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RESEARCH ARTICLE

Green Tea (Camellia sinensis) Protects Against Arsenic Neurotoxicity *via* Antioxidative Mechanism And Activation of Superoxide Dismutase Activity

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Abstract: ***Background*:** Chronic arsenic-exposure even at a low-dose results in the neural impairment and motor/cognitive dysfunction. However, several preventive approaches are made mainly against hepatic/gastrointestinal damages. Only a few investigations postulate therapeutic strategies for neural anomalies. Here, the protective role of Green tea (*Camellia sinensis or* CS; 10mg/ml aqueous) has been evaluated against arsenic-induced (0.6ppm/100g bw/28 days) cerebral/cerebellar tissue degeneration, oxidative-threats and neurotransmitter deregulation in female rats.

***Methods and Results*:** The Dunnett's *t* test and multiple-comparison ANOVA-test suggest that arsenic significantly decreased free thiol level with an increase in lipid-peroxidised product and damages to the tissue-structure. A significant decrease in serum urate accompanied by increases in C-reactive protein and TNF-α, an acute-phase inflammatory cytokine, strongly suggests a possible mechanism of oxidative-inflammatory tissue injury being supported by the increase in lactate-dehydrogenase activity. In addition, suppression in cytosolic superoxide-dismutase (Cu-Zn isoform/SOD1; NBT reduction-test) and an insufficient protection through catalase activity culminate free radical-related damages. *In-vitro*, H_2O_2 inactivated partially-purified (dialyzed/concentrated, 6-8kd cutoff-Millipore) rat liver SOD1 and that was markedly protected by 2-mercaptoethanol. Though significant signs of toxicities were noticed at biochemical/cellular level, the present treatment did not affect DNA (DNA-fragmentation assay) in the brain tissues. The CS supplementation significantly protected serum/tissue antioxidant-components, prevented inflammatory-responses and decreased lipid-peroxidation in brain resulting in increased tissue integrity. Moreover, arsenic-induced impairment of neurotransmitters *i.e.* glycine, glutamate and aspartate levels in cerebral tissue were significantly restored in CS-supplemented group.

***Conclusion*:** Taken together, this investigation indicates the potent neuroprotective and antioxidative efficiencies of *Camellia sinensis* against arsenic-induced oxidative threat.

ARTICLE HISTORY

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INTRODUCTION

Arsenic is a group-I toxicant and a co-carcinogen which has been shown to increase the cognitive dysfunction even at a lower concentration [1]. It has been shown to have a more intense effect in children [2]. Chronic arsenic exposure induces a significant deficit in the long term memory in children [3]. Inorganic arsenic modulates locomotor activity, and behavioral task, suggesting the impairment of sensory and motor nerves [4]. Epidemiological studies reveal that chronic arsenic exposure through drinking water causes cerebral infarction, microvascular diseases and impairment in neural conduction [5]. A significant elevation of malondialdehyde level in response to arsenic exposure is noticed with a decrease in mitochondrial- and cytosolic- superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase activities. These alterations were more pronounced in cortex compared with the cerebellum and hippocampus regions [6]. Subchronic level of arsenic exposure can affect the level of monoamine neurotransmitters in mice brains. Arsenic can downregulate the concentrations of norepinephrine (NE), dopamine (DA), mono-

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amine oxidase (MAO) and 5-OH tryptamine (5-HT) in the cerebrum or cerebellum of mice. Arsenic increases acetylcholinesterase in brain tissue [7, 8]. The oxidative damage results in structural deformities in the myelin sheath of nerve fibers and degradation in the terminals of the mossy-fibers impairing synaptic function and spatial memory [9]. Mechanistically, an elevated export of GSH and accelerated consumption of cellular glucose resulted in lactate production [10]. In addition, arsenic-treated astrocytes revealed a higher toxic potential of arsenite compared to arsenate, accompanied by a loss of total cellular glutathione by an increase in the cellular glutathione disulfide content [11]. It was evident from animal experiments that arsenic could pass through the blood-brain barrier and invades the brain parenchyma [12]. Arsenic exposure renders the brain tissue vulnerable to the free-radical attack resulting in apoptosis in the neural cells. The report suggests that arsenic exposure to mouse litters increased neuronal necrosis and mitotic impairment resulting in cerebellar immaturity [12]. However, arsenic induced neurotoxicity is not fully elucidated and its therapeutic outcome is out of reach [13]. Some inconclusive findings are available on therapeutic strategies against arsenic-related neurotoxicity. Arsenic-induced cholinergic deficits in rat brain could be protected by curcumin [14]. Report reveals a better therapeutic efficacy of nano-particulated (monoisoamyl dimercaptosuccinic acid) nano-MiADMSA in removing arsenic burden from the brain and liver [15]. Neuroprotective effect of Resveratrol, nanoparticulate-curcumin and Trichosanthes dioica fruit is reported on arsenic-induced oxidative stress in feline brain, kidney and brain of rats [16-18]. Therefore, it indicates that an adverse effect of arsenic on bio-macromolecule may be mitigated by the intervention of antioxidants. Tea polyphenols have chemopreventive properties against cancer [19, 20]. Recent reviews suggest protective role of natural products isolated from different herbs/plants in protection against heavy metals (cadmium, lead, arsenic, and mercury) mediated neurotoxicity [21, 22]. In this background, we aimed to determine the efficacy of *Camellia sinensis* (green tea) leaf extract against arsenic mediated cerebrum and cerebellum toxicity in experimental rat model.

