Abstract
This study was planned to determine the influence of age on arsenic (As; 10mg/kg body weight given through oral gavage) induced mitochondrial oxidative stress in three different age groups of rats; young (postnatal day [PND] 21), adult (3 months) and aged (18 months) at seven days post-acute exposure. Further, we also evaluated the therapeutic efficacy of essential metal Zinc (Zn; 0.02% through drinking water) and an antioxidant, α-Tocopherol (Vit-E; 125mg/kg body weight through oral gavage) against As-induced neurotoxicity. As exposure lead to a significant decrease in mitochondrial Superoxide Dismutase isoforms (Mn-SOD and Cu/Zn-SOD), Catalase (CAT), Glutathione reductase (GR), Glutathione peroxidase (GPx), non-enzymatic antioxidant levels of glutathione (GSH), total free sulfhydryl groups (TSG) with concomitant increase in lipid peroxidation and GST in different brain regions (cerebral cortex, cerebellum and hippocampus) of different age groups of rats. These effects were more pronounced in cerebral cortex followed by cerebellum and hippocampus. Among the three different age points, aged animals were found to be more vulnerable to the As-induced toxicity as compared to young and adult animals suggesting that As neurotoxicity is age dependent. These As-induced alterations were significantly reversed following supplementation with Zn or Vit-E. However, Vit-E was found to elicit greater protection as compared to Zn in restoring the altered neurochemical perturbations, providing evidence for As induced oxidative damage.

Key words: Age, Arsenic, Zinc, Vitamin-E, Oxidative stress, Brain regions

Introduction
The mechanism underlying aging, an unavoidable biological process that affects most living organisms, is still an area of significant controversy. One of the factors responsible for these diseases includes heavy metals accumulation. Metals are of special concern because of their presence in the environment, and the metals studied, exposure to arsenic (As) contaminated drinking water represents one of the largest public health poisonings in the history of human civilization, affecting tens of millions of people worldwide (1-3).

As is known to be toxic to different organs, including hepatic, hematopoietic, renal, reproductive, nervous system, etc (4). Several recent reports suggest that children and elderly population share higher susceptibility to the toxic effects of As (5-10). Differential toxicity of As may be due to difference in absorption, distribution, metabolism and elimination that varies with age (11).

The exact mechanisms by which As induces toxicity are still being elucidated (12). One possible molecular mechanism involved in the As induced neurotoxicity is the disruption of the prooxidant/antioxidant balance which is

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associated with increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that impair cellular antioxidant defense system and simultaneously damage the critical biomolecules, such as lipids, proteins and DNA (13).

Mitochondria have been a central focus of several theories of aging as a result of their critical role in bioenergetics, oxidants production, and regulation of cell death (14). Moreover, several studies have reported alterations in antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) which can trigger apoptosis in As exposed animals (15). Therefore, the present study was focused on mitochondrial oxidative stress marker enzymes.

Despite the well established toxicity, yet no safe and effective therapeutic management of arsenicosis is available. Essential trace metal such as zinc (Zn) has been studied for the protective values against As. Earlier reports from our laboratory have established the protective role of essential metals (Zn and Ca) in reducing the impact of Pb and As-induced developmental neurotoxicity (14, 16, 17).

Alpha (α) tocopherol is the most abundant and active form of vitamin E in vivo (18, 19) and is an important lipophilic radical scavenging antioxidant. α-tocopherol is a term that encompasses a group of potent, lipid soluble, chain-breaking antioxidants that prevents the propagation of free radical reactions. A number of studies have been carried out to determine the protective effects of vitamin E in different biological models of injury (20, 21).

We hypothesized that As induced neurotoxicity is age dependent and driven by oxidative damage. Therefore antioxidants and essential metals will provide protection against As induced neurotoxicity. In view of this, the present study was designed to study the impact of age on As induced alterations in mitochondrial enzymatic and non-enzymatic oxidative stress markers in the brain regions of albino rats at three different age points (PND [postnatal day] 21: young; 3 months: adult; 18 months: aged). Further, the present study was extended to compare the therapeutic efficacy of essential metal Zn and an antioxidant Vit-E against As induced neurotoxicity.

