Current Trends in Biotechnology and Pharmacy
Vol. 15 (4) 368 - 376, October 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.4.37

Essential Metals (Calcium and Znic) and Chelating Agent (Monoisoamyl 2, 3-dimercaptosuccinic acid) Attenuates Arsenic Induced Neurochemical Alterations in Rats

K. Praveen Kumar[1], S. Saritha[1], G. Rajarami Reddy[2,*]
[1]Department of Biotechnology, S.V.University, Tirupati-517502, India
[2]Department of Zoology, S.V.University, Tirupati-517502, India
*corresponding author : gottipolu2002@yahoo.com

Abstract

Arsenic (As) is ubiquitous in environment and well known developmental neurotoxicant. In the present study we examined the protective effect of calcium (Ca), zinc (Zn) and monoisoamyldimercaptosuccinic acid (MiADMSA), either individually or in combination against As induced alterations in catecholamine levels, acetylcholine (ACh), acetyl cholinesterase (AChE) and monoamine oxidase (MAO) in brain regions (cerebral cortex, hippocampus and cerebellum) of PND 21, PND 28 and 3 month old rats. Gestational and lactational (GD6 to PND 21) As exposure significantly decreased the levels of catecholamines (dopamine, epinephrine and nor epinephrine), AChE and MAO activities with an increase in ACh content in all selected age points. The observed alterations in these neurochemcical parameters were higher at PND 28. Among the brain regions, As induced alterations in catecholamines levels and cholinergic system were more pronounced in hippocampus than cortex and cerebellum. Individual supplementation of essential metals (Ca or Zn) or chelating agent (MiADMSA) significantly reversed the As induced neurochemical alterations. However, combined supplementation of essential metals with the chelating agent produced greater reversal effects in reducing As induced neurotoxicity.

Keywords: Arsenic developmental toxicity, essential metals, MiADMSA, brain regions

Introduction

Arsenic (As) is a toxic metalloid widely distributed in environment, particularly in soils, well water and contaminated food. Humans are exposed to As mainly from consumption of drinking water containing high amounts of inorganic arsenic (1, 2). Embryonic As exposure affects brain development in the form of neural tube defects, increase in neuronal apoptosis, disrupted neural outgrowth (3-5). Occupational and environmental exposure to arsenicals in adults and children has been shown to result in encephalopathy and impairments of neurological functions, such as memory (6), concentration (7), verbal comprehension and attention (8), decreased IQ (9), as well as peripheral neuropathy (10). Studies in rodents also showed impaired performance in learning tasks and altered behavior following sodium arsenite treatment (11, 12). Several experimental and epidemiological studies suggest that the neurotoxic effects of As are mediated through interference with the cholinergic and aminergic systems (13,14). Oxidative stress has been proposed to be another mechanism involved in As toxicity (15). Neurotoxicity of As exposure has been well established in adult rats but very little information is available on neuorchemical changes following gestational and lactational As exposure in early life. Till now, safe and effective therapeutic management of arsenicosis is also not available.

The Role of essential metals in reducing the impact of heavy metals has been reported (16). In animals, over 300 enzymes require zinc for their function. Zinc is necessary for DNA replication, transcription, protein synthesis and also significantly influences cell division and differentiation (17,18). Zinc deprivation may influence zinc homeostasis in the brain followed by brain dysfunction (19). Similarly, calcium is an important essential metal which play key role in neuronal functions (20). Calcium appears to partly inhibit As absorption via competition for common binding sites on intestinal binding proteins (21). Chelating agents are organic compounds capable of linking metal ions together to form complex ring-like structures called chelates. Sodium 2,3-dimercaptopropane 1-sulfonate (DMPS), DMSA (dimercaptosuccinic acid), and one of its analogues, monoisoamyl-DMSA (MiADMSA), are effective chelators, and the dithiol group in their moieties acts as an oxygen radical scavenger, which helps in inhibiting lipid peroxidation (22,23). In the present study, we examined the protective effect of calcium (Ca), zinc (Zn) or monoisoamyldimercaptosuccinic acid (MiADMSA), either individually or in combination against gestational and lactationalAs exposure induced alterations in brain regional catecholamines levels, cholinergic system and monoamine oxidase (MAO) activity at different early age points in rats.

Materials and Methods

Chemicals

Sodium arsenite (99% purity) used in this study was purchased from Sigma-Aldrich, (St Louis, MO, USA) and dissolved in sterile distilled water to the desired concentrations (parts per million). MiADMSA (purity 99.9%) was a gift from Division of Regulatory Toxicology,

DRDE, Gwalior, India. MiADMSA was dissolved in 5% sodium bicarbonate solution and solutions were prepared immediately before use. All other chemicals were purchased from Merck, India.

Study design

Healthy adult Wistar rats (140 ± 10 g) were purchased from Sri Venkateswara Traders, Bangalore and maintained in the animal house of Dept. of Zoology, Sri Venkateswara University. The protocol and animal use were approved by Animal ethical clearance committee, S.V. University. Rats were housed in an animal facility maintained in a 12-h (7:00-19:00) light/dark cycle, temperature of 24±2^{0}C and relative humidity of 55±15%. After a week of acclimatization, one male and two female rats were housed in the same cage every evening, and sperm plug in the female rat vagina was examined next morning. The day when sperm plug was confirmed was designated as day of gestation (GD0). The pregnant rats were randomly divided into eight groups of six animals. After birth, the litters of each group were culled on PND 4 to keep litter size four to six male pups per litter (each group containing six litters). Considering the similarities among littermates due to maternal effects, only one pup was selected from each litter in a group. Therefore, a total of 144 pups were used form eight different groups (18 per group; 6 per each age point). Pregnant rats were exposed to As and essential metal supplementation (calcium and zinc) form GD 6 till weaning (PND 21) and MiADMSA was given to pups from PND 18 to PND 20 (three consecutive days) as follows.