METHODS

Preparation of Green Tea (*Camellia sinensis* or CS) Aqueous Leaf Extract

The leaf dust of green tea was collected locally and dried in an incubator for 2 days at 40°C, crushed and then powdered in an electric grinder. Extraction was performed by taking 500 mg powder in 50 ml of distilled water in a Soxhlet apparatus, and a deep brown aqueous extract was obtained [23].

Animal Selection and Treatment

Female albino rats weighing 150 ± 10 g were acclimatized for 10 days at 12-hour light-dark cycle, 32°C ± 2°C temperature and in 50%-70% humidity in the institutional animal resource facility. Those were fed with a standard pellet diet (Hindustan Lever Ltd, Mumbai, India) and water ad libitum. Studies were carried out in accordance with the National Institutes of Health (NIH), USA guidelines and the Institutional Ethical concerns were maintained throughout the investigation. Ethical approvals were collected from the concerned institutes before the commencement of the investigation.

Rats were randomly distributed in 3 groups having 6 in each. Animals of Group-II and Group-III were fed with 0.5 ml drinking water containing sodium arsenite at a concentration of 0.6 ppm / 100 g b.w. / day for 28 days. After conducting several dose-response studies the present treatment schedule was employed [23]. The remaining group (I) designated as control was supplied with the same amount of drinking water for the stipulated duration. The Group-III animals were supplemented with lyophilized 1 ml extract of CS by gavages at a concentration of 10 mg/ml/100 g body weight / day for 28 days [24]. On the day 29, animals were exposed to light anesthesia (by ether) and blood was collected using disposable syringe (21-gauge needle) and the serum was separated. The cerebrum and cerebellum portion of the rat brain were carefully dissected and stored at -40°C until use.

Evaluation of General Toxicity

Serum lactate dehydrogenase (LDH), C-reactive protein and uric acid were measured from the rats of different groups by standard protocol with the assay kits (Ranbaxy, India or other reputed company).

Estimation of Malondialdehyde (MDA) Levels

The Cerebrum and cerebellum tissues were homogenized (10 % w/v) in the ice-cold phosphate buffer (0.1 mol /L, pH 7.4) and the homogenate was centrifuged at 10,000 x g at 4°C for 45 min. The supernatant was used for the estimation of MDA. The MDA assay was conducted following the protocol as described in Buege and Aust (1978) [25], with a slight modification [26]. The level of MDA was calculated utilizing the molar extinction coefficient of MDA (1.56×10^5 cm^2/ mmol).

Estimation of Amino Acid Neurotransmitter by Thin Layer Chromatography (TLC)

The supernatant of rat-cerebrum from different groups were run along with control amino acids to detect biogenic amines (neurotransmitter; glycine, glutamate and aspartate) on the TLC plate with the proper solvent (butanol-1:acetic acid:water = 3:1:1). The chromatogram was developed with 0.2% ninhydrine in acetone.

Assay of Cytosolic Super Oxide Dismutase (Cu-Zn, SOD1) and Catalase Activities

A tablet of nitro blue tetrazolium (NBT) was dissolved in 30 ml of water and the non-denaturing (10%) acrylamide gel was soaked in it for 30 minutes with shaking. The gel was then shaken in 40 ml SOD solution (0.028 M tetramethylethylenediamine (TEMED), 2.8×10^{-5} M riboflavin, and 0.036 M potassium phosphate at pH 7.8) for 15 min. The soaked gel was placed on a clean acetate sheet and was illuminated for 5 to 15 min. The gel became purple except at the position containing SOD1 [23]. The gel was scanned when the maximum contrast between the band and background has been achieved.

Catalase activity was assayed by a colorimetric method [27]. Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm. One unit of activity was expressed as one mole of H_2O_2 consumed/min/mg protein.

Estimation of Non Protein Soluble Thiol (NPSH)

The NPSH in cerebrum and cerebellum homogenates (prepared in 0.1M phosphate buffer, pH 7.4) were determined by the standard DTNB (5, 5'- dithiobis-2-nitrobenzoic acid) method with a slight modification [28]. In brief, the protein was precipitated by trichloroacetic acid and the clear cytosol was added to 0.1M sodium phosphate buffer containing 5 µM DTNB. The level of NPSH was determined against a GSH standard curve.

Estimation of Serum TNF-α Level

Serum TNF-α level was quantified by ELISA using a suitable antibody (BioLegend) following the supplier's and published protocol [29].