Materials and Methods

Procurement and maintenance of animals: Three months (adult) and 18 months (aged) old male albino rats (Wistar) were purchased from Sri Venkateswara Traders, Bangalore, whereas PND21 (young) rats were obtained from our animal house by maintaining pregnant rats. Animals of all age groups were maintained in the animal house of Sri Venkateswara University, Dept. of Zoology, Tirupati for at least one week for acclimatization before using for treatments. All the rats were kept in well cleaned, sterilized polypropylene cages lined with paddy husk (18” x 10” x 8”). The animals were maintained under a regulated light: dark 12 h (7:00–19:00) scheduled at 24 ±1°C and relative humidity of 55 ± 15%. Rats were provided standard rat chow (Sai Durga Feeds and Foods, Bangalore, India) and water ad libitum. The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India (CPCSEA, 2003) and approved by the Institutional Animal Ethical Committee (Resoulution No: 09/2012-2013/ia/ CPCSE/IAEC/ SVU/GRR-MRKdt.02-07-2012) at Sri Venkateswara University, Tirupati, India.

Chemicals: The chemicals used in this study were purchased from Sigma Chemicals (St. Louis, MO, USA) and Merck, India. Sodium arsenite (NaAsO₂, >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.

Animals exposure: After a week of acclimatization, rats of all age groups (PND21: young; 3 months: adult; 18 months: aged) were randomly divided into 4 groups and were treated

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for a period of one week as follows:

Group II: Arsenic as sodium arsenite (10 mg/kg dissolved in deionized water, given orally through gavage) (n=6)

Group III: As (10 mg/kg, orally) + Zinc as ZnCl₂ (0.02% through drinking deionized water) (n=6)

Group IV: As (10 mg/kg, orally) + Vit-E, (125 mg/kg body weight, orally through gavage) (n=6).

The control animals which served as group I, received equivalent volumes of deionized water through oral gavage. The dose of sodium arsenite used in this study was based upon the reports of Itoh et al., (22) and Rodriguez et al., (23) in rats and it represents ¼ of the LD₅₀ dose for rats (24). Sodium arsenite solutions were prepared fresh daily and dissolved in deionized water. The Vit-E dose used in this study has no known side effects and is considered a supplemental dose (25). After completion of treatment, animals were sacrificed through cervical dislocation and the brain regions (cerebral cortex, hippocampus and cerebellum) were quickly isolated and stored at -80°C for biochemical analysis.

Preparation of mitochondrial fractions: 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 mM Tris–HCl, and 1 mM EDTA, pH 7.4). The homogenate was first centrifuged at 800 x g for 10 min at 4°C, and then the supernatant was centrifuged at 10,000 x g for 20 min. Then the pellet of mitochondrial fraction was suspended in SET buffer.

Biochemical studies

Superoxide Dismutase (SOD) activity: Measurement of total SOD activity was performed according to Misra and Fridovich, (27) based on the inhibition of auto-oxidation of epinephrine. The total reaction mixture contained 880 μl of 0.05 M carbonate buffer (pH 10.2), 20 μl of 30 mM epinephrine and 100 μl of enzyme source and absorbance was recorded at 480 nm against reagent blank. The enzyme activity was expressed as micromoles of superoxide anion reduced/mg of protein. Mn–SOD activity was determined in the mitochondrial fractions by inhibiting the Cu/Zn–SOD with KCN and subtracting the values from total SOD activity.

Catalase (CAT) activity: CAT activity was measured by following the method of Chance and Maehly, (28). The reaction mixture contained 1.9 ml reagent grade water, 1.0 ml of 0.059 M hydrogen peroxide (H₂O₂) in buffer. The reaction mixture was incubated in spectrophotometer for 4–5 min to achieve temperature equilibration and to establish blank rate if any. 0.1 ml of enzyme (mitochondrial fraction) was added and decrease in absorbance was recorded at 240 nm for 2-3 min. "A₂₄₀/min from the initial (45s) linear portion of the curve was calculated. The enzyme activity was expressed as mmol of H₂O₂ decomposed/mg protein/min.