Group I : Control (received normal water)

Group II : MiADMSA (50 mg/kg.bt.wt)

Group III : Arsenic (100 ppm in sterile distilled water)

Group IV : Arsenic (Same as group III) + Calcium (10ppm in sterile distilled water)

Group V : Arsenic (Same as group III) + Zinc (10ppm in sterile distilled water)

Group VI : Arsenic (Same as group III) + Calcium (5ppm in sterile distilled water) + Zinc (5 ppm in sterile distilled water)

Group VII :Arsenic (Same as group III) + MiADMSA (same as group II)

Group VIII : Arsenic (Same as group III) + Calcium (same as group VI) + Zinc (same asgroup VI) + MiADMSA (25mg/kg. bt.wt)

The doses of As, essential metal supplementation and MiADMSA were selected on the basis of previously published studies (16,24). Rats were scarified by cervical decapitation on PND21, PND 28 and at 3 months age. After decapitation, the brain was removed onto ice-cold glass plate and different brain regions (cerebral cortex, hippocampus, and cerebellum) were quickly dissected and stored at -80^{0}Cuntil use.

Preparation ofmitochondiral and synaptosomal fractions in brain regions

Synaptosomes were prepared by following the method of Flora et al., (25).The tissues were homogenized in 10 volumes (w/v) of 0.32 M sucrose buffer (0.32 M sucrose, 10 mMTris-HCl, and 0.5 mM EDTA, pH 7.4). The homogenate was first centrifuged at 1000g for 10 min at 4°C, and then the supernatant was centrifuged at 12,000g for 20 min. The buffy layer of pelleted synaptosomes was suspended in a low K^+-HEPES buffer (125 mMNaCl, 5 mMKCl, 1.2 mM CaCl2, 1.2 mM Na2HPO4, 1.2 mM MgCl2, 5 mM NaHCO3, 10 mM HEPES, and 10 mM glucose, pH 7.4).

Mitochondrial fractions were prepared by following the method of Lai and Clark (26). The tissues were homogenized in 10 volumes (w/v) of SET buffer (0.25 M sucrose, 10 mMTris-HCl, and 1 mM EDTA, pH 7.4). The homogenate was first centrifuged at 800g for 10min at 4°C, and then the supernatant was centrifuged at 10,000g for 20 min. Then the pellet of mitochondrial fraction was suspended in SET buffer. Supernatant was used as cytosolic fraction

Determination of ACh levels

ACh levels were determined as described by Augustinssion (27). The synaptosomal fractions of cortex, hippocampus and cerebellum were placed in boiling water for 5 min to terminate the AChE activity and also to release the bound ACh. To the synaptosomal fractions,1 ml of alkaline hydroxylamine hydrochloride [equal volumes of 0.2 M hydroxylamine hydrochloride and 3.5 N sodium hydroxide (NaOH)] followed by 1 ml of 50% HCl was added. The contents were mixed thoroughly and centrifuged. To the supernatant, 0.5 ml of 0.37 M ferric chloride was added and the intensity of the color developed was read at 540 nm against a reagent blank in a spectrophotometer.

Determination of AChE activity

The specific activity of AChE was determined as described by Ellman et al. (28). The reaction mixture contained 3.0 ml of 0.1 M phosphate buffer (pH 8.0), 20 ml of 0.075 M acetylthiocholine iodide and 100 ml of 0.01 M 5,5-dithiobis-2-nitrobenzoic acid. The reaction was initiated with the addition of 100 ml of synaptosomal fraction. The contents were incubated for 30 min at room temperature and the color absorbance was measured at 412 nm in spectrophotometer (Hitachi, Model U-2000). The enzyme activity was expressed as mmol of ACh hydrolyzed/mg protein/h.

Estimation of catecholamines

The levels of catecholamines were determined according to the method of Kari et al. (29).Synaptosomal fractions were taken in acid butanol to give a final concentration of 50 mg/ml and centrifuged at 800 g for 10 min. Residues were discarded and to the supernatant 2.5 ml of distilled water and 2.5 ml of heptanes were added. The contents were thoroughly mixed and

centrifuged at 1000 g for 5 min. To the aqueous phase, 200mg of acidalumina was added followed by 1.5ml 2M sodium acetate. The contents were mixed thoroughly for 5 min, and the pH was adjusted to 8.0 with 1N sodium hydroxide. Then, the samples were centrifuged at 1000 g for 5 min. The supernatant was discarded, and the catecholamines were extracted from the alumina as described below. The acid alumina was washed by vortexing the tubes twice with 2.0 ml of distilled water and then, centrifuged at 1000 g for 5 min. The supernatant was discarded and the walls of the tubes were blotted with strips of filter paper. The alumina was then vortexed for 5 min with 2.0 ml of 0.2N acetic acid to elute the catecholamines. The contents were centrifuged at 1000 g for 5 min. The supernatants were transferred to 0.1 ml of 0.1 M EDTA, and the pH was adjusted to 6.3. This was followed by the addition of 0.1 ml of 0.1N iodine solution. The contents of tubes were mixed thoroughly and allowed to stand for 2 min. Then, 0.2 ml of alkaline sulphite solution was added. The contents were mixed and allowed to stand for 2 min at room temperature. Then, the pH of the solution was adjusted to 5.4 with 5N acetic acid. The samples with known amount of different amine standards were separately run to serve as internal standards. The fluorescence of epinephrine was read in a Shimatzu spectrophotometer (Model RF-500) with excitation and emission wavelengths of 410 and 500 nm, respectively, with a bandwidth of 10/10 nm. Norepinephrine was estimated by heating the same solution for 2 min in a boiling water bath. The tubes were cooled and the fluorescence of norepinephrine was read with excitation and emission wavelengths of 385 and 485 nm, respectively, with a slit width of 10/10 nm. After the estimation of norepinephrine, the same solution was again heated for 5 min in a boiling water bath. Then, the tubes were cooled and the fluorescence of dopamine was read with excitation and emission wave lengths of 320 and 370 nm, respectively, with a slit width of 10/10 nm. The amine content of each tissue sample was calculated by the method of Ansel and Beeson (30) and expressed as micrograms of amine per gram wet weight of tissue.