Histology and DNA Fragmentation Analysis

Cerebrum and cerebellum tissues were sectioned at 5 µM using cryocut (LEICA CM1850 CRYOSTAT) and stained with eosin, hematoxylin (Harris) and observed under polarized microscope (Nikon, Eclipse LV100, magnification 400 x) to study the histoarchitechture.

Cerebrum and cerebellum tissues were used for DNA preparation by standard protocol. The tissue was treated with 500 µl of lysis buffer (50 mM Tris pH 8.0, 20 mM EDTA, 10 mM NaCl, 1% SDS, 0.5 mg / ml proteinase K) for 20 min on ice (4°C) and centrifuged in cold at 12,000 g for 30 minutes. The supernatant was extracted with 1:1 mixture of phenol: chloroform with gentle agitation for 5 min followed by centrifugation and precipitated in two equivalence of cold ethanol and one-tenth equivalence of sodium acetate. After spinning down and decanting, the precipitate was resuspended in 30 µL of deionized water-RNase solution [0.4ml water + 5 µL of RNase] and 5 µL of loading buffer for 30 minutes at 37°C. A 0.8% agarose gel with ethidium bromide was run at 5V for 5 min before increasing to 100V and documented in gel documentation system [30].

In vitro Regulation of SOD1 Activity

The post-centrifuged (12,000×g) cytosolic fraction was prepared from control rat hepatic tissue homogenate, and that was cleaned by dialysis membrane (Spectrum Lab, USA) and concentrated with the Amicon centrifugal filter units (Millipore, USA, 6-8 kd MWCO) to negate small molecular interferences. The concentrated fraction was incubated with different concentration of only H_2O_2 (15mM - 100mM) or in combination with 2-mercaptoethanol (1µM - 30µM + H_2O_2). The cytosolic (Cu-Zn) SOD known as SOD1 activity was tested by NBT test in polyacrylamide gel as described earlier [23].

Statistical Analysis

Statistical analysis was performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA). Serum parameters and neural tissue thiol/free-radicals status and their comparison between several groups were tested by Dunnett's t test and ANOVA analysis. Data from supplemented group was compared to that of control by utilizing ANOVA followed by multiple comparison two tailed 't' test. Dunnett's t test was used to compare the characteristics of the different treatment categories and their relation to the control group. Comparative analysis of the catalase activity and serum TNF-α was done utilizing the students 't' test.

RESULTS

General Observations

No significant alteration of food and water consumption is noticed in all groups of animal during the experimental schedule. At the end of the experiment, general somatic or hepato somatic growth of arsenic-treated and CS co-administered groups of rat did not differ significantly from the control group (Table **1**). Here, organo-somatic indices refer to the organ weight in gram per 100g of animal body weight. This parameter represents the general health and toxicity status of the animal.

General Toxicity and Metabolic Inflammatory Outcome

Arsenic-induced increase in necrotic tissue marker LDH is markedly restored by CS up to the control level (Dunnett's t test $p<0.01$, $F = 4.031$ for ANOVA test). An important metabolic inflammatory marker CRP is significantly overexpressed (Dunnett's t test $p<0.01$, $F = 4.483$ for ANOVA test) by arsenic and that is prevented by CS (Fig. **1**). Serum uric acid (~50% decrease in arsenic group) and NPSH level were also regained by CS co-treatment, which was moderately decreased after arsenic treatment (Fig. **1**).

Table 1. Effect of green tea (GT) aqueous extract on somatic growth and hepato- and reno-somatic indices in arsenic exposed rats (mean+SE, n = 6).

Condition	Body Weight (gm)		Organosomatic indices (g %)	
	Initial	Final	Liver	Kidney
Control	138.75± 12.55	160± 10.55	3.423± 0.15	0.797± 0.02
Arsenic treated	142± 14.39	158± 16.42	3.353± 0.11	0.727± 0.04
Arsenic + GT	138± 9.54	162± 11.57	3.89± 0.09	0.752± 0.05

