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Arsenic toxicity induced endothelial dysfunction and dementia: Pharmacological interdiction by histone deacetylase and inducible nitric oxide synthase inhibitors

Bhupesh Sharma *, P.M. Sharma

Department of Pharmacology and Toxicology, School of Pharmacy, Bharat Institute of Technology, Partapur Bypass, Meerut, Uttar Pradesh, India

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ABSTRACT

Arsenic toxicity has been reported to damage all the major organs including the brain and vasculature. Dementia including Alzheimer's disease (AD) and vascular dementia (VaD) are posing greater risk to the world population as it is now increasing at a faster rate. We have investigated the role of sodium butyrate, a selective histone deacetylase (HDAC) inhibitor and aminoguanidine, a selective inducible nitric oxide synthase (iNOS) inhibitor in pharmacological interdiction of arsenic toxicity induced vascular endothelial dysfunction and dementia in rats. Arsenic toxicity was done by administering arsenic drinking water to rats. Morris water-maze (MWM) test was used for assessment of learning and memory. Endothelial function was assessed using student physiograph. Oxidative stress (aortic superoxide anion, serum and brain thiobarbituric acid reactive species, brain glutathione) and nitric oxide levels (serum nitrite/nitrate) were also measured. Arsenic treated rats have shown impairment of endothelial function, learning and memory, reduction in serum nitrite/nitrate & brain GSH levels along with increase in serum & brain TBARS. Sodium butyrate as well as aminoguanidine significantly convalesce arsenic induced impairment of learning, memory, endothelial function, and alterations in various biochemical parameters. It may be concluded that arsenic induces endothelial dysfunction and dementia, whereas, sodium butyrate, a HDAC inhibitor as well as aminoguanidine, a selective iNOS inhibitor may be considered as potential agents for the management of arsenic induced endothelial dysfunction and dementia.

Introduction

Arsenic is a naturally occurring toxic metalloid affecting millions of people worldwide (Pastoret et al., 2012). Exposure to arsenic is associated with an increased risk of several cancers, cardiovascular disease, and childhood neuro-developmental deficits. Arsenic is a known neurotoxin that has both neuro-developmental and neuro-cognitive consequences. Exposure to arsenic from drinking water has impact on mental abilities during the aging process and arsenic has been found significantly correlated to poorer scores in global cognition, processing speed and immediate memory (O'Bryant et al., 2011). The finding of a correlation between arsenic and the domains of executive functioning and memory is of critical importance as these are cognitive domains that reflect the earliest manifestations of dementia (O'Bryant et al., 2011). Prior research has shown that arsenic exposure induces changes that coincide with most of the developmental, biochemical, pathological, and clinical features of Alzheimer's disease (AD), VaD and associated disorders (Gong and O'Bryant, 2010).

In the last few decades the world has seen a tremendous shift in the medical needs of people. There has been a significant increase in average lifespan expectancy in the world, which will become a significant burden within the next two decades. The 80 + generation will increase by at least 30% in the next one decade and by at least 50% in the next two decades. One unambiguous clinical implication of getting older is the risk of experiencing age-related diseases like dementia (Wehling and Groth, 2011). In the recent times, we have reported induction of VaD in rats with the help of experimental hypertension, diabetes, hyperhomocysteinemia and hyperlipidemia (Sharma and Singh, 2010, 2011a,b,2012a,b,c,d). Presence of arsenic in drinking water is one of the major problems of the modern world and as we are working in the area of dementia (including AD & VaD), this research work has given us an important opportunity to work on another hypothesis which may involve induction of endothelial dysfunction and dementia (possibly VaD) due to arsenic.

HDAC inhibitors have been reported to improve Parkinson's, AD, and Huntington's diseases (Chuang et al., 2009). Arsenic has been reported to alter histone acetylation. Increased global histone acetylation related to decreased histone deacetylase activity in arsenic exposure has been reported (Ramirez et al., 2008). HDAC inhibition has been reported to exert neuroprotective effects on both in-vivo and in-vitro models of brain disorders (Chuang et al., 2009). Further it has been observed

* Corresponding author at: Department of Pharmacology and Toxicology, School of Pharmacy, Bharat Institute of Technology, Bypass Road, Partapur, Meerut 250103, Uttar Pardesh, India. Fax: +91 995 8219190.
E-mail address: drbhupeshresearch@gmail.com (B. Sharma).

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that, treatment with HDAC inhibitors markedly improved associative and spatial learning (Fischer et al., 2007) and memory (Ricobaraza et al., 2009) including contextual memory deficits (Kilgore et al., 2010). We have recently reported that sodium butyrate attenuates diabetes induced VaD (Sharma and Singh, 2011a). But the effect of HDAC inhibitor in arsenic induced endothelial dysfunction and dementia (possibly VaD) is still unknown.

Nitric oxide (NO) is an important regulatory molecule for the host defense that plays a fundamental role in the cardiovascular, immune, and nervous systems. NO is synthesized by the enzyme NO synthase (NOS), inducible nitric oxide synthase (iNOS) plays an important role in neuro-inflammation by generating high levels of brain nitric oxide (NO), a critical signaling and redox factor in the brain (Cai et al., 2008). Further many studies have implicated that up-regulation and over expression of iNOS is the most important aspect in dementia (Janda et al., 2011). We have recently reported the utility of aminoguanidine, a selective iNOS inhibitor in experimental hypertension induced VaD (Sharma and Singh, 2012d). Therefore, inhibitors of iNOS deserve investigation for their potential in arsenic induced endothelial dysfunction and dementia (possibly VaD).

Chelation therapy with chelating agents like dimercaprol (British anti-Lewisite — BAL), sodium 2,3-dimercaptopropane 1-sulfonate (DMPS), meso 2,3 dimercaptosuccinic acid (DMSA) etc., is considered to be the best known treatment against arsenic poisoning (Flora et al., 2007). Morse et al., (2004) have shown that dimercaprol suppressed amyloid precursor protein holoprotein levels and reduced amyloid beta secretion.

In the light of the above the present study has been undertaken to investigate the potential of sodium butyrate, a HDAC inhibitor as well as aminoguanidine, an iNOS in arsenic induced endothelial dysfunction and dementia in rats. Dimercaprol has been used as a positive control, as history suggests its beneficial role in the conditions occurred due to arsenic toxicity.