Glutathione Peroxidase (GPx) activity: GPx activity was determined by following the method of Rotruck et al., (29). Briefly, the reaction mixture contained 0.5 ml of 0.4 mol/l sodium phosphate buffer (pH 7.0), 0.1 ml sodium azide, 0.2 ml reduced glutathione, 0.1 ml H₂O₂, and 0.5 ml of 1:10 diluted aliquot of the enzyme extract and the total volume was made up to 2 ml with distilled water. The tubes were incubated at 37°C for 3 min and the reaction was terminated with 0.5 ml 10% TCA and centrifuged at 4°C for 10 min at 1500 rpm. The supernatant was collected and to this 4 ml of 0.3 mol/l disodium hydrogen phosphate and 1 ml DTNB (dithionitro benzoic acid) (0.004%) reagent were added. The color developed was read at 412 nm against the reagent blank containing only phosphate solution and DTNB reagent. The enzyme activity was expressed as μ moles of GSH oxidized/mg of protein/min.

Glutathione Reductase (GR) activity: GR activity in the mitochondrial fraction of brain regions was assayed as described by Staal et al.
al., (30). The reaction mixture in a final volume of 3.0 ml contained 1.0 ml of 0.3 M Sodium phosphate buffer (pH-6.8), 0.5 ml of 250 mM EDTA, 0.5 ml of 12.5 mM GSSG, 0.7 ml of distilled water, 0.2 ml of 30 mM NADPH and 0.1 ml of enzyme extract. Changes in absorbance were recorded at 340 nm in a spectrophotometer. The enzyme activity was expressed as μ moles of NADPH oxidized/mg protein/min.

Glutathione-S-Transferase (GST) activity: GST activity in the mitochondrial fraction of brain regions was assayed by using 1-chloro-2, 4-dinitro benzene (CDNB) (at 340 nm) as described by Habig et al., (31). The reaction mixture in a final volume of 3.0 ml contained; 150 mM phosphate buffer (pH 7.5), 1 mM CDNB, 5 mM glutathione (GSH) and an appropriate amount of enzyme source. The reaction was initiated by the addition of GSH and incubated at 37°C. The formation of a thioether by the conjugation of CDNB to GSH was monitored at 340 nm in a spectrophotometer (Hitachi model, U-2001). Thioether concentration was determined from the slopes of initial reaction rates. The activity was expressed as μ moles of thioether formed/mg protein/min.

Glutathione (GSH) content: GSH content was determined according to the method of Ellman, (32). The mitochondrial fraction (720 µl) was doubled diluted with distilled water and trichloroacetic acid (5%) was added to precipitate the protein content of the tissue homogenates. After centrifugation at 10000 g for 5 min, the supernatant was collected. To the supernatant, 5,5-dithiobis (2-nitrobenzoic acid solution (Ellman’s reagent)) was added and the absorbance was measured at 412 nm. The values were expressed as millimoles/gm tissue.

Total Sulphydryl Group (TSG) content: TSG content was determined by the method of Sedlak and Lindsay, (33). Briefly, to the homogenate (100 µl), 1 ml of tris-EDTA buffer (0.2 mol/L, pH 8.2) and 0.9 ml of EDTA solution (0.02 mol/L, pH 4.7) was added followed by the addition of 20 µl of Ellman’s reagent. After 30 min of incubation at room temperature, samples were centrifuged and the absorbance was read at 412 nm in spectrophotometer.

Lipid Peroxidation (LPx): The lipid peroxides were determined by the TBA method of Ohkawa et al., (34). The reaction mixture contained 0.1 ml of tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (TBA). The pH was adjusted to 3.5. The mixture was finally made up to 4.0 ml with distilled water and heated at 95°C for 60 min. After cooling under tap water, 0.1 ml of distilled water and 5.0 ml of a mixture of n-butanol and pyridine (15:1, V/V) were added and the mixture was shaken vigorously on a vortex mixture. After centrifugation at 2200 x g for 5 min the upper organic layer was separated and the absorbance was read at 532 nm in a spectrophotometer. The rate of lipid peroxidation was expressed as μ moles of malondialdehyde formed/gm wet wt. of tissue/hr.