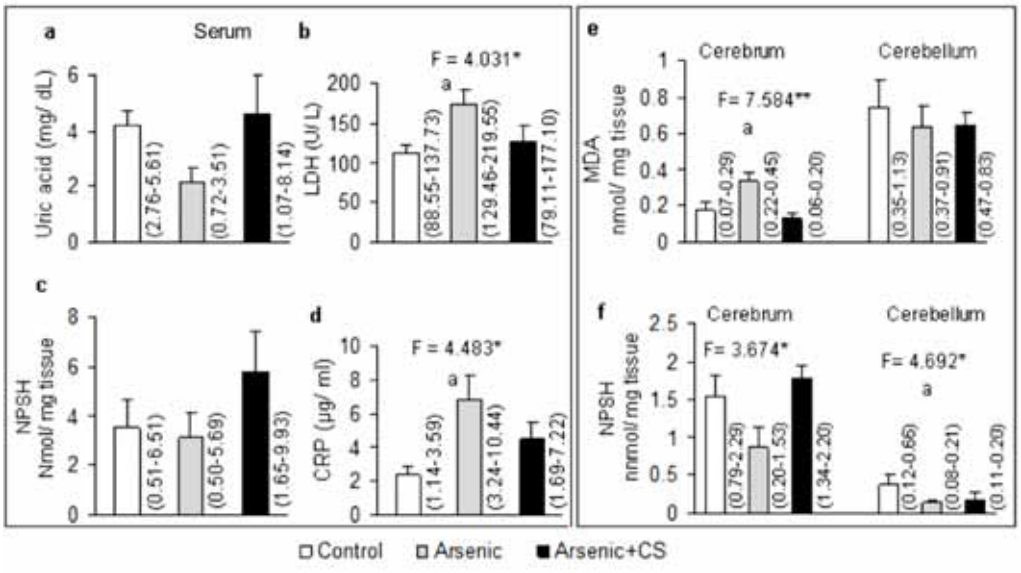

Fig. (1). Analysis of variance (ANOVA) for the multiple comparison (*p<0.05, **p<0.01) and Dunnett's t test ([a]p<0.05) were performed to characterize the comparison amongst different treatment groups of serum and brain parameters. Values in the parenthesis denote the 95% CI.

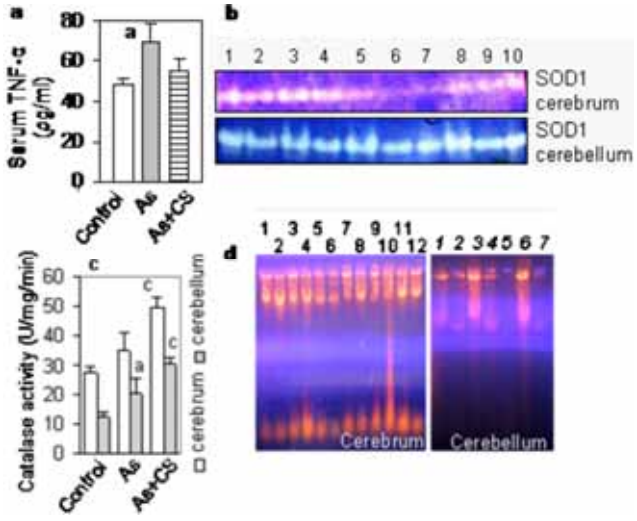

Fig. (2). Cytosolic SOD activity of cerebrum and cerebellum cells is shown on a polyacrylamide gel. The acrylamide gel containing concerned cytosol was soaked in NBT and is then shaken in SOD solution (TEMED, riboflavin, and potassium phosphate at pH 7.8) min. The soaked gel is placed on a clean acetate sheet and illuminated under UV, and the transparent SOD-activity band is visualized. The gel is scanned when the maximum contrast between the band and background has been achieved. (Please refer to the material methods section). (**b**) SOD- Lane distribution; 1 to 4- control, 5 to 7- arsenic treated, 8 to 10- arsenic + CS treated. (**d**) Effect of CS extract on DNA fragmentation in cerebrum and cerebellum tissue DNA. Lane distribution: Cerebrum: Lane; 1 to 4- control, 5 to 8- Treated with sodium arsenite, 9 to12- Sodium arsenite + CS. Cerebellum: Lane; 1 to 3- control rat, 4 to 6- Treated with sodium arsenite, 7 to 10- Sodium arsenite+CS. Picture shows that arsenic is not able to influence the DNA laddering pattern. (**a**) Serum TNF α was determined by a standard method. TNFα elevated in the As treated group compared to control and was recovered in As+ CS group. (**c**) Catalase activity was determined by a standard method. Catalase activity elevated in As group compared to control and was further increased in As+CS group compared to both Control and the As group.

Status of Oxidative Stress Markers

In the present study we examined the effects of arsenic on the antioxidant status of rat cerebral cortex and cerebellum. The MDA content in cerebrum increased significantly (Dunnett's t test $p<0.05$, $F = 7.584$ for ANOVA test) in the sodium arsenite-exposed rats. However, administration of CS extract in addition to arsenic prevented the elevation of MDA when compared to the arsenic only treated group. The NPSH level decreased in arsenic-treated group and it was regained markedly after the CS co-treatment (Fig. **1**).

The serum level of pro-inflammatory cytokine TNF-α significantly increased ($p<0.05$) in response to arsenic but that level was restored notably in the green tea supplemented group (Fig **2a**). The SOD1 gel-band picture (NBT reduction test, Fig. **2b**) reveals that there is a significant decrease SOD (Cu-Zn isoform, SOD1) activity in cerebrum and cerebellum in arsenic treated rats when compared to control group. Restoration of SOD1 and increase in catalase activities (Fig **2c**, $p<0.001$) are observed in the CS and arsenic combination group when compared to the control group. Results of agarose-gel electrophoresis of cerebrum and cerebellum DNA from different experimental groups showed no significant DNA ladder in any group with comparison to arsenic treated group. This suggests that the present schedule of arsenic treatment was not able to initiate DNA toxicity in rat neural tissues (Fig. **2d**)

In the *in vitro* study (Fig. **3**), a significant and gradual decrease in SOD1 activity is noticed in reponse to application of increasing concentration of H_2O_2 (upper panel, lane 3-5 and lower panel lane 3-5) which is markedly and progressively reversed (against highest concentration, 100 mM of H_2O_2) by the increasing concentration of thiol substance 2-ME (lower panel, lane 6-8).