Material and methods

Animals

Albino Wistar rats, weighing 200–250 g were employed in the present study and were housed in animal house with free access to water and standard laboratory pellet chow diet. The animals were exposed to natural light and dark cycle. The experiments were conducted between 9.00 and 18.00 h in a semi sound-proof laboratory. The animals were acclimatized to the laboratory condition five days prior to behavioral study. The protocol of the study was duly approved by Institutional Animal Ethics Committee (IAEC) and care of the animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

Drugs and reagents

All the drug solutions were freshly prepared before use. Sodium butyrate, aminoguanidine and 1,1,3,3 tetra methoxy propane were purchased from Sigma Aldrich, USA. Sodium arsenite and thiobarbituric acid were purchased from Loba Chemie, Mumbai, India. 5,5′-dithiobis (2-nitro benzoic acid) — DTNB, bovine serum albumin (BSA), glutathione reduced (GSH) standard and nitroblue tetrazolium (NBT) were purchased from Sisco Rresearch Laboratories Pvt Ltd., Mumbai, India. Dimercaprol was obtained from Abott, India. Sodium butyrate and aminoguanidine were dissolved in saline and administered intraperitoneally.

Arsenic toxicity induced endothelial dysfunction and dementia

To induce arsenic toxicity, the drinking water of the animals was replaced with sodium arsenite 100 ppm or 100 mg/L solution (equivalent to 57.3 ppm or 57.3 mg/L of arsenic). We have used the administration of arsenic through drinking water (for 60 days) as our aim was to mimic the natural conditions of arsenic exposure and its possible outcome on the vascular and neuronal health. Presence of arsenic above the permissible limits, in the drinking water in almost every part of the world, is imposing a great risk to the human health and thus we have used the exposure of arsenic to the animals through drinking water. Further, administration of sodium arsenite 100 ppm or 100 mg/L solution (equivalent to 57.3 ppm or 57.3 mg/L of arsenic) in drinking water has previously been used for induction of arsenic toxicity in animals (Bharti et al., 2012).

Drugs treatment

We have used 60 days protocol for the administration of sodium arsenite to induce endothelial dysfunction and dementia. We have started the drug treatment (sodium butyrate, aminoguanidine & dimercaprol) to arsenic administered animals from 21st day onward because our preliminary findings and previously published reports (Kaur et al., 2010) have suggested that arsenic induces endothelial dysfunction in 15–20 days. Thus, we have first allowed endothelial dysfunction to occur in initial 20 days, which was then followed by the drug administration. Further, it has been reported that arsenic toxicity requires at least 30–40 days, to induce dementia in rats (Yadav et al., 2011). Due to this reason we have continued the treatment of the drugs for the next 40 days, till the end of study (60 days of arsenic treatment).

Assessment of learning and memory by Morris water maze

Morris water maze (Sharma and Singh, 2010, 2011a, 2012a; Sharma et al., 2008a) is one of the most commonly used animal models to test memory. The MWM procedure was based on that principle, where the animal was placed in a large pool of water, as the animal dislike swimming, their tendency was to escape from the water by finding an escape platform. MWM consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 °C). The water was made opaque with white colored dye. A submerged platform (10 cm^2), painted white was placed inside the target quadrants of this pool, 1 cm below surface of water. Escape latency time (ELT) to locate the hidden platform in water maze was noted as index of acquisition or learning. The animal was subjected to acquisition trials for four consecutive days. On the fifth day, the mean time spent by the animal in target quadrant searching for the hidden platform is noted as index of retrieval.

Assessment of vascular endothelial function using isolated rat aortic ring preparation

Thoracic aorta was removed (as per the procedure defined above in Collection of sample), cut into a ring of 4 to 5 mm width, and mounted in organ bath containing Krebs–Henseleit bubbled with carbonated oxygen (95% O_2:5% CO_2), and maintained at 37.8 °C. The preparation was allowed to equilibrate for 90 min under 1.5 g tension. The isometric contractions were recorded with a force-displacement transducer (Ft-2147) connected to Physiograph (INCO, Ambala, India). The preparation was primed with 80 mmol L^{-1} KCl to check its functional integrity and to improve its contractility. The cumulative dose responses of acetylcholine (ACh; 10^{-8} to 10^{-4} mol L^{-1}) or sodium nitroprusside (SNP; 10^{-8} to 10^{-4} mol L^{-1}) were recorded in phenylephrine (3×10^{-6} mol L^{-1}) precontracted preparations (Sharma and Singh, 2010, 2011b, 2012b). The intimal layer of aortic ring was rubbed gently with a moistened filter paper for 30 s to obtain endothelium-free preparations. Loss of ACh (1×10^{-6} mol L^{-1}) induced relaxation confirmed the absence of vascular endothelium (Sharma and Singh, 2010, 2011b, 2012c).

Biochemical parameters

Collection of sample. Blood samples for biochemical estimation were collected by retro-orbital bleeding. The blood was kept at room temperature for 30 min and then centrifuged at 4000 rpm for 15 min to separate serum which was then used for biochemical estimation.

After retro-orbital bleeding, animals were euthanize by cervical dislocation; thoracic aorta and brain tissue were carefully removed. Thoracic aorta was used for endothelium dependent and independent relaxation, as well as for the estimation of superoxide anion, whereas the brains were subjected to various biochemical estimations (brain TBARS, GSH, AChE and proteins). The removed brains were homogenized in phosphate buffer (pH 7.4, 10% w/v) using Teflon homogenizer and centrifuged at 3000 rpm for 15 min to obtain the clear supernatant. This clear supernatant containing TBARS, GSH, AChE and proteins, was removed carefully from the centrifugation tube and it was then used for different biochemical estimations.

Estimation of serum nitrite/nitrate concentration. Serum nitrite concentration was measured spectrophotometerically (DU 640B Spectrophotometer, Beckman Coulter Inc., CA, USA) at 545 nm. Briefly, 400 μL of carbonate buffer (pH 9.0) was added to 100 μL of serum or standards sample followed by addition of small amount (0.15 g) of copper–cadmium alloy. The tubes were incubated at room temperature for 1 h to reduce nitrate to nitrite. The reaction was stopped by adding 100 μL of 0.35 M sodium hydroxide. Following this, 400 μL of zinc sulfate solution (120 mM) was added to deproteinate the serum samples. The samples were allowed to stand for 10 min and then centrifuged at 4000 g for 10 min. Greiss reagent (250 μL of 1.0% sulphanilamide prepared in 3 N HCl and 250 μL of 0.1% N-naphthylethylenediamine) (prepared in water) was added to aliquots (500 μL) of clear supernatant and serum nitrite was measured spectrophotometerically at 545 nm. The standard curve of sodium nitrite (5 to 50 μM) was plotted to calculate concentration of serum nitrite (Sharma and Singh, 2011a, 2012d; Sharma et al., 2008a).