Evaluation of As concentration in brain regions: Brain regional As levels were estimated according to the method of Ballentine and Burford, (35). To 50 mg of tissues, 0.5 ml of concentrated nitric acid was added, followed by 0.5 ml of perchloric acid. The sample was then digested over a sand bath until the solution turns yellow in color. If the color of the digest was brown, 0.25 ml each of nitric acid and perchloric acid were added and the oxidation was repeated. The digest was made up to 1 ml volume with deionized water. Aliquots of this were used to estimate arsenic by using atomic absorption spectrophotometer (AAS, Perkin Elmer model AAnalyst 100).

Statistical analysis of the data: Significance of each age group among different treatments was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test using Standard Statistical Software Package to compare the effects among various groups. The 0.05 level of probability was used as the criterion for significance.

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Results

Our results showed that mitochondrial oxidative stress markers such as SOD isoforms (Mn-SOD and Cu/Zn-SOD), Catalase (CAT), Glutathione reductase (GR), Glutathione peroxidase (GPx), glutathione (GSH), total free sulfhydryl groups (TSG) were decreased with concomitant increase in lipid peroxidation and GST in different brain regions (cerebral cortex, cerebellum and hippocampus) of As exposed rats of all the different age points (young: PND21; adult: 3 months; aged: 18 months).

The activities of Mn-SOD and Cu/Zn-SOD in control rats were increased with age documenting maximum activity in adult rats compared with young rats. However, these enzyme activities were decreased in aged rats (18 months). Among the different brain regions studied, the cerebral cortex showed greater activities followed by cerebellum and hippocampus.

Exposure to As significantly decreased the activities of Mn-SOD and Cu/Zn-SOD in all the three brain regions. Decrease in their activities were more pronounced in aged rats (67.36% - cortex; 64.67% - cerebellum; 68.16% - hippocampus) followed by young (61.77% - cortex; 60.43% - cerebellum; 61.25% - hippocampus) and adult rats (47.72% - cortex; 46.12% - cerebellum; 51.75% - hippocampus) with cortex documenting maximum decrease in enzyme activity than cerebellum and hippocampus. The observed decrease in Mn-SOD and Cu/Zn-SOD activities in different brain regions at all the age points was significant at P<0.05 (Fig.1a -2c).

Similar to SOD isoforms, exposure to As significantly decreased the CAT activity in all the three brain regions. Decrease in the CAT activity was more pronounced in aged rats (67.36% - cortex; 64.67% - cerebellum; 68.16% - hippocampus) followed by young (61.77% - cortex; 60.43% - cerebellum; 61.25% - hippocampus) and adult rats (47.72% - cortex; 46.12% - cerebellum; 51.75% - hippocampus) with cortex documenting maximum decrease in enzyme activity than cerebellum and hippocampus. The observed decrease in CAT activity in different brain regions at all the age points was significant at P<0.05 (Fig.3a – 3c).

The GPx activity in control rats was found to be maximum in adults followed by young and aged rats. Among the three brain regions, cortex...
showed maximum GPx activity followed by cerebellum and hippocampus (Fig.4) at all the age points studied. Exposure to As exhibited significant decrease in the specific activity of brain regional GPx and the decrease in activity was found to be greater in aged rats (60.67%, 61.44% and 59.49% decrease in cortex, cerebellum and hippocampus respectively) followed by young rats (55.07%, 55.20% and 54.86% decrease in cortex, cerebellum and hippocampus respectively) and adult rats (51.42%, 48.86% and 45.20% decrease in cortex, cerebellum and hippocampus respectively). From the data, it was found that cerebral cortex exhibited maximum decrease in enzyme activity than cerebellum and hippocampus. The observed decreases in

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enzyme activity in different brain regions of all the age points were significant at P<0.05 (Fig. 4a – 4c).