Status of Monoaminergic Neurotransmitters

Present result reveals a significant decrease in the level of three amino acid neurotransmitters; glutamate, aspartate and

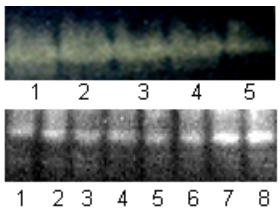

Fig. (3). Redox regulation of rat liver cytosolic superoxide dismutase (SOD1). *In vitro* H_2O_2 inactivation of rat cytosolic SOD1 and its protection by 2- mercaptoethanol. Lane distribution (upper panel): 2 hour incubation; Lane- 1 and 2-Control, 3- H_2O_2 (15 mM), 4- H_2O_2 (50 mM), 5- H_2O_2 (100 mM). Lower panel: Lane -1 and 2-Control, 3- H_2O_2 (15 mM), 4- H_2O_2 (50 mM), 5- H_2O_2 (100 mM), 6- H_2O_2 (100 mM) + 2-ME (1 μM), 7- H_2O_2 (100 mM) + 2-ME(10μM), 8- H_2O_2 (100 mM) + 2-ME (30μM).

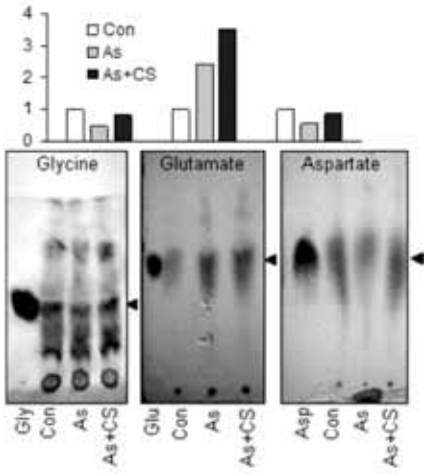

Fig. (4). TLC band of amino acid neurotransmitters with respect to standard amino acid in the cerebrum tissue extract. (**a**) Glycine, (**b**) Glutamate, (**c**) Aspartate.

glycine in cerebrum tissue following chronic arsenic (III) exposure which was restored in CS co-treatment group (Fig. **4**).

Cerebrum Tissue Architecture

Arsenic ingestion with the present dose and duration resulted in the disarrangement of cerebrum and cerebellum tissue. These histological sections were stained with eosin & hematoxilin. For the primary study this staining procedure is very efficient for the studies on tissue histoarchitecture, damages, pycnosis, nuclear condensation/de-condensation etc. Present histopathological data showed tissue swelling and vacuolar degeneration in cytoplasm, karyolysis and karyorrhexis, but the CS co-administration in arsenic-exposed animals shows partial but significant protection which is evident from the histoarchitechture picture (Fig. **5**). In the cortex picture the cell body part of the neurons are shown to be affected notably. The cell surface/ membranes and the origin of the dendrites are shown to be degenerated. The sharp edge of the membrane of cell body which is noticed in the control group are missing arsenic treated group, rather the edge become hazy loosing its sharp border line (higher magnifications are presented in the boxed window). But the GT treated group was found to be protected from this degeneration (Fig. **5**).