Estimation of aortic production of super oxide anion. The superoxide anion was determined spectrophotometerically at 540 nm. Briefly, the aorta was cut into transverse rings l0 mm in length and placed in 5 mL buffer at 37 °C containing 100 mM L^{-1} of nitroblue tetrazolium (NBT) for 1.5 h NBT reduction was stopped by addition of 5 mL of 0.5 mol L^{-1} HCl. The rings were minced and homogenized in a mixture of 0.1 M L^{-1} NaOH and 0.1% SDS in water containing 40 mg L^{-1} diethylentriaminepentaacetic acid. The mixture was centrifuged at 20,000 *g* for 20 min, the resultant pellet was resuspended in 1.5 mL of pyridine and kept at 80 °C for 1.5 h to extract formazon. The mixture was centrifuged at l0,000 *g* for 10 min and the absorbance of the formazon was determined spectrophotometerically at 540 nm. The amount of reduced NBT was calculated using the following formula

$$\text{Amount of reduced NBT} = A \times V/(T \times M \times e \times l)$$

where A is the absorbance, V is the volume of pyridine, T is the time for which the rings were incubated with NBT, M is the blotted wet mass of the aortic rings, e is the extinction coefficient (0.71 mM mm^{-1}), and l is the length of the light path (Sharma and Singh, 2010, 2011a, 2012b, 2013).

Estimation of thiobarbituric acid reactive substances (TBARS). The brain TBARS was measured spectrophotometerically at 532 nm. Supernatant of brain homogenate was pipette out in a test tube, followed by addition of 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 30% acetic acid (pH 3.5), 1.5 mL of 0.8% of thiobarbituric acid and the volume was made up to 4 mL with distilled water. The test tubes were incubated for 1 h at 95 °C, then cooled and added 1 mL of distilled water followed by addition of 5 mL of n-butanol-pyridine mixture (15:1 v/v). The tubes were centrifuged at 4000 *g* for 10 min. The absorbance of developed pink color was measured spectrophotometerically at 532 nm. A standard calibration curve was prepared using 1–10 nM of 1,1,3,3-tetra methoxy propane. The TBARS value was expressed as nanomoles per milligram of protein (Sharma and Singh, 2012c, 2013).

Estimation of reduced glutathione (GSH). The reduced glutathione (GSH) content was estimated at 412 nm (Sharma and Singh, 2010, 2011a, 2012a). Briefly, the supernatant of brain homogenate & serum was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio. The tubes were centrifuged at 1000 *g* for 10 min at 4 °C. The supernatant obtained (0.5 mL) was mixed with 2 mL of 0.3 M disodium hydrogen phosphate. Then 0.25 mL of 0.001 M freshly prepared DTNB [5, 5′-dithiobis (2-nitro benzoic acid) dissolved in 1% w/v sodium citrate] was added and absorbance was noted spectrophotometerically at 412 nm. A standard curve was plotted using 10–100 μM of reduced form of glutathione and results were expressed as micromoles of reduced glutathione per milligram of protein.

Estimation of brain total protein. The brain total protein was determined at 750 nm. The brain total protein was determined by using bovine serum albumin (BSA) as a standard. 0.15 mL of supernatant of tissue homogenate was diluted to 1 mL then 5 mL of Lowry's reagent was added. The contents were mixed thoroughly and the mixture was allowed to stand for 15 min at room temperature. Then 0.5 mL of Folin–Ciocalteu reagent was added and the contents were vortexed vigorously and incubated at room temperature for 30 min. The standard curve was plotted using 0.2–2.4 mg/mL of BSA. The protein content was determined spectrophotometerically at 750 nm. Protein concentration was expressed as mg/mL of supernatant (Sharma and Singh, 2011b, 2012c; Sharma et al., 2008a).

Experimental protocol. In total eleven (11) groups have been employed in the present study and each group was consisted of eight male Wistar rats (Table 1).

Group I — Control group: The animals were exposed to Morris water maze for acquisition trial from day 1 to day 4 and retrieval trial on day 5.

Group II — Vehicle control group (0.9% w/v saline): The animals were administered with saline (10 mL kg^{-1} i.p., daily) for 35 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 36th to 39th day) and retrieval trials (on 40th day) on Morris water maze.

Group III — Arsenic treatment group: The animals were administered with sodium arsenite 100 ppm or 100 mg/L solution (equivalent to 57.3 ppm or 57.3 mg/L of arsenic) in drinking water, for 55 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 56th to 59th day) and retrieval trials (on 60th day) on Morris water maze.

Group IV — Sodium butyrate per se: The animals were administered with sodium butyrate (100 mg kg^{-1} i.p., daily), for 35 days, rest of the procedure was the same as described in group II.

Group V — Aminoguanidine per se: The animals were administered with aminoguanidine (150 mg kg^{-1} i.p., daily), for 35 days, rest of the procedure was the same as described in group II.

Groups VI and VII — Arsenic and sodium butyrate dose 1/dose 2: Sodium butyrate (50/100 mg kg^{-1} i.p., daily) was administered to the arsenic treated rats, starting from 21st day of arsenic treatment followed by exposure to Morris water maze on 56th day of arsenic administration. The treatment was continued during acquisition (from 56th to 59th day) and retrieval trials (on 60th day) on Morris water maze.

Groups VIII and IX — Arsenic and aminoguanidine dose 1/dose 2: Aminoguanidine (75/150 mg kg^{-1} i.p., daily) was administered to the arsenic treated rats, rest of the procedure was the same as described in groups VI and VII.

Table 1
Tabular representation of the experimental protocol.

Group				Morris water maze					Biochemical estimations
				ELT				TSTQ	
Control				Day 1	Day 2	Day 3	Day 4	Day 5	
Saline	Day 1	----------		Day 36	Day 37	Day 38	Day 39	Day 40	
Arsenic	Day 1	----------		Day 56	Day 57	Day 58	Day 59	Day 60	
NaB per se	Day 1	----------		Day 36	Day 37	Day 38	Day 39	Day 40	
AG per se	Day 1	----------		Day 36	Day 37	Day 38	Day 39	Day 40	
BAL per se	Day 1	----------		Day 36	Day 37	Day 38	Day 39	Day 40	
			Drug treatment						

Arsenic + NaB D1	Day 1	----------	Day 21	Day 56	Day 57	Day 58	Day 59	Day 60	
Arsenic + NaB D2	Day 1	----------	Day 21	Day 56	Day 57	Day 58	Day 59	Day 60	
Arsenic + AG D1	Day 1	----------	Day 21	Day 56	Day 57	Day 58	Day 59	Day 60	
Arsenic + AG D2	Day 1	----------	Day 21	Day 56	Day 57	Day 58	Day 59	Day 60	
Arsenic + BAL	Day 1	----------	Day 21	Day 56	Day 57	Day 58	Day 59	Day 60	

Arsenic treatment	No treatment	Drug/vehicle treatment

ELT–; escape latency time; TSTQ–time spent in target quadrant; saline—0.9% sodium chloride solution; arsenic—sodium arsenite; NaB–sodium butyrate; AG–aminoguanidine; BAL–dimercaprol; D1–dose 1; D2–dose 2.

Group X — Dimercaprol per se: The animals were administered with dimercaprol (10 mg kg^{-1} i.p., daily), for 35 days, rest of the procedure was the same as described in group II.