The GR activity in control rats was found to be maximum in adults followed by young and aged rats. Among brain regions, cortex showed maximum GR activity followed by cerebellum and hippocampus at all the age points studied. Exposure to As exhibited significant decrease in the specific activity of brain regional GR and the decrease in activity was found to be greater in aged rats (51.51%, 50.52% and 49.46% decrease in cortex, cerebellum and hippocampus respectively) followed by young rats (54.87%, 51.96% and 49.21% decrease in cortex, respectively).
cerebellum and hippocampus respectively) and adult rats (39.27%, 41.31% and 39.50% decrease in cortex, cerebellum and hippocampus respectively). From the data, it was found that cerebral cortex exhibited maximum decrease in enzyme activity than cerebellum and hippocampus. The observed decreases in enzyme activity in different brain regions of all the age points were significant at P<0.05 (Fig. 5a – 5c).

Exposure to As exhibited significant increase in the brain regional GST activity levels and the increase in levels was found to be greater in aged rats (61.23%, 58.51% and 57.60% increase in cortex, cerebellum and hippocampus respectively). From the data, it was found that cerebral cortex exhibited maximum increase in enzyme activity than cerebellum and hippocampus. The observed increases in enzyme activity in different brain regions of all the age groups were significant at P<0.05 (Fig. 6).

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respectively) followed by young rats (56.85%, 54.95% and 53.41% increase in cortex, cerebellum and hippocampus respectively) and adult rats (42.06%, 43.79% and 40.94% increase in cortex, cerebellum and hippocampus respectively). From the results, it was found that cerebral cortex exhibited maximum increase in GST activity than cerebellum and hippocampus. The observed increases in GST activity in different brain regions at all the age points were significant at P<0.05 (Fig. 6a – 6c).

Similar to enzymatic antioxidants; the non-enzymatic antioxidants, GSH and TSG levels in control rats were found maximum in the brain regions of adult rats followed by young and aged rats. Among the three brain regions, cortex showed maximum GSH and TSG levels followed by cerebellum and hippocampus (Fig. 7a – 8c) at all the age points studied. Exposure to As exhibited significant decrease in the levels of brain regional GSH and TSG and the decrease was found to be maximum in aged rats (61.03%, 61.58%; 59.83%, 59.30% and 57.46%, 58.07% decrease in cortex, cerebellum and hippocampus respectively) followed by young rats (58.24%, 55.46%; 55.49%, 53.29% and 54.08%, 52.23% decrease in cortex, cerebellum and hippocampus respectively) and adult rats (42.61%, 43.32%; 41.54%, 42.14% and 41.09%, 40.93% decrease in cortex, cerebellum and hippocampus respectively). From the data, it was found that cerebral cortex exhibited maximum decrease in GSH and TSG levels than cerebellum and hippocampus. The observed decreases in enzyme activity in different brain regions of all the age points were significant at P<0.05 (Fig. 7a – 8c).

In the present investigation, thiobarbituric acid substances (TBARS) formation was recorded as a measure of lipid peroxidation in both control and experimental rat brain regions. The TBARS content increased with age in control brain regions with the cortex documenting the highest TBARS content followed by cerebellum and hippocampus at in aged (18 months old) rats. Exposure to As exhibited significant increase in the brain regional TBARS levels and the increase in levels was found to be greater in aged rats (78.42%, 78.09% and 76.92% increase in cortex, cerebellum and hippocampus respectively) followed by young rats (74.02%, 72.22% and 72.09% increase in cortex, cerebellum and hippocampus respectively) and adult rats (51.15%, 50.12% and 50.76% increase in cortex, cerebellum and hippocampus respectively) (Fig. 8a– 8c).

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in cortex, cerebellum and hippocampus respectively). From the results, it was found that cerebral cortex exhibited maximum increase in TBARS levels than cerebellum and hippocampus. The observed increase in TBARS levels in different brain regions at all the age points were significant at P<0.05 (Fig. 9a – 9c).