DISCUSSION

The mechanism of arsenic-induced neurotoxicity is not well defined. Several metabolites of arsenic are able to pass through the blood-brain barrier and accumulate in the hippocampus and some other locations [13]. This affects the intracellular Ca2+ homeostasis and ultrastructures of hippocampal neurons. Arsenic exposure can alter epigenetic regulations and the signaling of hypothalamus-pituitary-adrenal axis (HPA). However, in the present study arsenic was not able to manifest any significant alteration in the DNA laddering pattern in cerebral cortex and cerebellum tissues. Reports reveal that arsenic can initiate adult neurogenesis. It impairs monoaminergic neurotransmission, which is associated to Alzheimer-related pathologies [2]. In the present study both arsenic induced anomalies and degeneration of cell bodies in the cerebral cortex along with its decisive restrainment by green tea extract has been studied. Earlier Microscopic studies revealed a decreased number of pyramidal neurons in the superficial layers and the presence of polymorphic cells in the subgranular zone of ectal limb of the dentate gyrus [31]. Astrocyte damage has been shown to be associated with myelin disintegration in the central nervous system. The modulation in the levels of myelin basic protein (MBP), myelin-associated glycoprotein and neurofilament (NF) are reported to be associated with arsenite toxicity. It also upregulate vacuolated axons, especially those are present in the corpus-callosum region [32]. Oxidative stress associated neurodegeneration have been reported as the result of arsenic toxicity [33]. This is evident in our present investigation. Since, cerebral and cerebellar DNA is found to be less interfered by arsenic so the arsenic-induced toxicities are mostly attributable at the cellular level in the current study. In our study, decrease in cellular thiol content and the increase in MDA in both neural tissues support this fact. The strong stimulation of GSH export from astrocytes by arsenate may contribute to the arsenic-induced neurotoxicity [33]. However, in our study, we demonstrate strong anti-oxidant capacity of green tea by thiol restoration/upregulation against arsenic toxicity in neuronal tissues. Reports reveal that the glial remains unaffected by ROS mediated degeneration [34] because of its high content of antioxidant-enzymes and glutathione (GSH). This suggests that toxicity and protection both are mainly occurring at the cellular level [35]. Neurons are very much sensitive to oxidative stress for their high lipid content and low amount of protective enzymes and free-radical scavengers, *e.g.* catalase, SOD, GSH and vitamin E [36]. In the present study, arsenic treatment clearly induced cerebral tissue degeneration (Fig. **4**). The tri-valent arsenic produces reactive oxygen species on reaction with H2O2 and produces high level of lipid peroxides and conjugated di-ene [37]. Arsenic also decreases cellular NPSH concentration at a sub-chronic or chronic level of toxicity. The impairment of antioxidant enzymes like SOD and catalase activity has been reported to link with oxidative stress, and our study abides by the existing literature. Redox-regulated possible structural modification and inactivation of the SOD1 protein are much likely occurring in the neuronal tissues of arsenic-treated group. This has also been recently reported from our laboratory in rat liver tissues [38]. Arsenic exposure attributes to oxidative stress by generating ROS, which significantly decreased GSH content in liver, cultured lung epithelial cells