Group XI — Arsenic and dimercaprol: Dimercaprol (10 mg kg^{-1} i.p., daily) was administered to the arsenic treated rats, rest of the procedure was the same as described in groups VI and VII.

Statistical analysis. Statistical analyses were done using GraphPad Prism v5.01. All results were expressed as mean ± S.E.M. Data for isolated aortic ring preparation was statistically analyzed using repeated measures of analysis of variance (ANOVA) followed by Newman–Keuls test. All other results were analyzed using two way ANOVA followed by Bonferroni's post-test. $p < 0.05$ was considered to be statistically significant.

Results

Effect on escape latency time (ELT) and time spent in target quadrant (TSTQ), using Morris water maze (MWM)

Before subjecting the animals to MWM test, their motor coordination scores were measured by employing rotarod test. However, no significant difference was noted between arsenic treated and control animals (data not shown). In MWM test, control rats showed a downward trend in their ELT. There was a significant fall in day 4 ELT, when compared to day 1 ELT of these rats (Fig. 1), reflecting normal learning ability.

Further on day 5 a significant rise in TSTQ was observed, when compared to time spent in other quadrants (Fig. 2), reflecting normal retrieval as well. Administration of 0.9% w/v saline (10 mL kg^{-1} i.p., 40 days) did not show any significant effect on ELT and TSTQ. Administration of sodium butyrate (100 mg kg^{-1} i.p., 40 days) as well as aminoguanidine (150 mg kg^{-1} i.p., 40 days) did not show any significant per se effect on ELT and TSTQ (Figs. 1 and 2). Furthermore, sodium arsenite 100 ppm or 100 mg/L solution (equivalent to 57.3 ppm or 57.3 mg/L of arsenic in drinking water p.o., for 60 days) treated rats showed a significant increase in day 4 ELT (59th day of arsenic treatment), when compared to day 4 ELT of control animals (Fig. 1) indicating impairment of acquisition. Moreover, arsenic administration also produced a significant decrease in day 5 TSTQ (60th day of arsenic treatment), when compared to day 5 TSTQ of control animals (Fig. 2), indicating impairment of memory as well.

Daily administration of sodium butyrate (50 mg kg^{-1}/100 mg kg^{-1} i.p.); aminoguanidine (75 mg kg^{-1} i.p./150 mg kg^{-1} i.p.); dimercaprol (10 mg kg^{-1} i.p.), significantly prevented arsenic induced rise in day 4 ELT, indicating attenuation of arsenic induced impairment of acquisition (Fig. 1). Further treatment of these drugs also attenuated arsenic induced decreases in day 5 TSTQ in a significant manner, indicating attenuation of arsenic induced impairment of memory (Fig. 2).

Effect on endothelium dependent and independent relaxation

Thoracic aorta strips of rats have been used as the representative of blood vessels. Acetylcholine (ACh) and sodium nitroprusside (SNP) in a dose dependent manner produced endothelium dependent and independent relaxation in phenylephrine (3×10^{-6} M) precontracted isolated rat aortic ring preparation. Arsenic administration significantly attenuated acetylcholine induced endothelium dependent relaxation

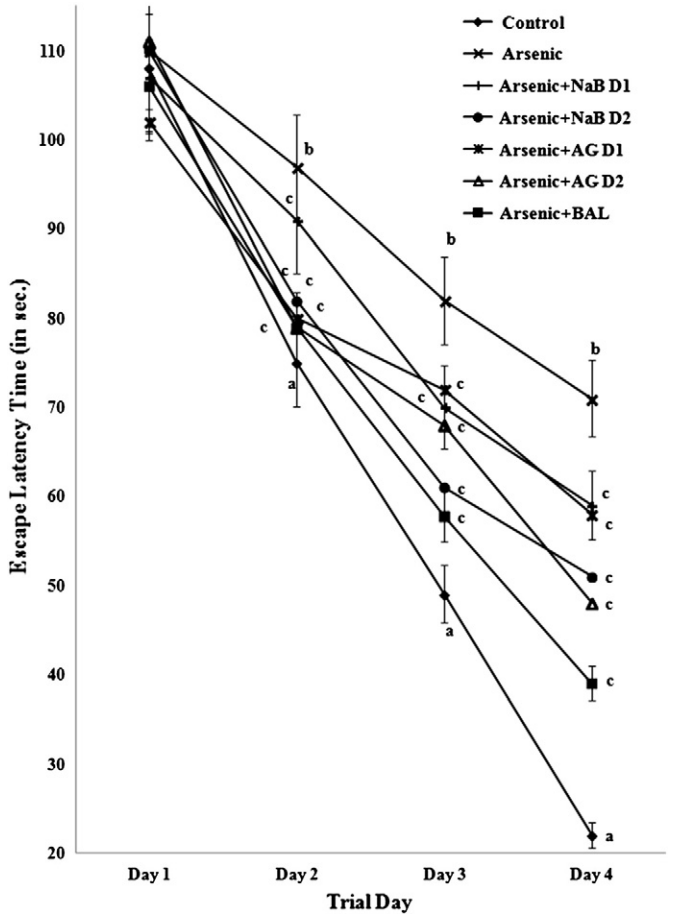

Fig. 1. Interdiction of sodium arsenite induced increases in day 4 escape latency time (ELT) of animals by sodium butyrate and aminoguanidine. Each group comprised of eight rats. As noted on Morris water maze, sodium arsenite administered rats have shown a significant increase in day 4 ELT, which was significantly reduced by sodium butyrate (doses 1 and 2), aminoguanidine (doses 1 and 2) and dimercaprol. All data of ELT is represented as mean ± standard error of means (S.E.M.) and was statistically analyzed using one way ANOVA followed by Tukey's multiple range test. $p < 0.05$ was considered to be statistically significant. $^{a}p < 0.05$ versus day 1 escape latency time in respective group; $^{b}p < 0.05$ versus day 4 escape latency time in control group; and $^{c}p < 0.05$ versus day 4 escape latency time in sodium arsenite treated group. Saline—0.9% sodium chloride solution; arsenic—sodium arsenite; NaB—sodium butyrate; AG—aminoguanidine; BAL—dimercaprol; D1—dose 1; D2—dose 2.

