipid peroxidation, protein carbonyls and decreased activity of superoxide dismutase and other enzymes involved in antioxidant defense in rat brain. Besides, arsenic has high affinity to GSH and thus enhances vulnerability towards oxidative stress by causing an imbalance between pro-oxidant and antioxidant homeostasis (Aposhian and Aposhian, 1989; Wang et al., 1996; Chen et al., 1998; Shila et al., 2005a, 2005b, 2005c). Chronic exposure to arsenic in rats was found to decrease the production of brain nitric oxide associated with an increase in the production of reactive oxygen species (Zarazua et al., 2006). In view of the continued exposure to arsenic in humans, there is a lot of interest in investigating if its neurotoxicity could be prevented.

Plant extracts and pharmacological agents have been used to investigate their neuroprotective efficacy in arsenic induced neurotoxicity with an aim to asses their antioxidant potential but with variable results (Gupta et al., 2005; Gupta and Flora, 2006; Flora and Gupta, 2007; Shila et al., 2005a; Sinha et al., 2008). Turmeric is extensively used as a spice, food preservative and coloring material in different parts of the world especially in Asian countries. It has been used as an additive by food industries in U.K. (Strimpakos and Sharma, 2008). Curcumin, present in turmeric, is an active ingredient and known to possess multiple pharmacological properties such as anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-ischemic, hypotensive and antioxidant (Arora et al., 1971; Kim et al., 1998; Polasa et al., 2004; Dikshit et al., 1995; Jones and Shoskes, 2000; Lee et al., 2005; Al-Omar et al., 2006; Aggarwal et al., 2006; Maheshwari et al., 2006; Shukla et al., 2007; Goel et al., 2008; Hatcher et al., 2008; Strimpakos and Sharma, 2008). Curcumin has been found to be effective in the treatment of Alzheimer's dementia, neuroleptic-induced tardive dyskinesia and chemical induced neurotoxicity including lead and cadmium (Garcia-Alloza et al., 2007; Bishnoi et al., 2008; Dairam et al., 2007; Shukla et al., 2003; Daniel et al., 2004). Due to high safety of curcumin in phase I trials on human volunteers, clinical trials are being done to assess its therapeutic potential in disease state (Chainani-Wu, 2003; Aggrawal and Sung, 2009). Recently, Yousef et al. (2008) observed that arsenic induced biochemical alterations in the brain and liver of rats could be protected by curcumin. Since enhanced oxidative stress has been reported to be one of the important mechanisms in arsenic neurotoxicity, the present study with curcumin has been carried out to investigate its neuroprotective efficacy because of its antioxidant potential focusing on the parameters related to oxidative stress. To further understand the potential of arsenic on dopaminergic alterations and neuroprotective efficacy of curcumin, if any, effect on dopamine receptors and related behaviors was studied. In view of the vulnerability of basal ganglia and associated risk of Alzheimer's disease to arsenic, studies were carried out on corpus striatum, a brain area controlling dopaminergic mechanisms and frontal cortex and hippocampus, functionally involved in Alzheimer's disease in the present investigation.

Materials and methods

Animals and treatment

Female rats (180 ± 20 g) of Wistar strain, obtained from the animal breeding colony of Indian Institute of Toxicology Research (IITR), Lucknow were used for the study. Rats were housed in an air conditioned room at a temperature 25 ± 2 °C with a 12-hour light/dark cycle under standard hygiene conditions and had free access to pellet diet (Ashirwad Industries, Chandigarh, India) and water. The animals were randomly divided into four groups. Rats in Group I were treated with arsenic as sodium arsenite (dissolved in distilled water, 20 mg/kg body weight, p.o., daily for 28 days). In Group II, rats were treated with curcumin 99% pure procured from Kancor, Kerala, India (suspended in 2% gum acacia, 100 mg/kg body weight, p.o., daily for 28 days). Rats in Group III were simultaneously treated with arsenic and curcumin in combination identically as in Groups I and II. In Group IV, rats were treated with 2% gum acacia dissolved in distilled water p.o. for the duration of the treatment to serve as controls.

Behavioral studies were carried out as per plan after the last dose of treatment. A set of five rats randomly selected from each treatment group was used to assess spontaneous locomotor activity 24 h after the last dose of treatment. The same set of rats was used to measure grip strength, 1 h after the spontaneous locomotor activity test. Rota-rod test was carried out in a separate set of five rats randomly selected from each treatment group. For neurochemical studies, rats were terminated by cervical decapitation around 24 h after the last dose of treatment. Brains were taken out quickly, washed in ice cold saline and dissected into regions (corpus striatum, frontal cortex and hippocampus) following the standard procedure (Glowinski and Iversen, 1966). Brain regions were processed immediately for the assay of parameters related with oxidative stress. For the assay of dopamine (DA) — D_2 receptors, corpus striatum was kept frozen at −20 °C and processed within 72 h. For TH immunohistochemistry, brains from the perfused rats from each treatment group were processed within 4–5 days following the standard protocol. Arsenic levels in the brain regions of treated rats were estimated within 6 days after the termination of treated animals. The study was approved by the Institutional Animal Ethics Committee (IAEC) of Indian Institute of Toxicology Research, Lucknow and all experiments were carried out in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Government of India), New Delhi, India.

Behavioral studies.

(i) **Spontaneous locomotor activity:** Spontaneous locomotor activity in rats was carried out using computerized Actimot (TSE, Germany) following the method as described by Ali et al. (1990). Effect on different parameters including total distance travelled, resting time, stereotypic time, time moving and rearing was studied in rats in the control and treated groups.

(ii) **Rota-rod performance:** Effect of arsenic and the protective effect of curcumin on motor in coordination were studied in rats using Rotomex (Columbus Instruments, USA) and the time of fall from the rotating rod was monitored following the standard procedure (Rogers et al., 1997).