Spectrophotometric assay of MAO

The activity of MAO was estimated by the method of Green and Haughton (31). The assay mixture containing of 1.0 ml of semicarbazide hydrochloride (0.05 M, pH 7.4), 1.6 ml of phosphate buffer (0.2 M, pH 7.4) and 0.4 ml of mitochondrial fraction was incubated for 20 min at 37^0Cin a water bath with a shaking device. The reaction was started by adding 0.4 ml of tyramine hydrochloride (0.1 M, pH 7.4). After 30 min of incubation, the reaction was stopped by adding 1.0 ml of 0.5N acetic acid and kept in boiling water bath for 30 min. The contents were centrifuged for 10 min at 1000 g. To 2.0 ml of supernatant, 2 ml of 2,4-dinitriphe-nylhydrazine (0.5 mg/ml in 2N HCl) was added. After keeping at room temperature for 15 min, 5 ml of benzene was added. The tubes were vortexed, and the aqueous layer was discarded. The benzene layer was washed with 4 ml of distilled water followed by the addition of 4 ml of 0.1N NaOH solution and the contents of the tubes were mixed thoroughly. The benzene layer was discarded, and the NaOH layer was allowed to stand at room temperature for 1 h. The absorbance of the samples was measured at 425 nm in a UV/ VIS spectrophotometer (Hitachi, Model U-2000). The activity of MAO was calculated using the molar extinction coefficient of 9500 and expressed as micromoles of p-hydroxy phenyl acetaldehyde formed per gram wet weight of tissue per hour.

Data Analysis

All values are mean ± SD of six observations in each group. The data were subjected to one way analysis of variance (ANOVA) followed by student Newman– Keuls (SNK) post hoc test. The 0.05 level of probability was used as the criterion for significance.

Results

In control rat brain regions, the AChE activity was found to be greater in three months age as compared to PND 21 and PND 28. Among the brain regions studied, the hippocampus showed greater AChE activity followed by cortex and cerebellum. Rats exposed to As exhibited significant reduction in the specific activity of AChE in all the brain regions of all age groups (Fig:1a-c). Decreasein AChE activity was more in PND 28 and more pronounced in hippocampus. As-exposure caused an increase in crude synaptosomalACh level in different brain regions of PND 21, PND 28 and 3 months old rats. Maximum increase in the levels of ACh was observed in hippocampus followed by cortex and cerebellum at PND 28 (Fig:2a-c).

The specific activity of MAO was determined in mitochondrial fraction of rat brain regions (cerebral cortex, hippocampus and cerebellum) at PND 21, PND 28 and 3 months old rats. In control rat brain regions,the MAO activity was gradually increased with age. As exposure significantly decreased the MAO activity in the brain region of rats and the three age groups. Maximum decrease in MAO activity was observed in cerebellum followed by hippocampus and cerebral cortex at PND 28 (Fig:3a-c).

To determine the catecholamines levels (dopamine, epinephrine and norepinephrine), the crude synaptosomal fractionsof brain tissues were used. Thecatecholamine levels showed a marginal increase with agein PND 21, PND 28 and 3-month-old control rat brain regions.Higher levels of dopamine and nor epinephrine were observed in cortex followed by hippocampus and cerebellumwhereas levels of epinephrine were observed in hippocampus than cortex and cerebellum.As exposure significantly decreased the catecholamine levels in the three brain regions and the decrease was found to be greater at PND 28 (Fig:4a-c; Fig:5a-c; Fig:6a-c). Among the brain regions, maximum decrease in catecholamines levels was observed in

hippocampus as compared to cortex and cerebellum.

Supplementation ofCa, Zn or chelation therapy with MiADMSAindividually or together in combination significantly reduced the As induced alterations incatecholamine levels, ACh content andactivities of MAO and AChEin all the three brain regions of all the three age groups of rats. Among individual treatments, MiADMSA showed greater recovery in Asinduced alterations in rat brain regions. However, the recovery was much greaterand significant with combined supplementation of Ca and Zn along with MiADMSA.

Discussion

Results of the present study showed that individual and combined supplementation of Ca, Zn or MiDMSA significantly reversed the gestational and lactationalAs exposure induced neurochemical alterations in all selected age points. Biogenic amines are important regulators of various physiological and pharmacological functions in the biological system. Fluctuations in the levels of biogenic amines may affect the signaling process and behavioral functions (32-34). In this study, As exposure significantly decreased the levels of catecholamines (Dopamine, Epinephrine and Norepinephrine) in all the three brain regions of different age groups of rats. Decrease in tyrosine hydroxylase and dopamine beta hydroxylase activities might be partly responsible for the decreased levels of the Norepinephrine, Dopamine and Epinephrine (14,35). Neurotransmitters are mainly stored in synaptic vesicles and ultrastructural changes of these vesicles in the synapses might also be responsible for decrease in the level of the neurotransmitters in the brain regions (14).

AChE, an enzyme involved in the metabolism of acetylcholine and a neuromodulator at the cholinergic synapsesplays a major role in synaptic plasticity, specifically in learning and memory (36-38). The cholinergic synapses are more in hippocampus compared to cerebral cortex and cerebellum (39). This may be the region for the higher AChE activity observed in hippocampus. In the current study, it was observed that As exposure significantly decreased the AChE activity in the three brain regions of all age groups. Decrease inAChEactivity leads to accumulation of acetylcholine excessively at synaptic cleft and causes desensitization of brain muscarinic cholinergic receptors (40).MAO, as a catabolic enzyme, regulates monoamine transmitter levels in the central nervous system (41). We observed a significant decrease in MAO activity in all agepoints of Asexposed brain regions. As has high affinity for –SH groups in enzymes and proteins, and its binding could alter the functions of AChE and MAO(42).

As can transverse the placental barrier and reaches to the conceptus in the case of maternal exposure and can also cross the blood-brain barrier (BBB) easily (43). Early in gestation, arsenic selectively accumulates in the neuroepitheliumin brain tissue and retained for longer periods of time (5). In the present study, we found that As induced alterations were morepronounced inPND 28 as compared to adults (3months). This may be due to highest accumulation of As which was observed in our earlier study (15) or involvement of As in biochemical and cellular process (15). Among the brain regions, As induced alterations in catecholamine levels and cholinergic system were more in hippocampus. The neurotoxic effects of As on different sites of brain can be related to local differentiation in their formation and maturation as well as development of neurotransmitters system.