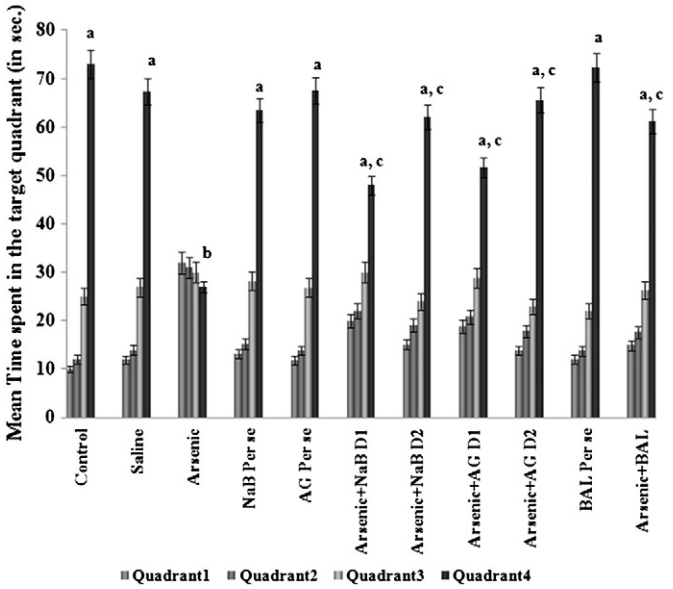

Fig. 2. Interdiction of sodium arsenite induced reductions in mean time spent in target quadrant (TSTQ) of animals by sodium butyrate and aminoguanidine. Each group comprised of eight rats. As noted on Morris water maze, sodium arsenite administered rats have shown a significant reduction in day 5 TSTQ, which was significantly increased sodium butyrate (doses 1 and 2), aminoguanidine (doses 1 and 2) and dimercaprol. All data of TSTQ is represented as mean ± standard error of means (S.E.M.) and was statistically analyzed using one way ANOVA followed by Tukey's multiple range test. $p < 0.05$ was considered to be statistically significant. $^{a}p < 0.05$ versus mean time spent in other quadrants in respective group; $^{b}p < 0.05$ versus mean time spent in target quadrant in control group; and $^{c}p < 0.05$ versus mean time spent in target quadrant in sodium arsenite treated group. Saline—0.9% sodium chloride solution; arsenic—sodium arsenite; NaB—sodium butyrate; AG—aminoguanidine; BAL—dimercaprol; D1—dose 1; D2—dose 2.

(Fig. 3), however it did not affect SNP induced endothelium independent relaxation (Fig. 4).

Treatment of sodium butyrate (dose 1 and dose 2); aminoguanidine (dose 1 and dose 2); and dimercaprol, significantly abolished the effect of arsenic on endothelial dependent relaxation. However, sodium butyrate; aminoguanidine; and dimercaprol did not show any per se effect on endothelium dependent relaxation.

Effect on serum nitrite and oxidative stress levels

Administration of arsenic produced a significant decrease in serum nitrite (Fig. 5) & brain levels of reduced form of glutathione (GSH) (Fig. 6) with significant increase in aortic superoxide anion level (Fig. 7), brain thiobarbituric acid reactive species (TBARS) (Fig. 8), when compared to control rats. Treatment with sodium butyrate (dose 1 and dose 2); aminoguanidine (dose 1 and dose 2); and dimercaprol, prevented arsenic induced impairment of these biochemical parameters, in a significant manner (Figs. 5–8). Further, sodium butyrate and aminoguanidine, did not show any significant per se effect on any of the biochemical parameters (Figs. 5–8).

Discussion

Sodium arsenite has been used for the induction of arsenic toxicity in rats (Yadav et al., 2009). In the present investigation, sodium arsenite induced arsenic toxicity has resulted in vascular endothelial dysfunction, impairment of learning & memory, serum nitrite/nitrate levels, and oxidative stress. Morris water maze is the most widely used and well accepted model to test learning and memory of rodents (Sharma and Singh, 2011a, 2012a; Sharma et al., 2008a,b). Sodium arsenite treated rats, performed poorly on Morris water maze test which indicates impairment in the learning abilities and memory capacities of sodium arsenite treated rats. Arsenic is a known neurotoxin that has both neuro-developmental and neuro-cognitive consequences, which are significantly related to poor language, visuo-spatial skills, executive functioning, global cognition, processing speed and immediate memory (O'Bryant et al., 2011). We have not found any significant change in the motor coordination scores of arsenic treated rats, when compared to the control animals. However, Yadav et al. (2009) have reported significant decreases in locomotor activity and rotarod performance in rats exposed to arsenic toxicity. After careful analyses of this work we have found that Yadav and colleagues have used a very high dose of arsenic and thus, possibly the higher dose of arsenic is responsible for the said changes in locomotor activity and rotarod performance of the animals. But as we have used a much lower dose as compared to them, therefore such changes in motor activity were not observed in the present study. Furthermore, prior research has shown that arsenic exposure induces changes that coincide with most of the developmental, biochemical, pathological, and clinical features of AD and other dementia (Gong and O'Bryant, 2010). It has also been reported that arsenic toxicity induces hyper-phosphorylation of protein tau and over transcription of the amyloid precursor protein, which is involved in the formation of neurofibrillary tangles and brain amyloid plaques (Gong and O'Bryant,