(iii) **Grip strength:** A computerized grip strength meter (TSE, Germany) was used to measure the forelimb grip strength in the control and treated rats following the standard procedure as described by Terry et al. (2003).

Neurochemical studies. Assay of dopamine receptors and other parameters related to oxidative stress was carried out following the standard protocol to understand the protective efficacy of curcumin in arsenic induced neurotoxicity.

(i) **Dopamine receptor binding assay:** Radioligand receptor binding technique was employed to assay dopamine (DA) — D_2 receptors in crude synaptic membranes of corpus striatum following the standard procedure described in detail by Khanna et al. (1994). ^{3}H-spiperone (18.5 Ci/mmoles, 1×10^{-9} M, Perkin Elmer, USA) was used as a radioligand while haloperidol (1×10^{-6} M) as a competitor to assess the extent of non-specific binding. Specific binding has been expressed as pmoles ligand bound/g protein. Scatchard analysis was carried out at varying concentrations of ^{3}H-spiperone ($0.1–4\times10^{-9}$ M) to ascertain whether change in the binding is due to alteration in the affinity (Kd) or number of receptor binding sites (Bmax).

(ii) **Assay of lipid peroxidation:** Lipid peroxidation as a measure of malonaldehyde (MDA) formation was estimated following the method of Ohkawa et al. (1979) and the intensity of pink color formed during the reaction was read at 532 nm.

(iii) **Assay of protein carbonyl content:** Protein carbonyl content in brain regions was measured following the method of Levine et al. (1990) using 2,4-dinitrophenylhydrazine (DNPH) as a substrate.

(iv) **Assay of reduced glutathione:** Reduced glutathione (GSH) levels in brain regions were measured spectrophotometrically using 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) as the color reagent following the method of Hasan and Haider (1989). A range of glutathione (2–10 μg) was also run in parallel to plot the standard curve. The values are expressed as μg GSH/g tissue weight.

(v) **Assay of superoxide dismutase activity:** Activity of superoxide dismutase (SOD) was assayed following the method of Kakkar et al. (1984) using NADH as a substrate in the post mitochondrial fraction of different brain regions. The SOD activity has been expressed in units/min/mg protein.

(vi) **Assay of catalase activity:** Activity of catalase in brain regions was assayed following the method of Aebi (1984) spectrophotometrically in post mitochondrial fraction using hydrogen peroxide (H_2O_2) as substrate. The activity is expressed in μmole/min/mg protein.

(vii) **Assay of glutathione peroxidase activity:** Glutathione peroxidase (GPx) activity in brain regions was measured following the procedure of Flohe and Gunzler (1984).

(viii) **Protein estimation:** Protein content in samples was measured following the method of Lowry et al. (1951) using bovine serum albumin as a reference standard.

Estimation of arsenic levels in brain regions. Arsenic levels in brain regions were estimated by hydride system 60 atomic absorption spectrophotometer (HSAAS, ZEEnit 700) following the method of Ballentine and Burford (1957). A calibration curve was constructed by adding known amounts of arsenic standard (Sigma) to calculate arsenic levels in brain regions.

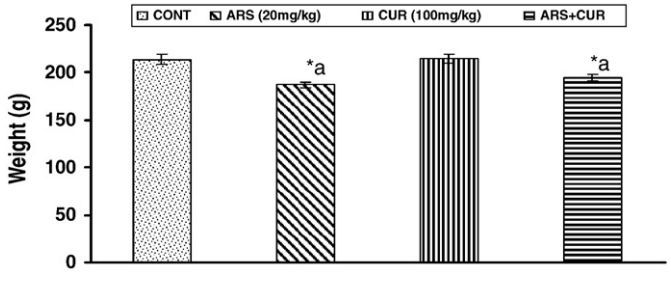

Fig. 1. Effect on body weight of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of ten animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group. *Significantly differs ($p<0.05$).

Immunohistochemical studies. Immunohistochemical studies were carried out following the method of Goslin et al. (1990). Briefly, rats were anesthetized and perfused with 150 ml of phosphate-buffered saline (PBS, 0.1 M, pH 7.4) followed by 250 ml of ice cold 4% paraformaldehyde in PBS for fixation of tissues. Brains were removed and post fixed in 10% paraformaldehyde in PBS and samples were kept in 10%, 20%, and 30% (w/v) sucrose in PBS. Serial coronal sections of 20-μm thickness were cut on a cryomicrotome (Microm HM 520, Labcon, Germany), incubated with primary (Tyrosine hydroxylase, Sigma, USA, 1:200) and secondary antibodies (biotinylated peroxidase linked, Sigma USA, 1:400) and processed as per protocol. The intensity of tyrosine hydroxylase (TH) positive neurons in striatal region of brain was determined using a computerized image analysis system (Leica Qwin 500 image analysis software) as described by Shingo et al. (2002). Computerized analysis enabled to asses the percent area of a selected field that was occupied by TH positive neurons.

Statistical analysis. Statistical analysis was carried out by one way analysis of variance (ANOVA) involving Newman–Keuls test for post-hoc comparisons. The level of significance was accepted at $p<0.05$.

Results

Effect of arsenic, curcumin and their co-treatment on body and brain weight of rats

Exposure to arsenic in rats caused a significant decrease (12%) in body weight as compared to rats in the control group (Fig. 1). No significant effect on body weight was observed in rats treated with curcumin compared to controls. Simultaneous treatment with arsenic and curcumin in rats caused a marginal change in body weight as compared to those treated with arsenic alone however, body weight in the simultaneously treated rats remained decreased as compared to controls (Fig. 1).

No significant change in brain weight was observed in rats treated with arsenic or curcumin or those treated simultaneously with arsenic and curcumin as compared to controls (Fig. 2).