Our results showed that As exposure alters the catecholamine levels, ACh content and activities of AChE and MAO at different age points and this could be significantly reversed by combined supplementation of calcium and zinc together with MiADMSA. Essential trace elements can affect toxicity of heavy metals by interacting at the primary site of action and these trace elements could reduce the gastrointestinal absorption of As. Zn could be competing for binding sites and effectively reducing the availability of binding sites for metal uptake (21,16). Periods of rapid growth such as pregnancy and infancy are the most susceptible to dietary zinc deficiency (44). More zinc may be necessary for the development of the cerebellum than for other regions in the brain at a relatively early stage, because the growth and differentiation of the cerebellum is rapid after birth. On the other hand,Wolf et al (45)reported that the Zn level in the synaptosomal fraction of the hippocampus increased during postnatal development. In the hippocampus, Zn is localized in the mossy neuropil of the dentate gyrus and performneurochemical functions(46).The secretion of neurotransmitters and neurohormones is triggered by a rise in intracellular calcium (47). Metals and calcium interact at several sites in the body, including cell membranes through mechanisms that regulate ion transport (48). Animal studies have also shown higher retention of Pb in animals fed low-calcium diets, raising the possibility that low-calcium diets could affect the blood Pb levels of humans (49-51).The supplementation with Ca reversed the Pb-induced alterations in aminergic system of developing mouse brain (16). The mechanism by which calcium interferes with arsenic absorption is not clear. However, several interesting studies suggest that calcium intake rather than calcium status modulates metal absorption in animals (51).Our previous study also showed that supplementation of calcium during gestation and lactation reduces the As induced oxidative stress in rat brain regions (15). Mono isoamyl ester of DMSA (MiADMSA) is a C_5 branched chain ester comprising straight and branched chain amyl group which helps in increasing its lipophilicity. Lipophilicity and molecular size of this drug might be important factors for the removal of As from both intra and extra cellular sites leading to better therapeutic efficacy (52). From the results, it is concluded thatAs induced neurotoxicity in brain regions can bereduced with essential metal supplementation and chelation therapy. However, combined supplementation

of Ca and Zn along with chelatorMiADMSA seems more effective in reducing As induced neurotoxicity in developing brain.

Fig. 1a.

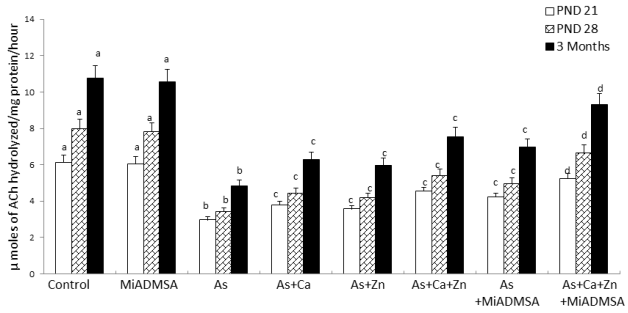


Fig. 1b.

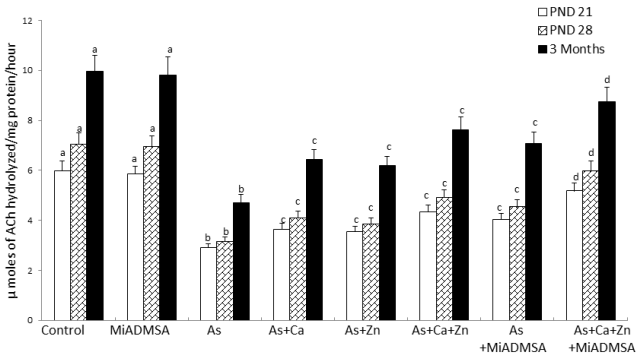


Fig. 1c.

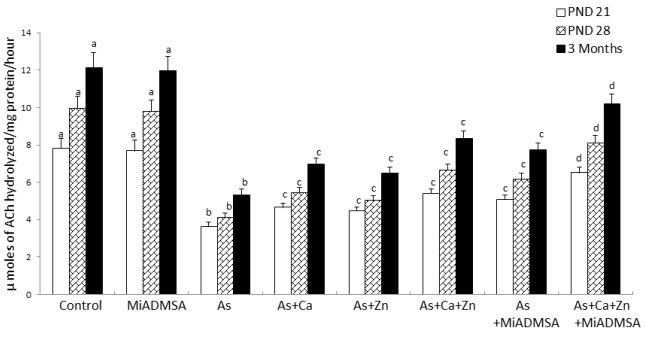


Fig.1. Effect of arsenic exposure on acetylcholine esterase activity in different brain regions (cerebral cortex (a), cerebellum (b), hippocampus (c)) of PND 21, PND 28 and 3 months old rats and the reversal effetcs of calcium, zinc or MiADMSA, either individually or in combination. Rats were exposed to either deionized water (control) or As (100 ppm) or supplementation of calcium and zinc (individual 10 ppm each or combined 5 ppm each) together with As from gestation day 6 to PND 21. MiADMSA was given to pups from PND 18 to PND 20, either individually (50 mg/kg b.wt) or in combination (25 mg/kg b.wt) with calcium and zinc to As. Values represent Mean ± S.D (n=6). The 0.05 level of probability was used as the criterion for significance. Mean values in a row that share the same superscript do not differ significantly at P <0.05.

Fig. 2a.

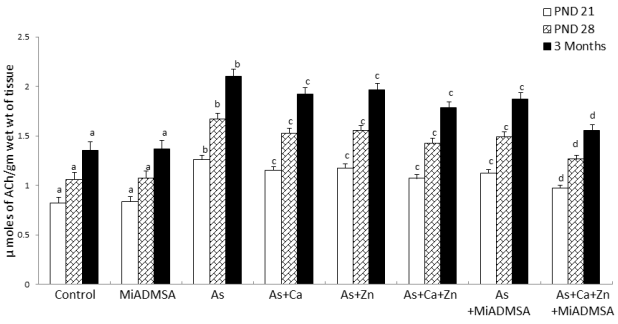


Fig. 2b.

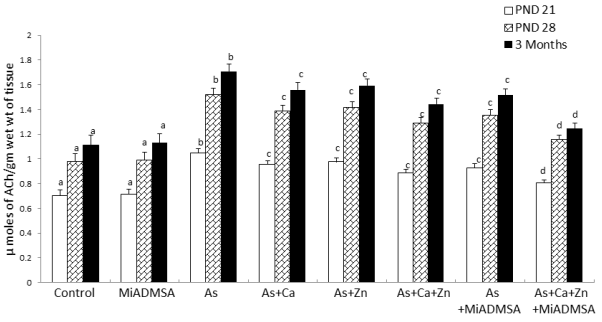


Fig. 2c.