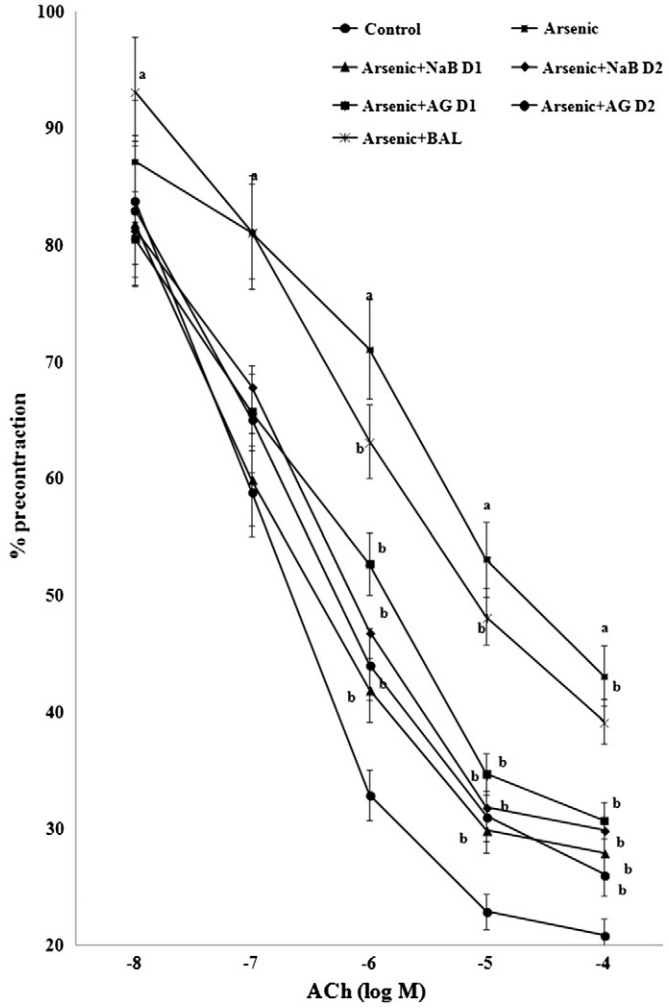

Fig. 3. Interdiction of sodium arsenite induced impairments of Acetylcholine induced endothelium dependent relaxation by sodium butyrate and aminoguanidine. Each group comprised of eight rats and all the responses are expressed as percentage of precontraction induced by 3×10^{-6} M phenylephrine. As noted on aortic ring preparation using student physiograph, sodium arsenite administered rats have shown a significant reduction in acetylcholine induced endothelium dependent relaxation, which was significantly reduced by sodium butyrate (doses 1 and 2), aminoguanidine (doses 1 and 2) and dimercaprol. All the data represented as mean ± standard error of means (S.E.M.) and was statistically analyzed using repeated measure analysis of variance (ANOVA) followed by Newman–Keuls test. $p < 0.05$ was considered to be statistically significant. $^{a}p < 0.05$ versus control; and $^{b}p < 0.05$ versus sodium arsenite treated group. Arsenic—sodium arsenite; NaB—sodium butyrate; AG—aminoguanidine; BAL—dimercaprol; D1—dose 1; D2—dose 2.

2010). Thus, reduced learning and memory in this study are in accordance with the previous findings.

Arsenic administration has been reported to induce endothelial dysfunction and reduce serum nitrate/nitrite levels (Kaur et al., 2010) and in accordance to this, present research work has also found reduction in endothelium dependent relaxation and serum nitrite/nitrate levels, thus endothelial dysfunction. Arsenic exposure plays a key role in the pathogenesis of vascular endothelial dysfunction as it inactivates endothelial nitric oxide synthase, leading to reduction in the generation and bioavailability of nitric oxide. In addition, the chronic arsenic exposure induces high oxidative stress, which may affect the structure and function of endothelium (Balakumar and Kaur, 2009). Long-term exposure to arsenic by drinking water has been shown to reduce nitric oxide production in endothelial cells, due to an "uncoupling" of endothelial NO synthase evoked by decreased levels of (6R)-5,6,7,8-tetrahydro-L-biopterin (BH(4)), a cofactor of the enzyme, leading to endothelial dysfunction (Kumagai, 2009). It has also been reported that arsenic induces

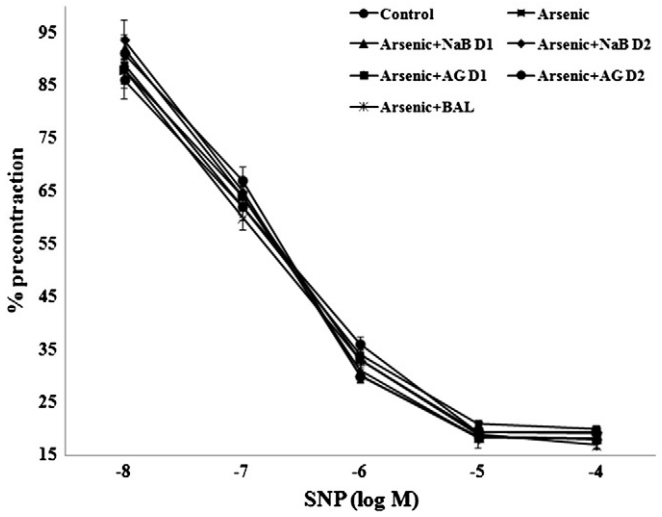

Fig. 4. Effect of various treatments on sodium nitroprusside induced endothelium independent relaxation. Each group comprised of eight rats and all the responses are expressed as percentage of precontraction induced by 3×10^{-6} M phenylephrine. As noted on aortic ring preparation using student physiograph, there was no effect of any of the treatments on endothelium independent relaxation. All the data represented as mean ± standard error of means (S.E.M.) and was statistically analyzed using repeated measure analysis of variance (ANOVA) followed by Newman–Keuls test. $p < 0.05$ was considered to be statistically significant. Arsenic—sodium arsenite; NaB—sodium butyrate; AG—aminoguanidine; BAL—dimercaprol; D1—dose 1; D2—dose 2.

apoptosis and subsequent dysfunction of endothelium also involves an alteration of intracellular calcium homeostasis (Suriyo et al., 2012).

Furthermore, arsenic treatment in this study has shown a significant rise in brain TBARS, aortic superoxide anion along with a fall in brain GSH levels. Free radical generation with subsequent oxidative stress in the biochemical and molecular mechanisms of arsenic toxicity has previously been reported (Prabu and Muthumani, 2012). A markedly

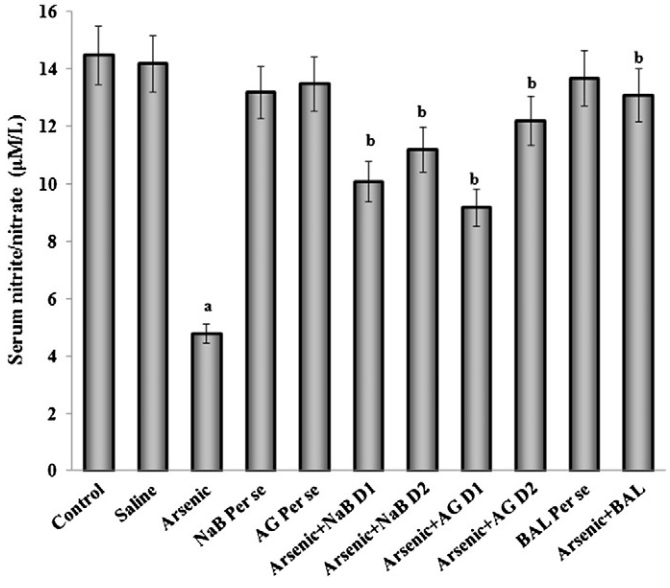

Fig. 5. Interdiction of sodium arsenite decreases in serum nitrite/nitrate levels by sodium butyrate and aminoguanidine, as measured on day 1 of Morris water maze exposure. Each group comprised of 8 rats. Sodium butyrate (doses 1 and 2), aminoguanidine (doses 1 and 2), and dimercaprol significantly reverse the sodium arsenite administered induced reduction in serum nitrite/nitrate levels. Data is represented as mean ± standard error of means (S.E.M.) and was statistically analyzed using one way ANOVA followed by Tukey's multiple range test. $p < 0.05$ was considered to be statistically significant. $^{a}p < 0.05$ versus control group; $^{b}p < 0.05$ versus sodium arsenite treated group; saline—0.9% sodium chloride solution; arsenic—sodium arsenite; NaB—sodium butyrate; AG—aminoguanidine; BAL—dimercaprol; D1—dose 1; D2—dose 2.