Behavioral studies

(i) **Effect on spontaneous locomotor activity:** Exposure to arsenic in rats caused a decrease in total distance travelled (48%), stereotypic time (50%), time moving (43%), rearing (42%) and an increase in resting time (14%) as compared to rats in the control group (Table 1). Simultaneous treatment with arsenic and curcumin in rats increased the total distance travelled (38%), stereotypic time (17%), time moving (32%), and rearing (17%) and decreased the resting time (5%) as compared to rats treated with arsenic alone. Although distance travelled, stereotypic time and time moving increased in the co-treatment group compared with arsenic treatment alone, these parameters remained decreased as compared to controls.

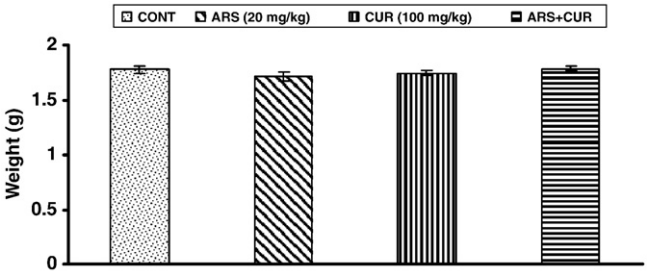

Fig. 2. Effect on brain weight of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of five animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin.

Table 1
Effect on different parameters of locomotor activity in rats following exposure to arsenic, curcumin and their co-treatment for 28 days.

Parameters	Treatment groups			
	Control	Arsenic (20 mg/kg)	Curcumin (100 mg/kg)	Curcumin + arsenic
Total distance travelled (cm)	1886 ± 119	973 ± 105[a,*]	1763 ± 131	1348 ± 98[a,b,*]
Resting time (s)	224 ± 2.96	256 ± 5.67[a,*]	224 ± 5.25	242 ± 4.04[a,*]
Stereotypic time (s)	189 ± 21.32	93 ± 8.78	173 ± 55.57	109 ± 17.11
Time moving (s)	75.8 ± 3.00	43.17 ± 5.67[a,*]	75.25 ± 5.32	57.17 ± 3.99[a,b,*]
Rearing	17.67 ± 1.94	10.17 ± 0.47[a,*]	12.83 ± 2.12	12.0 ± 2.79

Values are mean ± SE of five animals in each group.
[a] Compared to control group.
[b] Compared to arsenic treated group.
* Significantly differs ($p<0.05$).

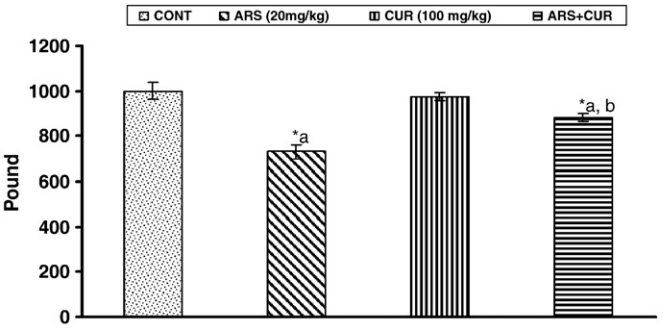

Fig. 4. Effect on fore limb grip strength of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of five animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group, b — compared to arsenic treated group. *Significantly differs ($p<0.05$).

No significant change on any of these parameters was observed in rats treated with curcumin as compared to controls (Table 1).

(ii) **Effect on rota-rod performance:** A significant impairment (82%) in motor coordination was observed in rats treated with arsenic since these rats fell quickly from the rotating rod on rota-rod performance test compared to controls (Fig. 3). Treatment with curcumin in rats had no significant effect on the time of fall from the rotating rod as compared to controls. It was interesting to note that the rats simultaneously treated with arsenic and curcumin stayed on the rotating rod for a longer period of time as compared to those treated with arsenic alone. However, these rats fell earlier from the rotating rod as compared to controls (Fig. 3).

(iii) **Effect on grip strength:** The grip strength was found to be significantly decreased in rats treated with arsenic (26%) compared to controls (Fig. 4). An improvement in grip strength (20%) was observed in rats simultaneously treated with arsenic and curcumin in comparison to those treated with arsenic alone as assessed by their potential to hold the bar (Fig. 4). No significant change on the grip strength was observed in rats treated with curcumin alone compared with control rats (Fig. 4).

Neurochemical studies

(i) **Effect on dopamine receptors in rats:** A significant decrease in the binding of ^{3}H-spiperone to striatal membranes, known to label dopamine receptors (32%) was observed in rats treated with arsenic compared to controls (Fig. 5). Scatchard analysis revealed that the decrease in the binding was due to decreased affinity as evident by increased Kd and no significant effect on the number of binding sites (Bmax) in the arsenic treated rats as compared to those in the control group (Table 2). Simultaneous treatment with arsenic and curcumin in rats caused an increase in the binding of ^{3}H-spiperone to striatal membranes (35%) associated with increased affinity as compared to those treated with arsenic alone. No significant change in the binding of dopamine receptors was observed in rats treated with curcumin alone as compared to controls (Fig. 5).

(ii) **Effect of arsenic and curcumin on lipid peroxidation and protein carbonyl content in rat brain:** A significant increase in malonaldehyde (MDA) levels in frontal cortex (43%), corpus striatum (61%) and hippocampus (50%) was observed in rats following exposure to arsenic as compared to control (Fig. 6). Rats exposed to arsenic also exhibited increased level of protein carbonyl content in frontal cortex (18%), corpus striatum (68%) and hippocampus (58%) in comparison to control (Fig. 7). Simultaneous treatment with curcumin and arsenic in rats decreased the MDA levels in frontal cortex (36%), corpus striatum (28%) and hippocampus (4%) in comparison to rats treated with arsenic alone (Fig. 6). Levels of protein carbonyl in frontal cortex (22%), corpus striatum (20%) and hippocampus (9%) were also decreased in rats treated with arsenic and curcumin simultaneously as compared to those treated with arsenic alone (Fig. 7). No significant change both in the MDA levels and protein carbonyl content was observed in any of the brain regions in rats treated with curcumin as compared to rats in the control group (Figs. 6, 7).