Brain regional accumulations of As at different age points were shown in Fig. 10. As exposure lead to a significant increase in As content in all the three brain regions at all age points. Maximum increase in As content was observed at PND 21 followed by aged and adult rats. Among the brain regions maximum increase in As levels was found in cerebral cortex.

**Fig. 9.** Effect of arsenic on lipid peroxidation in three different brain regions (a) cerebral cortex, (b) cerebellum and (c) hippocampus and its response to administration of Zn and Vit E in young, adult and aged rats. Rats of all age groups were given deionized water (control), or arsenic (10mg/kg orally), zinc (0.02% through drinking water) and Vit E (125mg/kg orally) for a period of one week. All the values are mean ± SD of six individual observations. All significant values are marked with asterisk (*); non-significant values are marked with (ns) as evaluated by the ANOVA followed by Bonferroni multiple comparisons test (P<0.05).

**Fig. 10.** Arsenic levels in three different brain regions (a) cerebral cortex, (b) cerebellum and (c) hippocampus of young, adult and aged rats. Rats of all age groups were given deionized water (control), or arsenic (10mg/kg orally), zinc (0.02% through drinking water) and Vit E (125mg/kg orally) for a period of one week. All the values are mean ± SD of six individual observations. All significant values are marked with asterisk (*); non-significant values are marked with (ns) as evaluated by the ANOVA followed by Bonferroni multiple comparisons test (P<0.05).

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compared to cerebellum and hippocampus (Fig. 10a – 10c).

We have also evaluated the protective effect of Zn / Vit E against the influence of age on As induced alterations in mitochondrial oxidative stress and brain As levels. Supplementation with Zn / Vit E, significantly reversed the As induced alterations in all the selected enzymatic antioxidant activities (Mn-SOD, Cu/Zn-SOD, CAT, GPx, GR, GST) non-enzymatic antioxidant levels (GSH and TSG), TBARS levels and As content in all brain regions (cerebral cortex hippocampus and cerebellum) of young (PND 21), adult (3 months) and aged (18 months) rats (Figures 1 to 10). However, among Zn and Vit E, Vit E produced greater recovery against As induced alterations in mitochondrial oxidative stress marker enzymes, non-enzymatic antioxidants and brain As levels compared to Zn, suggesting oxidative damage as the principal mechanism underlying As induced neurotoxicity.

**Discussion**

The current study was designed to determine the influence of age on As-induced alterations in brain regional (cerebral cortex, hippocampus, cerebellum) antioxidant system at different age group rats and further to compare the therapeutic efficacies of Zn and Vit-E against As induced neurotoxicity. As is known to cross blood–brain barrier and produce neurotoxic effects (23, 36). In the present study, As exposed rats showed a significant accumulation of As in brain compared to controls leading to possible neurotoxic effects. These increased As levels in rat brain regions exposed to As indicate that As and its metabolites are able to pass through the blood brain barrier (BBB), which is consistent with the results of Zheng et al., (37).

Among various anti-oxidative mechanisms in the body, SOD is thought to be one of the major enzymes which protects against tissue damage caused by the potentially cytotoxic activities of radicals (38, 39). In our current study, As exposure resulted in a decrease in brain regional SOD isoforms activity at all age groups compared with respective controls. The decrease in SOD isoforms activity may be attributed to enhanced superoxide radical production during As metabolism (40, 41). Moreover the effect of As on SOD has also been attributed to (i) altered SOD expression (ii) modification of cellular antioxidant uptake, GSH and vitamin depletion or (iii) alteration in an antioxidant activity by affecting their structure (oxidation/reduction of thiol group and displacement of essential metals) (42).

Among the three different age groups studied, the activity levels of SOD-isoforms were significantly decreased in aged group rats followed by young and adult rats. This age-related decrease in SOD-isoforms activity in brain specific regions documented in the present study was in corroboration with the findings of Carrillo et al., (38). The possible mechanism suggested is that, an increase in arachidonic acid turnover (e.g. increase in prostaglandin synthase activity) may play a role in the increased oxygen radical load (43). Sawada and Carlson, (44) reported that superoxide radical formation increases with age, therefore a decreased protection against toxic radicals may have serious consequences for the aging brain.