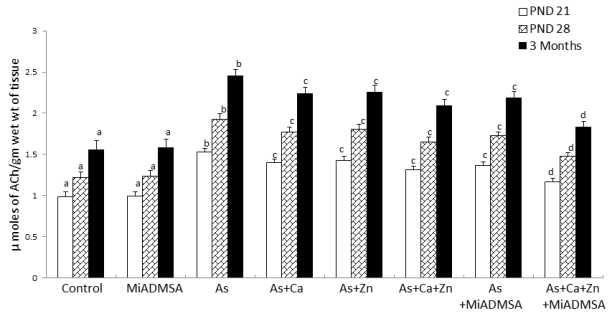


Fig.2. Effect of arsenic on acetylcholine content in different brain regions (cerebral cortex (a), cerebellum (b), hippocampus (c)) of PND 21, PND 28 and 3 months old rats and the reversal effects of calcium, zinc or MiADMSA, either individually or in combination. Rats were exposed to either deionized water (control) or As (100 ppm) or supplementation of calcium and zinc(individual 10 ppm each or combined 5 ppm each) together with As from gestation day 6 to PND 21. MiADMSA was given to pups from PND 18 to PND 20, either individually (50 mg/kg b.wt) or in combination (25 mg/kg b.wt) with calcium and zinc to As. Values represent Mean ± S.D (n=6). The 0.05 level of probability was used as the criterion for significance. Mean values in a row that share the same superscript do not differ significantly at P <0.05.

Fig.3. Effect of arsenic on monoamine oxidase activity in different brain regions (cerebral cortex (a), cerebellum (b), hippocampus (c)) of PND 21, PND 28 and 3 months old rats and its response to calcium, zinc or MiADMSA, either individually or in combination. Rats were exposed to either deionized water (control) or As (100 ppm) or supplementation of calcium and

Current Trends in Biotechnology and Pharmacy
Vol. 15 (4) 368 - 376, October 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.4.37

Fig. 3a.

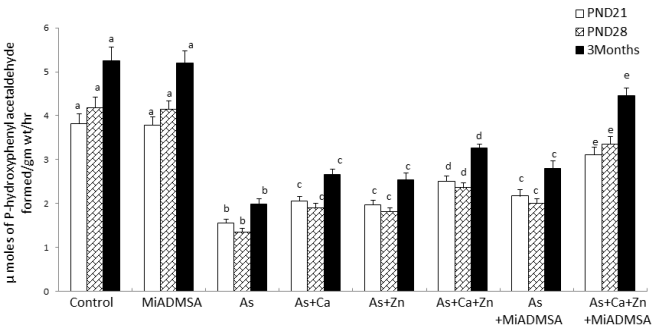


Fig. 3b.

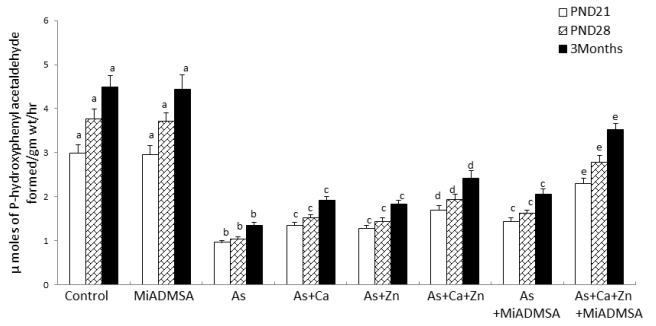
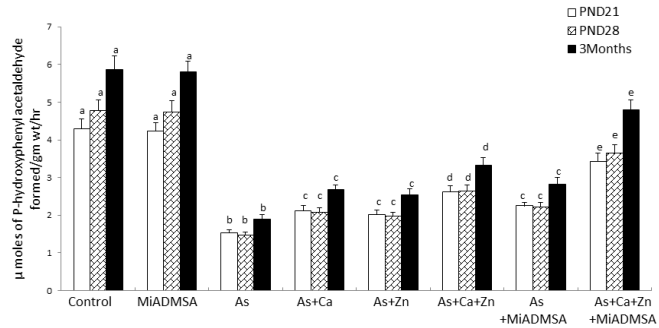


Fig. 3c.



zinc (individual 10 ppm each or combined 5 ppm each) together with As from gestation day 6 to PND 21. MiADMSA was given to pups from PND 18 to PND 20, either individually (50 mg/kg b.wt) or in combination (25 mg/kg b.wt) with calcium and zinc to As. Values represent Mean ± S.D (n=6). The 0.05 level of probability was used as the criterion for significance. Mean values in a row that share the same superscript do not differ significantly at P <0.05.

Fig. 4a.

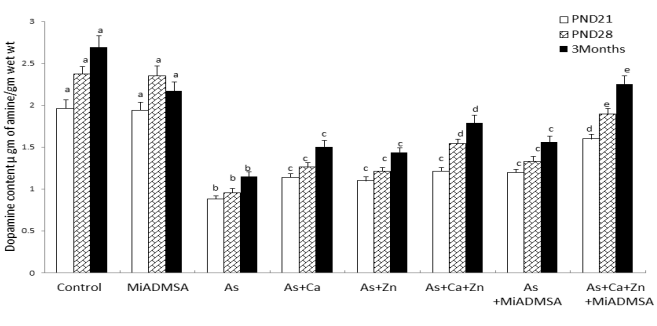


Fig. 4b.

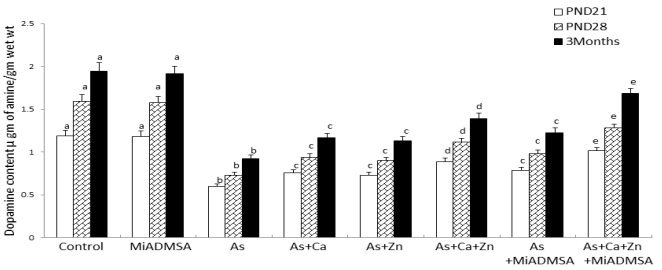


Fig. 4c.