(iii) **Effect on reduced glutathione levels:** A significant decrease in reduced glutathione (GSH) levels in frontal cortex (30%), corpus striatum (28%) and hippocampus (27%) was observed in arsenic treated rats as compared to those in the control group (Fig. 8). Interestingly, co-treatment with curcumin and arsenic in rats caused an increase in the GSH levels in frontal cortex (48%), corpus striatum (20%) and hippocampus (29%) as compared to rats treated with arsenic alone. No significant change in the GSH

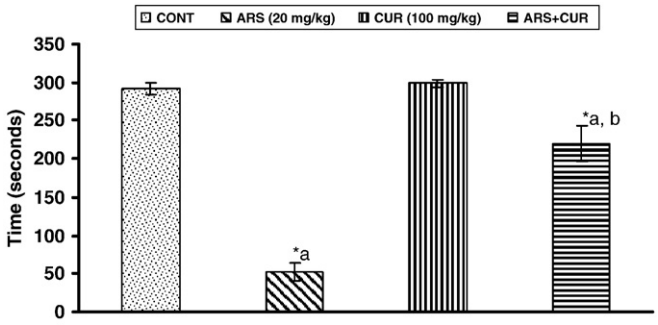

Fig. 3. Effect on rota-rod performance of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of five animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group, b — compared to arsenic treated group. *Significantly differs ($p<0.05$).

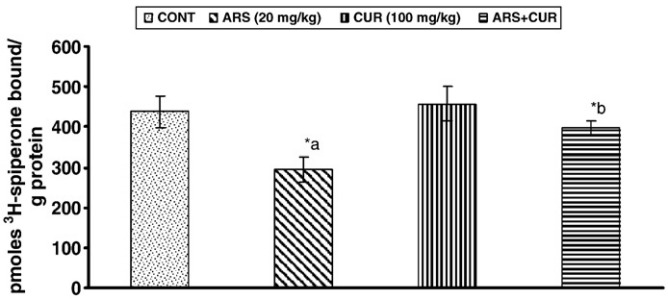

Fig. 5. Effect on ^{3}H-spiperone binding to striatal membrane of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of five animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group, b — compared to arsenic treated group. *Significantly differs ($p<0.05$).

Table 2
Scatchard analysis of ^{3}H-spiperone binding to striatal membranes of rats following exposure to arsenic, curcumin and their co-treatment for 28 days.

Parameters	Treatment groups			
	Control	Arsenic (20 mg/kg)	Curcumin (100 mg/kg)	Curcumin + arsenic
Kd	1.39 ± 0.11	2.41 ± 0.16[a,*]	1.31 ± 0.12	1.81 ± 0.12[a,b,*]
Bmax	911 ± 69	752 ± 51	891 ± 54	835 ± 72

Values are mean ± SE of five animals in each group. Kd — dissociation constant expressed in nM. Bmax — maximum number of binding sites expressed in pmoles bound/g protein.
[a] Compared to control group.
[b] Compared to arsenic treated group.
* Significantly differs ($p<0.05$).

levels was observed in any of the brain regions of rats treated with curcumin as compared to control rats (Fig. 8).

(iv) **Effect on superoxide dismutase and catalase activities:** A decrease in the activity of superoxide dismutase (SOD) in frontal cortex (45%), corpus striatum (29%) and hippocampus (9%) was observed in arsenic treated rats as compared to controls (Fig. 9). The arsenic treated rats also showed a decrease in the activity of catalase in frontal cortex (46%), corpus striatum (43%) and hippocampus (31%) as compared to rats in the control group (Fig. 10). Simultaneous treatment with arsenic and curcumin caused an increase in the activity of SOD in the frontal cortex (73%), corpus striatum (51%) and hippocampus (14%) as compared to rats treated with arsenic alone (Fig. 9). Activity of catalase in frontal cortex (38%), corpus striatum (83%) and hippocampus (27%) was also found to be increased in rats simultaneously treated with arsenic and curcumin as compared to those treated with arsenic alone (Fig. 10). No significant change in the activity of SOD and catalase was observed in any of the brain regions of the rats treated with curcumin as compared to controls (Figs. 9, 10).

(v) **Effect on glutathione peroxidase activity.** A significant decrease in glutathione peroxidase (GPx) activity in frontal cortex (17%), corpus striatum (17%) and hippocampus (15%) was observed in arsenic treated rats compared to those in the control group (Fig. 11). Interestingly, co-treatment with curcumin and arsenic in rats caused an increase in the activity of glutathione peroxidase in frontal cortex (10%), corpus striatum (9%) and hippocampus (23%) as compared to rats treated with arsenic alone (Fig. 11). No significant change in the GPx activity was observed in any of the brain regions of rats treated with curcumin as compared to controls (Fig. 11).

Arsenic levels

Effect on arsenic levels in brain regions: A significant increase in the levels of arsenic in frontal cortex (6.3 fold), corpus striatum (6.5 fold) and hippocampus (7.0 fold) was observed in rats exposed to arsenic for 28 days as compared to controls (Table 3). Levels of arsenic were found to be significantly decreased in frontal cortex (2.3 fold), corpus striatum (1.2 fold) and hippocampus (1.6 fold) in rats simultaneously treated with arsenic and curcumin as compared to rats treated with arsenic alone. No significant change in arsenic levels in any of the brain regions was observed in rats treated with curcumin in comparison to controls (Table 3).