By analysis of the Mn-SOD activity of the three brain regions at different ages, we found that the activity was significantly decreased in the cerebral cortex of aged rats. These results indicate that the cerebral cortex may suffer from higher oxidative stress during aging. Benzi et al., (45) and Carrillo et al., (38) also demonstrated that the activities of antioxidant enzymes, total SOD, and Mn-SOD decreased with aging in the cerebral cortex followed by cerebellum.

CAT has been shown to be responsible for the detoxification of significant amounts of $\text{H}_2\text{O}_2$. From the present data, it is evident that As exposure significantly decreased CAT activity at all age groups compared with their respective controls. CAT requires NADPH for its regeneration from its inactive form. The activity
of glucose-6-phosphate dehydrogenase (G6PDH) decreases with advancing age. As the level of NADPH depends on that of G6PDH, a decrease in the activity of the latter affects the levels of the former. The paucity of NADPH production along with increased superoxide radicals during As exposure also decreases catalase activity (46, 47). The decreased NADPH production during As exposure also decreases catalase activity (46, 47). Among the different age groups, As exposure lead to a significant reduction in catalase activity of aged rats, followed by young and adult rats. The gradual decrease in catalase with increasing age appears to be due to an age-dependent change in the expression of the related genes (48).

Glutathione-related enzymes, such as glutathione peroxidase (GPx) and glutathione reductase (GR), function as antioxidants either directly or indirectly, whereas glutathione-S-transferase (GST) plays an important role in metabolic detoxification. GPx metabolizes peroxides such as H₂O₂ and protects cell membranes from lipid peroxidation. GPx is a Se-dependent enzyme and effect of As on synthesis and activity of selenoenzymes has been previously reported. Similarly, in the present study, a significant reduction in GPx activity was observed upon exposure to As at all age groups studied. As may directly interact with Se and form insoluble and inactive As–Se complex (49, 50) rendering it unavailable and ultimately resulting in the inhibition of GPx activity or alter the expression and synthesis of selenoproteins like GPx (51, 52).

The activity of GPx was found to be less in brain regions of aged rats suggesting that aged are most sensitive to oxidative stress than young and adult controls. Our results are in agreement with Brannan et al., (53) who observed a similar decrease in the activity of GPx as well as SOD during aging. In addition, during aging considerable increase in the production of H₂O₂ and a decrease in the activity of GPx were reported. The lowered GPx activity in aging may also be attributed due to the decline in glutathione (GSH) concentration as observed in the present study.

GR is an important enzyme for maintaining the intracellular concentration of reduced glutathione. From the results, it is evident that, As exposure lead to a significant decrease in GR activity at all age groups compared with respective controls. Both arsenite and its methylated metabolites have been known to be potent inhibitors of GR in vitro (54-56); thus, exposure to arsenicals would compromise the antioxidant mechanisms by utilizing GSH and inhibiting the enzyme responsible for its recycling and eventually leading to cell death.

Our data on decrease of GR further gets support from the fact that trivalent arsenicals are potent inhibitors of GR (54), which can cause inhibition by the interaction of As with critical thiol groups in these enzymes (57). However, in the present study, a significant decrease in GR activity was observed in the brain regions of aged rats compared to the young and adult rats. The significant reduction in the activity of GR in the brain of aged rats may be associated with age-related depletion of GSH in various brain regions (58).

GST is believed to participate in the detoxification process by catalyzing the conjugation of electrophilic xenobiotics to GSH, by binding various ligands covalently and non-covalently and by expressing GPx activity towards lipid hydro-peroxides. Exposure to As lead to an increase in GST activity in all the brain regions of all the age groups studied. Increased GST activity following As exposure suggests a counteracting mechanism adopted by system to eliminate As. In one of the study conducted by Kim et al., (59), GST activity increased 1.7 fold in the brains of 9-month-old rats compared with those of 5-week-old rats. In our current study, GST was increased significantly in all the three brain regions of aged animals compared to other two age groups. Elevation in GST activity is assumed to respond to aging as an adaptive mechanism secondary to increases in oxidative stress.