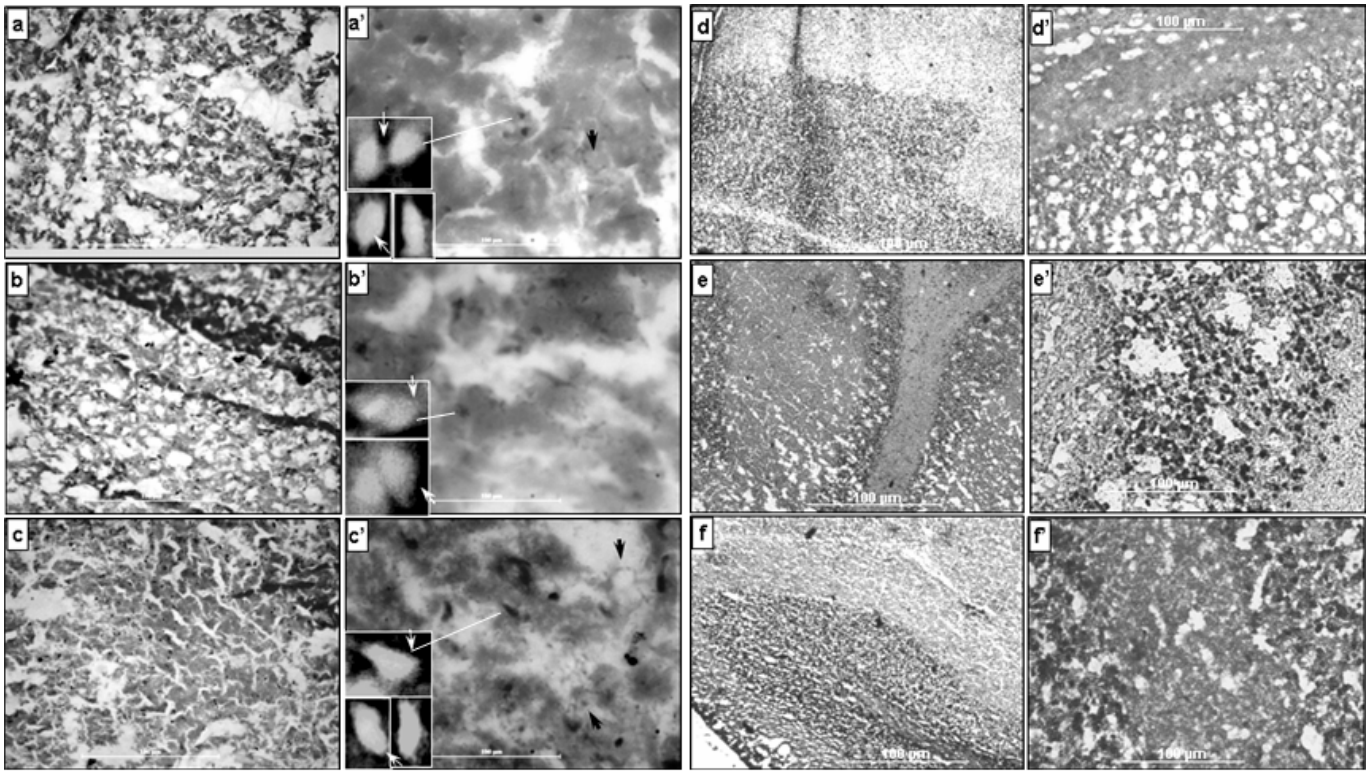

Fig. (5). Cerebrum and cerebellum tissue were sectioned by cryostat microtome, serially sectioned at 5 mM, stained with eosin and hematoxylin (Harris) and observed under a microscope (magnification X 40 and X 100) to study the histoarchitechture (**a-f**). Cerebrum (X40): (**a**) Control rat, (**b**) Treated with Sodium arsenite, (**c**) Sodium arsenite + CS. Cerebrum (X100): (**a´**) Control, (**b´**) Sodium arsenite, (**c´**) Sodium arsenite + CS respectively. Cerebellum (X40): (**d**) Control, (**e**) Treated with sodium arsenite (**f**) Sodium arsenite + CS. Cerebellum (X100) : (**d´**) Control, (**e´**) Treated with sodium arsenite, (**f´**) Sodium arsenite + CS.

and in discrete brain areas [39-41]. These findings corroborate with our present result of NPSH, MDA content as well as catalase and SOD activities. Thus it is clearly evident from both earlier and our present study that arsenic induces oxidative stress. And our study clearly shows that this oxidative stress can be potentially restrained by green tea extract in brain tissue. Natural polyphenols present in tea serve as excellent antioxidants. Green tea quenches the excessive production of ROS, reduce the elevated levels of lipid peroxidation and enhance the activity of antioxidant enzymes such as catalase, SOD and GPx [37].

The oxidative stress, increased the expression of pro-apoptotic proteins and decreased anti-apoptotic proteins in frontal cortex and hippocampus [42]. Alteration in the antioxidant components like SOD and catalase, as noticed in this study has been linked to the cellular apoptotic signaling introduced by transcriptional factors and/or mitochondrial factors. The ratio of Bax/Bcl-2 in the high dose arsenic group (50mg/L) was significantly higher than that of the control group, thus initiating apoptosis in liver and brain tissues [43]. Reports reveal that inorganic arsenic-induced oxidative stress can cause cellular apoptosis in the cerebrum and cerebral locations [44]. These may be the reason behind the cerebrum and cerebellum tissue disarrangement and degeneration, in our present study. Green tea extract was found to be efficient even in the restoration of the cellular morphology and tissue histoarchitechture.

Reports reveal that arsenic can initiate inflammatory responses by increasing proinflammatory cytokines (IL-6 and TNF-α), [45] which has been noticed in the current study. But the green tea supplementation remarkably restored the TNF-α level. Moreover, the inflammatory marker, c-reactive protein (CRP) originating from metabolic dysregulations has also been linked to the tissue degeneration, necrosis and carcinogenesis. ROS-induced significant elevation of CRP has been noticed *in vivo* in mouse and *in vitro* in human in response to arsenite exposure [46]. In our present study, a significant elevation of serum CRP in arsenic-exposed rats might initiate sustained inflammatory responses which are decisively counteracted by CS exposure resulting in the cessation of ROS-related stress. The result suggests that ROS plays an important role in neuronal differentiation. Report reveals that both ROS and CRP can activate the important transcription factor like NF-κ-β [46]. Beside arsenic induction of NF-κ-β, several MAPKs and AKT are also activated by arsenic in cellular degeneration process which is visible in the present study (Fig. **4**) [47]. In addition to the ROS role in CRP or NF-κ-β activation, ROS also induce apoptotic degenerative signaling *via* mitochondrial-membrane instability followed by the cytochrome-c release to the cytosol and related reaction cascade [48]. The LDH result of our present study infers the occurrence of necrotic tissue lesions which strongly support our hypothesis on oxidative-inflammatory factors in arsenic induced nervous toxicity. The present toxicity has been decisively restrained by the green tea supplementation.