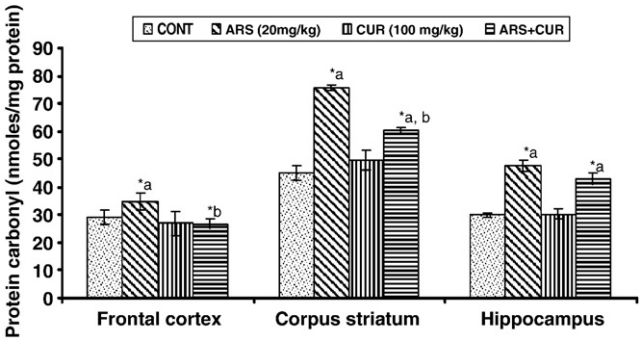

Fig. 7. Effect on protein carbonyl content in different brain regions of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of five animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group, b — compared to arsenic treated group. *Significantly differs ($p<0.05$).

Immunohistochemical studies

Effect on tyrosine hydroxylase (TH) Immunoreactivity: Quantitation of immunoreactivity (IR) using image analysis exhibited decreased TH expression in striatal sections in arsenic treated rats (Fig. 12). A 19% decrease in percent area was observed in these rats in comparison to controls. Interestingly, TH immunoreactivity was found to be increased (14%) in rats simultaneously treated with curcumin and arsenic in comparison to rats treated with arsenic alone suggesting a trend of recovery. No significant change in TH immunoreactivity was observed in rats treated with curcumin as compared to controls (Fig. 12).

Discussion

Enhanced oxidative stress has been suggested to be an important mechanism in arsenic induced neurotoxicity. Arsenic exposure has been found to cause oxidative damage to the biological system by enhancing generation of free radical species (Yamanaka et al., 1991, 1997; Flora et al., 2005) which in turn may be responsible for increased lipid peroxidation, protein carbonyl and decreased GSH levels (Flora et al., 2005, Shila et al., 2005a, 2005b, 2005c). Glutathione is an important biomolecule involved in the defense against toxicants (Nordmann 1994). The decrease in GSH levels

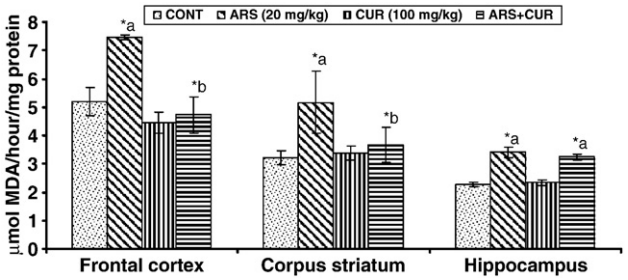

Fig. 6. Effect on lipid peroxidation in different brain regions of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of five animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group, b — compared to arsenic treated group. *Significantly differs ($p<0.05$).

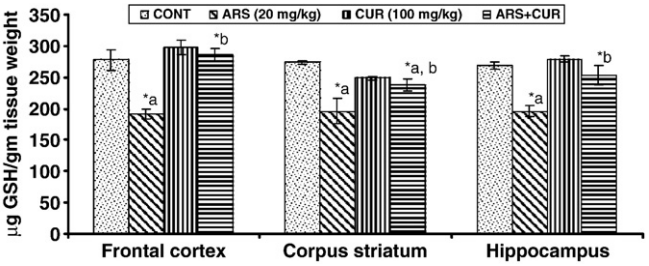

Fig. 8. Effect on reduced glutathione in different brain regions of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of five animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group, b — compared to arsenic treated group. *Significantly differs ($p<0.05$).

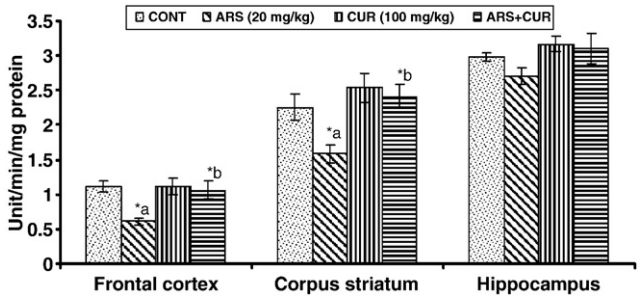

Fig. 9. Effect on superoxide dismutase activity in post mitochondrial fraction of different brain regions of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of five animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group, b — compared to arsenic treated group. *Significantly differs ($p<0.05$).

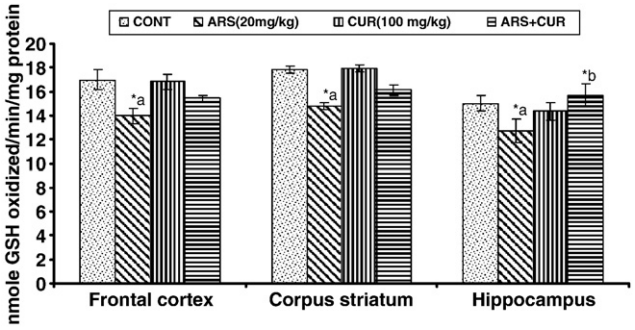

Fig. 11. Effect on glutathione peroxidase activity in different brain regions of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of five animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group, b — compared to arsenic treated group. *Significantly differs ($p<0.05$).