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Reduced glutathione (GSH), a non-protein thiol, plays an important role in the detoxification via facilitating removal of As from the cellular sites and stimulating excretion of methylated As (60), which is responsible for As-induced cytotoxicity. From the experimental results, it is evident that As exposure significantly depleted GSH levels at all age groups compared with their respective controls. Among the different age groups, As exposure lead to a significant reduction in GSH levels of aged rats, followed by young and adult rats. As-induced GSH depletion may be attributed to its direct binding with GSH due to its high thiol binding affinity and electrophilic nature of GSH. Indirect depletion of GSH by As may also due to its utilization in scavenging of As-induced free radical generation. This could be due to the utilization of GSH as electron donor for As metabolism or direct binding due to thiol preference. An age-associated decline in GSH level has been reported in the brain tissue of various species (61-63), which are consistent with the findings of current study. Maiti and Chatterjee (64) also suggested that depletion of GSH may also be caused due to the GPx mediated excess utilization of GSH.

Glutathione and total thiols (sulfhydryl groups) non-enzymatically reduce peroxides and/or prevent occurrence of peroxidation. In the present study, a significant decrease in the sulfhydryl groups was noticed in aged rats. The recycling of GSH from GSSG (oxidised glutathione) is catalyzed by the enzyme glutathione reductase using NADPH as a cofactor. NADPH is generated in a pathway involving the enzyme, G6PDH. Age-associated decline in the activity of G6PDH leading to diminished production of NADPH may be the cause for the observed decrease in sulfhydryl groups.

LPx measured as TBARS in the present study forms the most common indices to assess oxidative stress. These peroxidation products of lipids, usually polyunsaturated fatty acids are susceptible to the attack of free radicals forming an important biomarker (65). In the present study, significant increase in LPx was observed in young and old rats, compared to the adult rats. Increased lipid peroxidation in response to As is thought to be a consequence of oxidative stress, which occurs when the dynamic balance between peroxidant and antioxidant mechanism is impaired. The results of this study also support the correlation of increased TBARS level with the decreased antioxidant defense system due to arsenic toxicity.

Among the brain regions studied, the activity levels of oxidative stress marker enzymes were found to be depleted to a greater extent in cortex, followed by cerebellum and hippocampus, indicating cortex is highly sensitive to oxidative stress. Certain brain regions such as cortex and striatum are highly enriched with non-heme iron, which is catalytically involved in the production of free radicals (66). The relative sensitivity of cortex when compared to cerebellum and hippocampus may be attributed to the differences in the iron content which influence the generation of ROS.

The present investigation, however, showed that these alterations in mitochondrial oxidative stress markers induced by As could be reversed by administration of essential metal, Zn or antioxidant, Vit E. This may be attributed due to the fact that this essential trace element can affect toxicity of heavy metals by interacting at the primary site of action and/or this trace element 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 (16, 67). Treatment with vitamin E reduced As induced alterations, probably through its capacity to quickly and efficiently scavenge lipid peroxyl radicals before they attack membrane lipids. The reaction of \( \alpha \)-tocopherol with free radicals generates tocopherol radicals, which can be reduced by vitamin C or GSH (68), thereby reducing ROS. Since Vit-E is primarily located in cell membranes, it is possible that it modifies the kinetics of distribution of As in cell membranes.
such that its delivery to neuronal cells may be impeded.

Among Zn and vitamin E, Vitamin E supplementation produced significant reversal effect and found to be a better therapeutic agent against As induced neuronal oxidative damage at all the age points. Our findings are consistent with the view that protective effects of Vit-E against As exposure might be due to its antioxidant properties, and this may have a major impact on improving the quality of life of individuals suffering from neurotoxicity caused by As exposure. However, further studies have to be carried out to see whether this type of therapy can be advocated as safe and effective treatment for As induced neurotoxicity at all the age points of life.

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