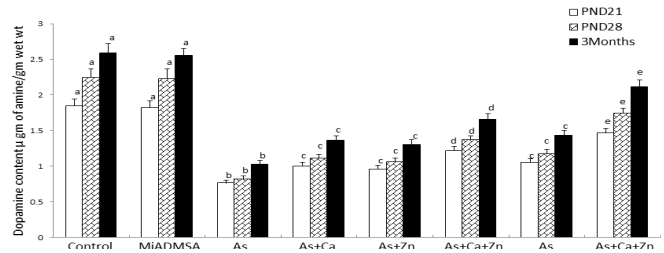


Fig.4. Effect of arsenic on dopamine content in different brain regions (cerebral cortex (a), cerebellum (b), hippocampus (c)) of PND 21, PND 28 and 3 months old rats and the reversal effects of calcium, zinc or MiADMSA, either individually or in combination. Rats were exposed to either deionized water (control) or As (100 ppm) or supplementation of calcium and zinc (individual 10 ppm each or combined 5 ppm each) together with As from gestation day 6 to PND 21. MiADMSA was given to pups from PND 18 to PND 20, either individually (50 mg/kg b.wt) or in combination (25 mg/kg b.wt) with calcium and zinc to As. Values represent Mean ± S.D (n=6). The 0.05 level of probability was used as the criterion for significance. Mean values in a row that share the same superscript do not differ significantly at P <0.05.

Fig. 5a.

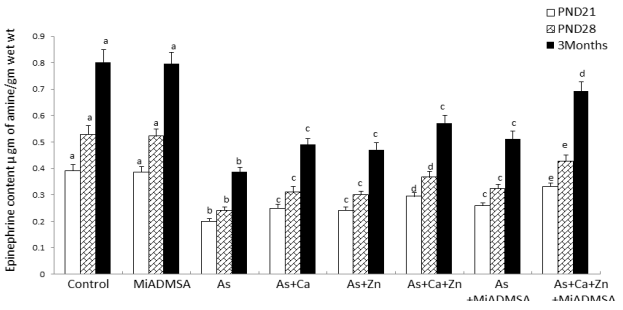


Fig. 5b.

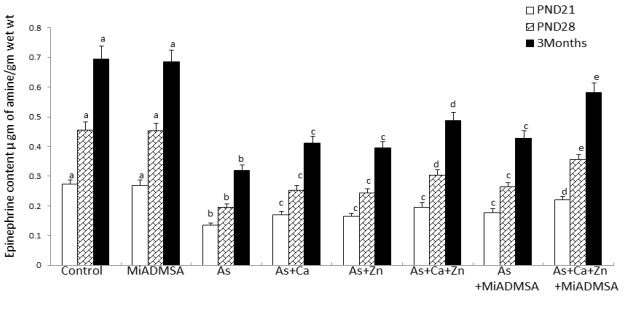


Fig.5c.

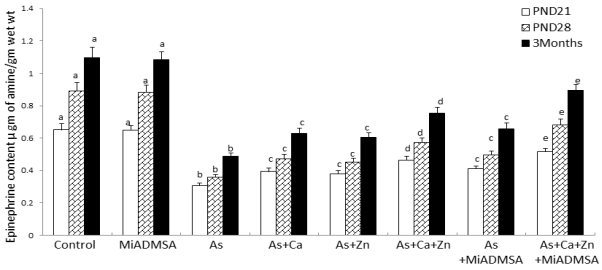


Fig.5. Effect of arsenic on epinephrine in different brain regions (cerebral cortex (a), cerebellum (b), hippocampus (c)) of PND 21, PND 28 and 3 months old rats and the reversal effects of calcium, zinc or MiADMSA, either individually or in combination. Rats were exposed to either deionized water (control) or As (100 ppm) or supplementation of calcium and zinc (individual 10 ppm each or combined 5 ppm each) together with As from gestation day 6 to PND 21. MiADMSA was given to pups from PND 18 to PND 20, either individually (50 mg/kg b.wt) or in combination (25 mg/kg b.wt) with calcium and zinc to As. Values represent Mean ± S.D (n=6). The 0.05 level of probability was used as the criterion for significance. Mean values in a row that share the same superscript do not differ significantly at P <0.05.

Fig. 6a.

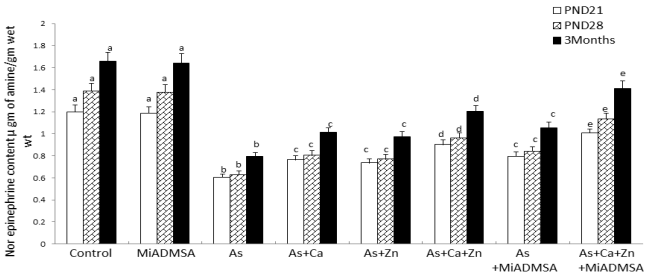


Fig. 6b.

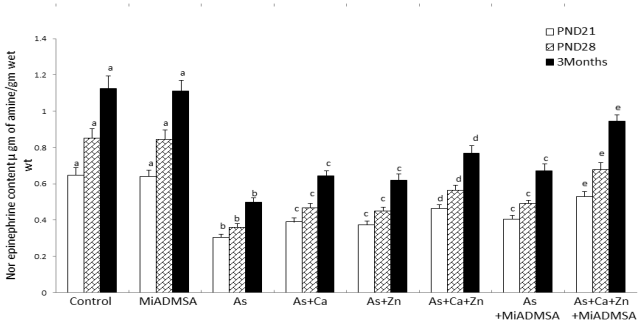


Fig. 6c.

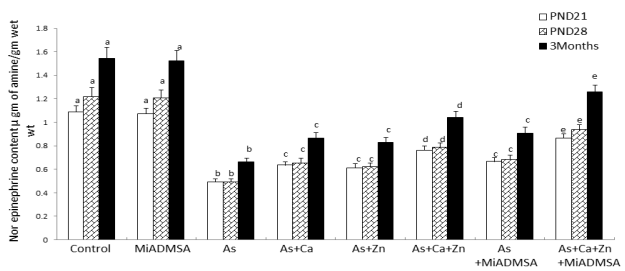


Fig.6. Effect of arsenic on nor-epinephrine in different brain regions (cerebral cortex (a), cerebellum (b), hippocampus (c)) of PND 21, PND 28 and 3 months old rats and the reversal effects of calcium, zinc or MiADMSA, either individually or in combination. Rats were exposed to either deionized water (control) or As (100 ppm) or supplementation of calcium and zinc (individual 10 ppm each or combined 5 ppm each) together with As from gestation day 6 to PND 21. MiADMSA was given to pups from PND 18 to PND 20, either individually (50 mg/kg b.wt) or in combination (25 mg/kg b.wt) with calcium and zinc to As. Values represent Mean ± S.D (n=6). The 0.05 level of probability was used as the criterion for significance. Mean values in a row that share the same superscript do not differ significantly at P <0.05.