following arsenic exposure has been associated with high affinity of arsenic with GSH (Aposhian and Aposhian, 1989). Decreased GSH levels in the brain regions following arsenic exposure could also be due to its increased utilization by toxic radicals and intermediates formed. A decrease in the activity of superoxide dismutase, catalase and GPx in the brain has also been observed in arsenic exposed rats (Gupta et al., 2005; Gupta and Flora, 2006; Flora and Gupta, 2007; Shila et al., 2005a). In the present study, an increase in MDA and protein carbonyl levels was observed in frontal cortex, corpus striatum and hippocampus regions of the brain in arsenic treated rats. Also, a decrease in glutathione levels and in the activity of superoxide dismutase, catalase and glutathione peroxidase observed in these brain regions is consistent with earlier reports and suggests enhanced oxidative stress following arsenic exposure in these rats. Although basal ganglia has been suggested to be a target of arsenic (Ghafgazi et al., 1980), the metalloid is distributed into the brain regions after crossing the blood brain barrier. Shila et al. (2005c) found that arsenic exposure in rats has pronounced effect on striatum, cortex and hippocampus. Recently, arsenic exposure has been reported to be a risk factor for Alzheimer's disease (Gharibzadeh and Hoseini, 2008). In view of these facts, studies were focused on corpus striatum, frontal cortex and hippocampus regions of brain.

Oxidative effects of arsenic have been found to be counteracted by plant extracts and pharmacological agents due to their antioxidant potential (Gupta et al., 2005; Gupta and Flora, 2006; Flora and Gupta, 2007; Shila et al., 2005a; Sinha et al., 2008). Due to multiple pharmacological properties including strong antioxidant effect, curcumin has been suggested to be a good neuroprotectant. The antioxidant property of curcumin is largely associated with its classical structure containing phenolic and methoxy group on the phenyl ring and 1,3-diketone in its structure (Kapoor and Priyadarsini, 2001) and potential to remove hydroxyl radical, singlet oxygen and reactive nitrogen species (Reddy and Lokesh 1994b, Rao et al., 1995; Unnikrishann and Rao, 1995; Sreejayan and Rao, 1997) and by inhibiting the generation of superoxide radicals (Ruby et al., 1995; Sreejayan and Rao, 1996; Ohara et al., 2005; Priyadarsini et al., 2003). Curcumin has been shown to inhibit lipid peroxidation in rat liver, erythrocytes and the brain (Reddy and Lokesh, 1994a, Rajeswari, 2006) and maintain the activity of antioxidant enzymes and therefore effectively modulates the redox reactions in the biological system (Rajeswari, 2006). Zhao et al. (1989) found that curcumin is several times more potent than vitamin E as a free radical scavenger.

The classical finding that curcumin crosses the blood brain barrier (Yang et al., 2005) provided further support to its neuroprotective potential in preclinical models of neurodegenerative disorders including Parkinson's and Alzheimer's diseases (Lim et al., 2001; Cole et al., 2004; Strimpakos and Sharma, 2008). Garcia-Alloza et al. (2007) observed that curcumin crosses blood brain barrier and labels senile plaques and cerebrovascular amyloid angiopathy (CAA) and can prevent and reduce amyloid deposition *in vivo*. Curcumin treatment was able to reverse neuroleptic-induced tardive dyskinesia in rats (Bishnoi et al., 2008). Curcumin also exhibited significant neuroprotection in animal model of focal cerebral ischemia by inhibiting lipid peroxidation and modulating antioxidant defense enzymes (Thiyagarajan and Sharma, 2004; Shukla et al., 2007). It was also found to be effective in reducing the peroxynitrite formation in cerebral ischemia (Thiyagarajan and Sharma, 2004).

Neuroprotective potential of curcumin in neurotoxicological manifestations has also been investigated. Interestingly, neurotoxic effects of lead and cadmium have been found to be restored following curcumin treatment (Shukla et al., 2003; Daniel et al., 2004; Dairam et al., 2007). The studies suggested that curcumin besides having strong antioxidant property also has metal binding property that may possibly reduce the neurotoxicity of lead (Shukla et al., 2003; Daniel et al., 2004). Dairam et al. (2008) recently demonstrated antioxidant and iron binding properties of spice ingredients including curcumin and linked it with the prevention and treatment of Alzheimer's

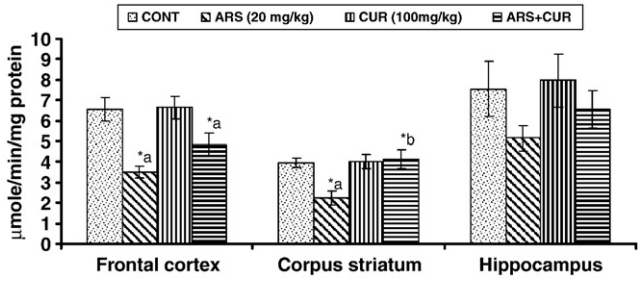

Fig. 10. Effect on catalase activity in different brain regions of rats following exposure to arsenic, curcumin and their co-treatment for 28 days. Values are mean ± SE of five animals in each group. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group, b — compared to arsenic treated group. *Significantly differs ($p<0.05$).

Table 3
Effect on arsenic levels in different brain regions of rats following exposure to arsenic, curcumin and their co-treatment for 28 days.

Brain regions	Treatment groups			
	Control	Arsenic (20 mg/kg)	Curcumin (100 mg/kg)	Curcumin + arsenic
Frontal cortex	499 ± 47.88	3185 ± 210.5[a,*]	480 ± 56.86	1331 ± 194.6[a,b,*]
Corpus striatum	492 ± 31.02	3235 ± 162.6[a,*]	447 ± 48.47	2645 ± 143.8[a,b,*]
Hippocampus	530 ± 80.09	3745 ± 383.4[a,*]	542 ± 78.93	2230 ± 392.8[a,b,*]

Values are mean ± SE of five animals in each group.
Values are expressed as ng/g tissue weight.
[a] Compared to control group.
[b] Compared to arsenic treated group.
[*] Significantly differs ($p<0.05$).