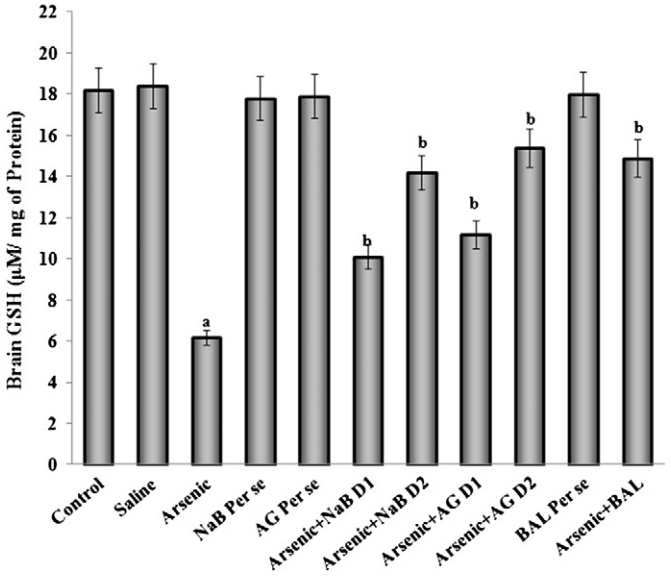

Fig. 6. Interdiction of sodium arsenite induced decreases in brain glutathione (GSH) levels by sodium butyrate and aminoguanidine, as measured on day 1 of Morris water maze exposure. Each group comprised of 8 rats. Sodium butyrate (doses 1 and 2), aminoguanidine (doses 1 and 2), and dimercaprol significantly reverse the sodium arsenite administered induced reduction in brain GSH levels. Data is represented as mean ± standard error of means (S.E.M.) and was statistically analyzed using one way ANOVA followed by Tukey's multiple range test. $^{a}p < 0.05$ versus control group; $^{b}p < 0.05$ versus sodium arsenite treated group. Saline—0.9% sodium chloride solution; arsenic—sodium arsenite; NaB—sodium butyrate; AG—aminoguanidine; BAL—dimercaprol; D1—dose 1; D2—dose 2.

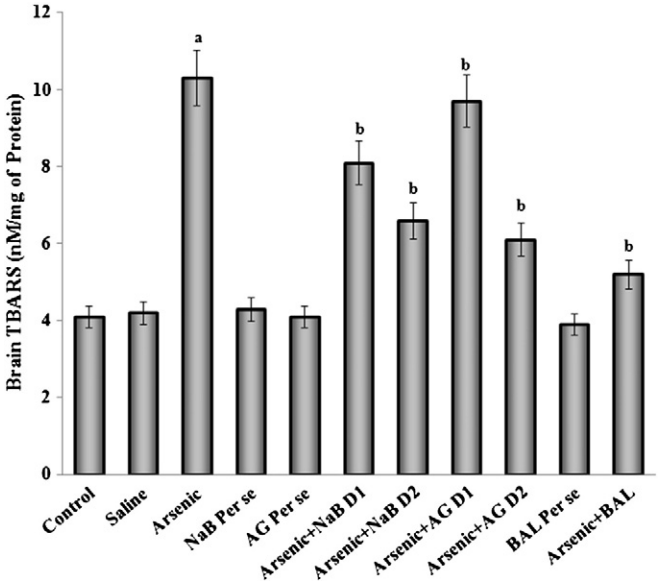

Fig. 8. Interdiction of sodium arsenite induced increases in brain thiobarbituric acid reactive species (TBARS) levels by sodium butyrate and aminoguanidine, as measured on day 1 of Morris water maze exposure. Each group comprised of 8 rats. Sodium butyrate (doses 1 and 2), aminoguanidine (doses 1 and 2) and dimercaprol significantly reverse the sodium arsenite administered induced increase in brain TBARS levels. Data is represented as mean ± standard error of means (S.E.M.) and was statistically analyzed using one way ANOVA followed by Tukey's multiple range test. $p < 0.05$ was considered to be statistically significant. $^{a}p < 0.05$ versus control group; $^{b}p < 0.05$ versus sodium arsenite treated group. Saline—0.9% sodium chloride solution; arsenic—sodium arsenite; NaB—sodium butyrate; AG—aminoguanidine; BAL—dimercaprol; D1—dose 1; D2—dose 2.

increased levels of lipid peroxidation markers (thiobarbituric acid reactive substances and lipid hydroperoxides) and protein carbonyl contents with decrease in non-enzymatic antioxidants (total sulfhydryl groups, reduced glutathione, vitamin C and vitamin E) and enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase), glutathione metabolizing enzymes (glutathione reductase and glutathione-6-phosphate dehydrogenase) and membrane bound ATPases were also observed in arsenic treated rats (Kramárová et al., 2012; Prabu and Muthumani, 2012). Thus arsenic induced increases in oxidative stress in brain, blood and endothelium are in line with the previous reports.

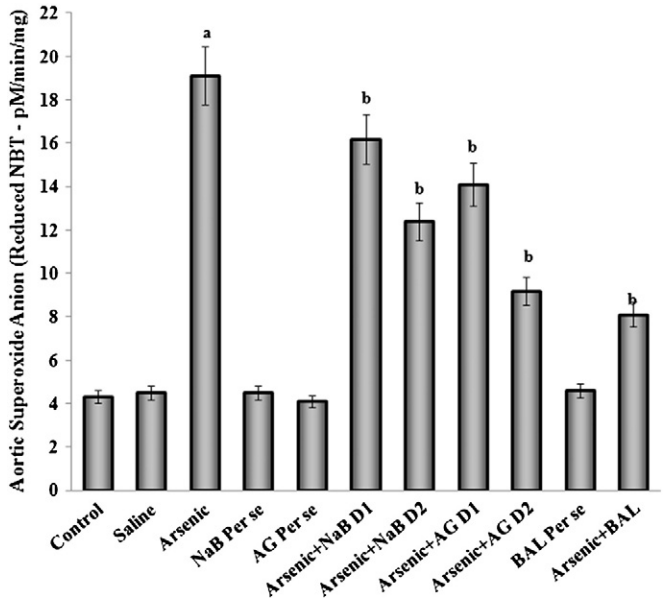

Fig. 7. Interdiction of sodium arsenite induced increases in aortic superoxide anion generation by sodium butyrate and aminoguanidine, as measured on day 1 of Morris water maze exposure. Each group comprised of 8 rats. Sodium butyrate (doses 1 and 2), aminoguanidine (doses 1 and 2), dimercaprol significantly reverse the sodium arsenite administered induced increase in aortic superoxide anion generation. Data is represented as mean ± standard error of means (S.E.M.) and was statistically analyzed using one way ANOVA followed by Tukey's multiple range test. $p < 0.05$ was considered to be statistically significant. $^{a}p < 0.05$ versus control group; $^{b}p < 0.05$ versus sodium arsenite treated group. Saline—0.9% sodium chloride solution; arsenic—sodium arsenite; NaB—sodium butyrate; AG—aminoguanidine; BAL—dimercaprol; D1—dose 1; D2—dose 2.