References

1. Adil, M., Kandhare, A. D., Ghosh, andBodhankar, S. L. (2016). Sodium arsenite-induced myocardial bruise in rats: Ameliorative effect of naringin via TGF-b/Smad and Nrf/HO pathways. Chemico-Biological Interactions. 253, 66-77.

2. Hitoshi Mochizuki., KhinPhyuPhyu., Myo Nanda Aung., PhyoWaiZin., Yasunori Yano., Moe ZawMyint., Win Min Thit., Yuka Yamamoto., Yoshitaka Hishikawa., KyawZin Than., Masugi Maruyama., and Yoshiki Kuroda. (2019). Peripheral neuropathy induced by drinking water contaminated with low-dose arsenic in Myanmar. Environmental Health and Preventive Medicine.24-23.

3. Chaineau, E., Binet, S., Pol, D., Chatellier, G., and Meininger, V. (1990). Embryotoxic effects of sodium arsenite and sodiumarsenateonmouse embryos in culture. Teratology.41, 105–12.

4. Li, D., Lu, C., Wang, J., Hu, W., Cao, Z., and Sun, D. (2009). Developmental mechanisms of arsenite toxicity in zebrafish (Daniorerio) embryos. AquatToxicol. 91, 229–37.

5. Lindgren, A., Danielsson, B.R.G., Dencker, L., and Vahter, M., (1984). Embryotoxicity of arsenite and arsenate: distribution in pregnant mice and monkeys and effects on embryonic cell in vitro. ActaPharmacolToxicol. 54, 311–320.

6. Danan, M., Dally, S., and Conso, F. (1984). Arsenic-induced encephalopathy.Neurology, 34, 1524.

7. Mukherjee, S.C., Rahman, M.M., Chowdhury, U.K., Sengupta, M.K., Lodh, D., Chanda, C.R., Saha, K.C., and Chakraborti, D. (2003). Neuropathy in arsenic toxicity from groundwater arsenic contamination in West Bengal. India. J Environ Sci Health A Tox Hazard Subst Environ Eng. 38, 165–183.

8. Gerr, F., Letz, R., Ryan, P.B., and Green, R.C.

(2000). Neurological effects of environmental exposure to arsenic in dust and soil among humans. Neurotoxicology. 21, 475–487.

9. Wasserman, G.A., Liu, X., Parvez, F., Ahsan, H., Factor-Litvak, P., Van Geen, A., Slavkovich, V., LoIacono, N.J., Cheng, Z., Hussain, I., Momotaj, H., and Graziano, J.H. (2004). Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect. 112, 1329–1333.

10. Fincher, R.M., and Koerker, R.M. (1987). Long-term survival in acute arsenic encephalopathy.Follow-up using newer measures of electrophysiologic parameters.Am J Med. 82, 549–552.

11. Rodriguez, V.M., Carrizales, L., Jimenez-Capdeville, M.E., Dufour, L., and Giordano, M. (2001). The effects of sodium arsenite exposure on behavioral parameters in the rat. Brain Res Bull. 55, 301–308.

12. Rodriguez, V.M., Carrizales, L., Mendoza, M.S., Fajardo, O.R., and Giordano, M. (2002). Effects of sodium arsenite exposure on development and behavior in the rat.NeurotoxicolTeratol. 24, 743–750.

13. Yadav, R.S., Chandravanshi, L.P., Shukla, R.K., Sankhwar, M.L., Ansari, R.W., Shukla, P.K., Pant, A.B., and Khanna, V.K. (2011).Neuroprotective efficacy of curcumin in arsenic induced cholinergic dysfunctions in rats**.** Neurotoxicology.32(6), 760-8.

14. Zhang, J., Liu, X., Zhao, I., Hu, S., Li, S., and Piao, F. (2013). Subchronic exposure to arsenic disturbed the biogenic amine neurotransmitter level and the mRNA expression of synthetase in mice brains. Neuroscience. 241, 52–58.

15. Praveen Kumar, K., Saritha, S., and Rajarami Reddy, G. **(**2013). Alterations in apoptotic caspases and antioxidant enzymes in arsenic exposed rat brain regions: reversal effect of essential metals and a chelating agent.Environ ToxicolPharmacol. 36(3), 1150-66.

16. Prasanthi, R.P.J., Reddy, G.H., Chetty, C.S., and Reddy, G.R. (2006). Influence of calcium and zinc on lead-induced alterations in ATPases in the developing mouse brain.J PharmacolToxicol. 1 (3), 245–252.

17. Vallee, B.L., and Falchuk, K.H. (1981). Zinc and gene expression.Philos Trans R SocLond B Biol Sci. 294, 185–197.

18. Coleman, J.E. (1992). Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. Annu Rev Biochem. 61, 897–946.

19. Kasarskis, E.J. (1984). Zinc metabolism in normal and zinc-deficient rat brain. Exp Neurol. 85, 114–127.

20. Geer, P.L., and Greenberg, M.E. (2008). From Synapse to Nucleus: Calcium-Dependent Gene Transcription in the Control of Synapse Development and Function. Neuron.846-860.

21. Kalia, K., and Flora, S.J.S. (2005). Strategies for safe and effective treatment for chronic arsenic and lead poisoning.J Occup Health. 47, 1-21.

22. Gersl, V., Hrdina, R., Vavrova, J., Holeckova, M., Palicka, V., Voglova, J., Mazurova, Y., and Bajgar, J. (1997). Effects of repeated administration of dithiol chelating agent sodium 2,3-dimercapto-1-propanesulphonate (DMPS) on biochemical and haematological parameters in rabbits. Acta Med (Hradec Kralove). 40, 3–8.