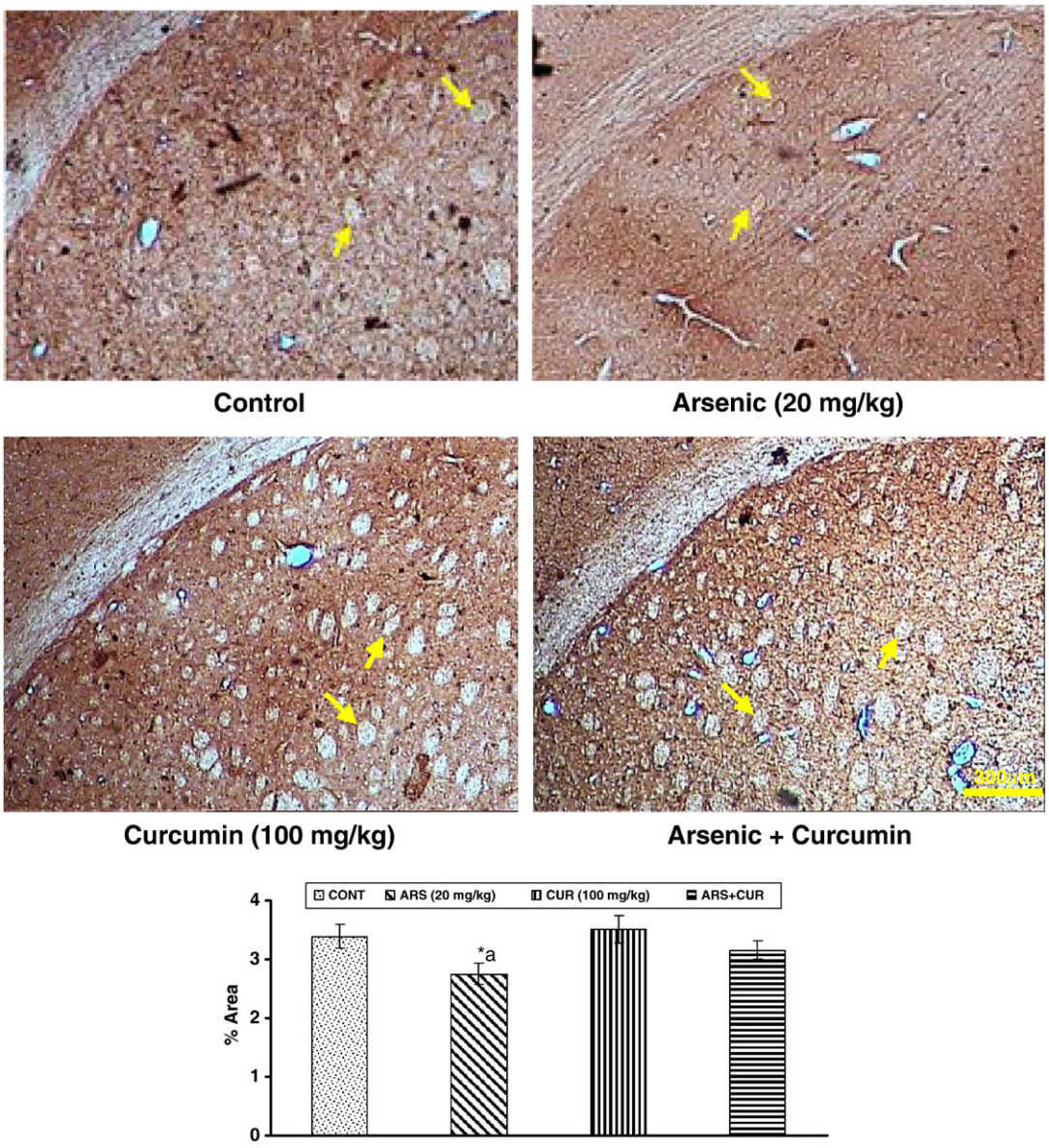

Fig. 12. Photomicrographs of rat striatal sections illustrating tyrosine hydroxylase (TH) immunoreactivity following exposure to arsenic, curcumin and their co-treatment for 28 days. Exposed rats showed diminished TH-expression as compared to control group. Arrow indicates immunoreactivity for TH. Scale bar = 300 μm. CONT — Control, ARS — Arsenic, CUR — Curcumin, a — compared to control group. *Significantly differs ($p<0.05$).

disease. It is of interest to note that Yousef et al. (2008) found that simultaneous treatment with sodium arsenite and curcumin in rats could ameliorate sodium arsenite induced decrease in brain acetylcholinesterase activity and liver transaminases and phosphatases. The dose of arsenic exposure (5 mg/kg body weight, p.o., 30 days) in the study by Yousef et al. (2008) was low as compared to that used by us (20 mg/kg body weight) in the present study. A number of studies have been carried out using low to high doses of arsenic that range from 20 to 100 ppm (Flora and Gupta, 2007; Shila et al., 2005a, 2005b; Gupta et al., 2005) and 5 to 20 mg/kg body weight (Schulz et al., 2002; Sinha et al., 2008; Rodriguez et al., 2001) involving different exposure routes including oral, i.p. and through drinking water. Most of the studies have been carried out at 100 ppm dose of arsenic in rats exposed through drinking water to understand the mechanism of neurotoxicity (Shila et al., 2005a, 2005b; Gupta et al., 2005). This dose is nearly equivalent to the dose of arsenic used in the present study (20 mg/kg body weight). Further, to avoid variation in arsenic exposure through drinking water, rats were administered arsenic orally in the present study. The dose of curcumin used in the present study by us (100 mg/kg body weight, p.o., 28 days) is high as compared to that (15 mg/kg body weight, p.o., 30 days) used by Yousef et al. (2008). Neuroprotective efficacy of curcumin has been studied at different doses (40 to 200 mg/kg body weight, p.o.) in animal models of disease conditions and neurotoxicity (Jagatha et al., 2008; Al-Omar et al., 2006; Rajeswari, 2006). Duration and route of exposure vary with the experimental design in these studies. Preliminary dose range studies were carried out with curcumin (20–200 mg/kg body weight, p.o.) earlier in our laboratory. The dose (100 mg/kg, p.o.) of curcumin selected in the present study is based on our published data and was found effective in preventing lead induced neurotoxicity (Shukla et al., 2003).