Arsenic exposure induces the overproduction of reactive nitrogen species in brain tissue and results in nucleic acid damage to the nerve cells (Ma et al., 2010). Reactive oxygen species mediated oxidative damage is a common denominator in arsenic pathogenesis. Arsenic exposure has shown to increased formation of ROS/RNS, including peroxyl radicals (ROO•), the superoxide radical, singlet oxygen, hydroxyl radical (OH•) via the Fenton reaction, hydrogen peroxide, the dimethylarsenic radical, the dimethylarsenic peroxyl radical and/or oxidant-induced DNA damage. Arsenic induces the formation of oxidized lipids which in turn generates several bioactive molecules (Jomova et al., 2011). Further it has been reported that arsenic induces disassembly of cellular microtubules and inhibition of microtubular polymerization (Zhao et al., 2012).

Treatment of sodium butyrate (a HDAC inhibitor); aminoguanidine (an, iNOS inhibitor); and dimercaprol (a chelating agent) has attenuated arsenic induced endothelial dysfunction, impairment of learning & memory, serum nitrite/nitrate, and oxidative stress levels.

Inhibition of histone deacetylases by HDAC inhibitors (such as sodium butyrate) causes increase in histone acetylation, chromatin remodeling (Fischer et al., 2007), transcriptional dysfunction (Rexhaj et al., 2011), enhanced neurogenesis, and decreased infarct volume (Faraco et al., 2006). Further HDAC inhibition has also been reported to decrease neuro-inflammation (Kimura et al., 2010), decrease neurological deficits (Yildirim et al., 2008) and increase synaptic plasticity (Guan et al., 2009) that make these agents as suitable therapeutic avenue for neurodegenerative diseases. HDAC inhibition has been reported to prevent neuronal apoptosis in excitotoxic and hypoxic conditions (Dietz and Casaccia, 2010). It has also been shown that inhibition of HDACs reduces

inflammatory responses (Moreira et al., 2003). It has been reported that HDAC inhibition modulates memory in the hippocampus via cyclic-AMP response element binding protein (Haettig et al., 2011). Sodium butyrate has been reported to completely restore contextual memory in APPswe/PS1dE9 mice and maintained newly consolidated memories (Kilgore et al., 2010). Furthermore, HDAC inhibitors have previously been reported to reverse memory deficits and improve spatial learning in a transgenic mouse model (Tg2576) of AD (Ricobaraza et al., 2009) and of neurodegeneration (CK-p25TG) (Fischer et al., 2007). Moreover Stefanko et al. (2009) has also reported that in memory compromised mice, HDAC inhibition generates a type of long-term memory that persists beyond a point at which normal memory for novel object recognition fails. Thus in the present study improvement of learning and memory of animals are in line with the previous findings. Moreover, HDAC inhibitors have also been documented to protect cortical neurons in culture from oxidative stress-induced death (Kozikowski et al., 2007). Advani et al. (2011) has recently reported that administration of HDAC inhibitors reduced the oxidative stress. Our previous study with diabetic animals also suggests that sodium butyrate administration reduces aortic superoxide anions, serum and brain thiobarbituric acid with significant enhancement in brain glutathione levels (Sharma and Singh, 2011a). Thus reduction in oxidative stress by sodium butyrate is in accordance to previous studies from our lab and others.

Rexhaj et al. (2011) has reported that HDAC inhibitors have attenuated vascular endothelial dysfunction in offspring of restrictive diet during pregnancy (RDP) of mothers. As per the report of Mahpatra et al. (2010), HDAC inhibitor induces self-repair, vascular tissue regeneration, controlled angiogenesis and endothelial dysfunction in endothelial cells. We have also reported recently that administration of sodium butyrate in diabetic animals has controlled the endothelial dysfunction (Sharma and Singh, 2011a). Results of this study further emphasize the endothelial function preservation by HDAC inhibitor. Hence, there are multiple actions of sodium butyrate which could be attributed to its beneficial effects in arsenic induced VaD in the present study. Perhaps this is the first report highlighting usefulness of sodium butyrate in arsenic induced VaD.

It has been suggested that inducible nitric oxide synthase (iNOS) is upregulated by arsenic exposure (Prabu and Muthumani, 2012), which may contribute to the inflammatory response, increased reactive oxygen species, vascular remodeling, decreased aortic blood flow attenuation of endothelium dependent relaxation, endothelial cell damage or cell death, reduced NO bioavailability etc. (Steed et al., 2010). Reactive oxygen species cause structural membrane damage, induce inflammation, and scavenge NO to yield peroxynitrite ($ONOO^-$). This activates the inducible NO synthase, which further compounds $ONOO^-$ formation (Berg et al., 2011). Reactive oxygen species and $ONOO^-$ cause mitochondrial dysfunction by inhibiting the mitochondrial electron transport chain and uncoupling oxidative phosphorylation, which ultimately leads to neuronal bioenergetic failure. Furthermore, in certain 'at risk' areas of the brain, free radicals may induce neuronal apoptosis (Berg et al., 2011; Steed et al., 2010).

Recently it has also been reported that blockage of iNOS by aminoguanidine caused enhancement of cytoprotective mechanisms, reduction of iNOS activity and oxidative stress, and an increase in blood L-arginine level (Sklyarov et al., 2011). It has previously been reported that aminoguanidine attenuates memory impairment and reductions in brain nitrite in global cerebral ischemia, hypoxia, neurotoxicity and in various other neurodegenerative disorders (Stevanović et al., 2010; Udayabanu et al., 2008). Therefore, beneficial effect of aminoguanidine in the present study is may be attributed to its inhibitory activity on iNOS, preventive action on oxidative stress and neuro-protective activity.

Dimercaprol has long been the mainstay of chelation therapy for lead or arsenic poisoning (Flora and Pachauri, 2010). Dimercaprol treatment enhanced the elimination of arsenic in two ways: by decreasing the tissue-to-blood partitioning which mobilizes arsenic into the bloodstream, and by increasing the clearance of arsenic (Snider et al., 1990). We have used dimercaprol as a standard treatment for arsenic toxicity. Dimercaprol has attenuated arsenic induced endothelial dysfunction, impaired learning and memory, serum nitrite/nitrate and oxidative stress levels due to the chelation of administered arsenic in the animals.

Thus it may be concluded that arsenic toxicity has induced endothelial dysfunction and dementia in rats. Treatments with sodium butyrate (a HDAC inhibitor) and aminoguanidine (an iNOS inhibitor) have interdicted arsenic induced experimental endothelial dysfunction and dementia. As this is the first study of its kind which suggests induction of endothelial dysfunction as well as dementia due to arsenic toxicity, which may be improved by modulation of HDAC and iNOS, thus further studies are required to explore the benefit of HDAC and iNOS modulation in arsenic induced endothelial dysfunction and dementia.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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