23. Flora, S. J. S., and Pachauri, V. (2010). Chelation in metal intoxication.Int J Environ Res Public Health. 7, 2745–2788.

24. Flora, S.J.S., Bhadauria, S., Pachauri, V., and Yadav, A. (2012). Monoisoamyl 2, 3-dimercaptosuccinic Acid (MiADMSA) demonstrates higher efficacy by oral route in reversing arsenic toxicity: a pharmacokinetic approach. Basic &ClinPharmacol&Toxicol. 110, 449–459.

25. Flora, S.J.S., GeetuSaxena., and Ashish Mehta. (2007). Reversal of Lead-Induced Neuronal Apoptosis by Chelation Treatment in Rats: Role of Reactive Oxygen Species and Intracellular $Ca2^{+}$. The Journal of Pharmacology and Experimental Therapeutics. 322, 108–116.

26. Lai, J.C., and Clark, J.B. (1979). Preparation of synaptic and nonsynaptic mitochondria from mammalian brain. Methods Enzymol.55, 51–60.

27. Augustinssion, K.B. (1963). Cholinesterase and anticholinesterase agents, Berlin Springer Verlag. 5, 89–128.

28. Ellman, G. L., Courtney, K. D., and Andres, V. Jr., and Feath-erstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Bio-chem. Pharmacol.7, 88-95.

29. Kari, H.P., Davidson, P.P., Herbert, H.H., and Kochbar, M.H. (1978). Effects of Ketoamine on brain monoamine levels in rats. Res CommChem Path Pharamacol. 20, 475–488.

30. Ansell, G.B., and Beeson, M.F. (1968). A rapid and sensitive procedure for the combined assay of

noradrenalin, dopamine and serotonin in a single brain sample.Anal Biochem.23, 196– 206.

31. Green, A.L., and Haughton, T.M. (1961). A colorimetric method for the estimation of monoamine oxidase.Biochem J. 78, 172–175.

32. Hritcu, L., Clicinschi, M., and Nabeshima, T. (2007). Brain serotonin depletion impairs short-term memory, but not long term memory in rats. PhysiolBehav. 91(5), 652–657.

33. Cools, R. (2008). Role of dopamine in the motivational and cognitive control of behavior. Neuroscientist.14(4), 381–95.

34. Schank, J.R., Liles, L.C., and Weinshenker, D. (2008). Norepinephrine signaling through beta-adrenergic receptors is critical for expression of cocaine-induced anxiety. Biol Psychiatry. 63(11), 1007–1012.

35. Bardullas, U., Limón-Pacheco, J.H., Giordano, M., Carrizales, L., Mendoza-Trejo, M.S., and Rodríguez, V.M. (2009). Chronic low-level arsenic exposure causes gender-specific alterations in locomotor activity, dopaminergic systems, and thioredoxin expression in mice. ToxicolApplPharmacol. 239, 169–77.

36. Law, A., Gauthier, S., and Quirion, R. (2001). Say NO Alzheimer's disease: the putative links between nitric oxide and dementia of the Alzheimer's type. Brain Res Rev. 35, 73–96.

37. Giacobini, E. (2003). Cholinesterases: new roles in brain function and in Alzheimer's disease. Neurochem Res. 28, 515–522.

38. Lane, R.M., Kivipelto, M., and Greig, N.H. (2004). Acetylcholinesterase and its inhibition in Alzheimer disease. ClinNeuropharmacol. 27, 141–9.

39. Ehlert, F.J., Kolska, N., and Fairhurs,t A.S. (1980). Altered (3H)-quinuclidnylbenzilate binding in the striatum of rats following chronic cholinesterase inhibition with diisopropyl fluorophosphates. MolPharmacol. 17, 24–30.

40. Zheng, Q., Olivier, K., Wan, Y.K., and Pope, C.N. (2000). Comparative cholinergic neurotoxicity of oral chlorpygrifos exposures in preweanling and adult rats. Toxicol Sci. 55(1), 124–32.

41. Cao, X.J., Wang, M., Chen, W.H., Zhu, D.M., She, J.Q., and Ruan, D.Y. (2009). Effect of chronic administration of melatonin on spatial learning ability and long term potentiation in lead-exposed and control rats. Biomed Environ Sci. 22, 70-75.

42. Aposhian, H.V. (1989). Biochemical toxicology of arsenic. In: Reviews in Biochemical Toxicology (Eds: Hodgson, E., Bend, J.R., Philpot, R.M). Elsevier, New York, 265–299.

43. Xi, S., Jin, Y., Lv, X., and Sun, G. (2010). Distribution and speciation of arsenic by transplacental and early life exposure to inorganic arsenic in offspring rats. Biol Trace Elem Res. 134(1), 84-97.

44. Favier, A.E. (1992). Hormonal effects of zinc on growth in children, Biol Trace Elem Res. 32, 383–398.

45. Wolf, G., Schutte, M., and Romhild, W. (1984). Uptake and subcellular distribution of zinc in brain structures during the postnatal development of the rat.NeurosciLett. 51, 277–280.

46. Takeda A., Sawashita, J., and Okada, S. (1995). Biological half-lives of zinc and manganese in rat brain. Brain Res. 695, 53–58.

47. Cohen, I., and Kloot, V.W. (1985) Calcium and transmitter release. Int Rev Neurobiol. 27, 299–336.

48. Pound, J.G., Long, M., and Rosen, J.F. (1991). Cellular and molecular toxicity of lead in bone. Environ Health. Perspect. 91, 17-32.

49. Six, K.M., and Goyer, R.W. (1970). Experimental enhancement of lead toxicity by low dietary calcium. J Lab Clin Med. 76, 933–942.

50. Mahaffey, K.R., Goyer, R., and Haseman, J.K. (1973). Dose-response to lead ingestion in rats fed low dietary calcium. J Lab Clin Med. 82(1), 92–100.

51. Barton, J.C., Conrad, M.E., Harrison, L., and Nuby, S. (1978). Effects of calcium of the absorption and retention of lead. J Lab Clin Med. 91, 366–376.

52. Flora, S.J.S., Sekhar, K. (2004). Chronic Arsenic Poisonig: Diagnosis and Treatment. In Pharmacological Perspectives of some Toxic Chemicals and Antidotes (S. J. S. Flora and James A Romano, Eds).Narosa Publications, New Delhi.271-302.