In the present study, treatment with curcumin had no significant effect on lipid peroxidation, protein carbonyl and glutathione levels in any of the brain regions. Also, no effect on the activity of superoxide dismutase, catalase and glutathione peroxidase was observed in these rats in comparison to controls. Interestingly, increased lipid peroxidation and protein carbonyl in different brain regions following arsenic exposure were decreased in rats co-treated with arsenic and

curcumin. Levels of GSH and activity of superoxide dismutase, catalase and glutathione peroxidase, involved in the antioxidant defense were found increased with differential effects in rats co-treated with arsenic and curcumin as compared to those treated with arsenic alone. Such a protective effect of curcumin may be attributed to its potential to neutralize/counteract free radicals (Bishnoi et al., 2008) and may have prevented enhanced oxidative stress. Further, enhanced arsenic levels in corpus striatum, frontal cortex and hippocampus could be correlated with sodium arsenite treatment in these rats. A decrease in arsenic levels in these brain regions in rats simultaneously treated with curcumin and sodium arsenite could be attributed to the metal binding/chelating property of curcumin (Daniel et al., 2004) that may have possibly decreased the load of arsenic.

A decrease in body weight has been reported in rats following exposure to arsenic at different doses and time (Shila et al., 2005b; García-Chávez et al., 2006; Schulz et al., 2002; Dhar et al., 2005). The decreased body weight indicates the general toxic effect of the chemical and has been associated with decreased food consumption and water intake (García-Chávez et al., 2006). A decrease in body weight in arsenic exposed rats as observed in the present study is consistent with these reports. No significant effect on body weight was observed in rats treated with curcumin alone suggesting its non toxic effect. In the present study, a marginal change in body weight was observed in rats simultaneously treated with arsenic and curcumin in comparison to those treated with arsenic alone. The body weight in these rats however remained decreased as compared to controls. Although the brain is a soft target of environmental chemicals, no significant effect on brain weight was observed in rats treated with arsenic or curcumin or those treated simultaneously with arsenic and curcumin as compared to controls in the present investigation.

Exposure to arsenic in humans has been reported to cause both central and peripheral neuropathy (Vahidnia et al., 2007). Alterations in motor behavior, impaired learning and concentration are common CNS manifestations of arsenic exposure in humans (Bolla-Wilson and Bleecker, 1987). Both an increase and decrease in motor activity have been observed following arsenic exposure in rats and mice (Itoh et al., 1990, Rodriguez et al., 2001). It was suggested that dose of arsenic and duration of treatment are important determinants in altered response in locomotor activity (Rodriguez et al., 2003). A decrease in locomotor activity observed in the present study is consistent with earlier reports (Rodriguez et al., 2001, Chattopadhyay et al., 2002). Rota-rod is a preferred test to evaluate the performance of motor coordination in experimental studies. Exposure to arsenic also affected the rota-rod performance of rats as they fell early suggesting impaired motor coordination. Interestingly, treatment with curcumin in arsenic treated rats could protect the arsenic induced alterations in motor activity as reflected by changes in distance travelled, resting time, time moving and rota-rod performance.

As neurotransmitters play an important role in modulating an array of behavior and signaling cascade, a number of experimental studies have been carried out to investigate the effect of arsenic on their levels and metabolites (Rodriguez et al., 2003). Alterations in dopaminergic, cholinergic, serotonergic and glutamatergic systems have been reported in rats and mice exposed to arsenic (Tripathi et al., 1997, Kannan et al., 2001; Valkonen et al., 1983; Nagaraja and Desiraju, 1994; Nagaraja and Desiraju, 1993; Itoh et al., 1990). Consistent changes in the levels of brain neurotransmitters have not been observed due to variations in the dose of arsenic used, duration and route of exposure. However, a decrease in dopamine levels in striatum and whole brain has been reported largely following prolonged exposure to arsenic (Kannan et al., 2001; Nagaraja and Desiraju, 1993). In an interesting study, decreased release of dopamine and its metabolite in striatum was observed in rats exposed to mining waste containing high contents of arsenic and other metals (lead, manganese, and copper) (Rodriguez et al., 1998). In the present study, decreased dopamine receptors in striatum as reflected by a decrease in the binding of ^{3}H-spiperone to striatal membranes, suggest alterations in dopaminergic system. Treatment with curcumin had no significant effect on striatal dopamine receptors, locomotor activity and rota-rod performance in rats. However, binding of dopamine receptors in the striatum increased in rats simultaneously treated with arsenic and curcumin as compared to those treated with arsenic alone. More interestingly, the decrease in locomotor behavior and impaired motor coordination in arsenic treated rats and recovery of these parameters in rats simultaneously treated with arsenic and curcumin could be correlated with alterations in dopamine receptors. A decrease in the expression of tyrosine hydroxylase (TH) immunoreactivity in striatum in arsenic exposed rats and its protection in those simultaneously treated with arsenic and curcumin suggests specificity of changes in dopaminergic system. At present it is difficult to explain the neuroprotective efficacy of curcumin in arsenic induced dopaminergic alterations as observed in the present study. As curcumin has metal binding property, simultaneous treatment with arsenic and curcumin may decrease the load of arsenic in striatum and other brain regions and associated with protective effect. Although the results of the present study exhibit neuroprotective efficacy of curcumin, further studies are required to understand the complete mechanism of neuroprotection.

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