Moreover, the immuno-inflammatory potentiation of TNF-α by arsenic make the tissue more sensitive [49]. TNF α is a homotrimer, binding of TNF-α trimer to the extracellular domain of TNFR1 induces receptor trimerization and recruitment of the adaptor protein TNF receptor-associated death domain (TRADD) which then recruits additional adaptor proteins: receptor-interacting protein (RIP), TNF receptor-associated factor 2 (TRAF2), and Fas-associated death domain (FADD). Recruitment of TRAF2 and FADD activates caspases 8 and 10 that initiate a protease cascade that leads to apoptosis [50]. Studies found a significant higher level of TNFα in the brain and CSF (cerebrospinal fluid) in Parkinsonian pateints than those in controls and suggest its relation to the neuronal degeneration in Parkinsonian disease [51]. Being an important signal transducer of the immune system with cytotoxic properties, it may be one of the cause of tissue degeneration (Fig. **4**) in the brain in our study. The (-)-epigallocatechingallate (EGCG) performs as an efficient preventive agent against neural toxicity by targeting a signal pathway for the cellular terminal differentiation. Such properties could impact on cell chemotaxis through the blood-brain barrier and prevent cancer-related neuroinflammation [52]. Reports reveal that neuroglobin involves during arsenic toxicity in rat cerebellar granular apoptosis. Apoptotic effects could be partially reversed by the antioxidant N-acetyl-L-cysteine supporting our present result of green tea mediated thiol protection and decrease in MDA and neuronal damages in arsenic intoxicated rats [49]. In relation to the amino acid neurotransmission, report reveals that arsenic-induced excessive glutamate accumulation in the extracellular space induces excitotoxicity [53]. Excitatory amino acid neurotransmitters are glutamate and aspartate, while GABA (γ-aminobutyric acid), glycine (aminoacetic acid), and taurine are inhibitory. Excitotoxicity refers to a process of neuronal death caused by excessive or prolonged activation of receptors such as AMPA-type glutamate receptors (AMPARs) [54]. Excitotoxicity has a role in the progression of many human acute or chronic neurodegenerative diseases, including ischemia, AD, PD, MS, and ALS have been proposed [55]. Glycine and aspartate was found to be reduced in the As treated group, whereas, glutamate was increased in the As treated group. Thus, an imbalance between the excitatory and inhibitory neurotransmitters may cause excitotoxicity. Studies report that TNF-α was able to potentiate excitotoxicity in human neuronal cultures [56]. TNF-α also potentiated glutamate neurotoxicity and that effect could be blocked by competitive (2-APV) and noncompetitive (MK-801) NMDA receptor antagonists [57]. Both glycine and aspartate were restrained in the As+CS group. Whereas, glutamate was further elevated in As+CS treated group. The neuronal, cerebrum and cerebellum degeneration in our study may also be attributed by excitotoxicity. The cells being unable to resolve the triggers imposed by inflammatory markers and oxidative stress undergoes degeneration. There is a negative correlation between the levels of amino acid neurotransmitters and the contents of arsenic in brain tissues which is an important provision for neurotoxicity [58]. The proportion of the apoptotic cells gradually increased with the increasing level of arsenic. In our present study, a significant increase in oxidative stress along with altered monoaminergic neurotransmitter was noticed. Arsenic may downregulate the gene expression and synthesis of important neurotransmitters [8] which is reflected in our experimental results. Further, a protection by CS supplementation is also evident.

Arsenic significantly depleted serum urate level in the present study, which has been reported in our earlier rat experiment and human sample studies [59,60]. The uric acid, being an anti-oxidant has been regarded in delaying the senescence and ageing process [61]. The hypouricemic condition may facilitate diseases like multiple sclerosis [62]. The long term effects in the decrease in serum urate may sustain an increase in free radical product like MDA level, which may be linked to the neural-senescence promoting ability of arsenic [63]. The blocking of the molybdenum (Mo) center of xanthine oxidase by arsenic minimizes O_2 reduction to form H_2O_2 during the formation of hypoxanthine to xanthine and xanthine to uric acid [64]. This incident results in the deleterious effects like decline in antioxidant capacity as a result of low urate level and decrease in rate of oxidation of more toxic arsenite (+3) to less toxic arsenate (+5) [64]. Hydrogen peroxide is reduced to H_2O by antioxidant enzymes like peroxidase and catalase [65]. In the present study, green tea has been decisively shown to restore the serum urate level with a concomitant decline in serum and tissue MDA level. The anti-ageing and life-span enhancing role of urate and SOD1 (cytosolic Cu-Zn SOD) are evident [61,66]. In the current study, for the first time we demonstrate the role of green tea in promoting urate and SOD1 function in neural tissues to resist oxidative stress in arsenic-intoxicated rat model. This finding is further justified by our *in vitro* study, where H_2O_2-mediated inactivation of partially purified SOD1 is decisively circumvented by the thiol substance 2-ME. Role of important Cys residues in the catalytic/substrate-binding domain of SOD1 is evident [23].

CONCLUSION

In conclusion, we demonstrated that the present duration and dose of arsenic was able to initiate toxicity more at cellular level in cerebral tissues. Here, we demonstrated for the first time that green tea is efficient to initiate strong antioxidant and anti-inflammatory responses against arsenic-induced neurotoxicity. Future investigations are required to predict more conclusively regarding the green tea protection mechanism.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Naujokas, M. F.; Anderson, B.; Ahsan, H.; Aposhian, H. V.; Graziano, J. H.; Thompson, C.; Suk, W. A. The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. *Environ Health Perspect*, **2013**, 121(3), 295-302.

[2] Tyler, C. R.; Allan, A. M. The Effects of Arsenic Exposure on Neurological and Cognitive Dysfunction in Human and Rodent Studies: A Review. *Curr Environ Health Rep*, **2014**, 1, 132-147.

[3] Calderon, J.; Navarro, M. E.; Jimenez-Capdeville, M. E.; Santos-Diaz, M. A.; Golden, A.; Rodriguez-Leyva, I.; Borja-Aburto, V.; Diaz-Barriga, F. Exposure to arsenic and lead and neuropsychological development in Mexican children. *Environ Res*, **2001**, 85(2), 69-76.

[4] Rodriguez, V. M.; Limon-Pacheco, J. H.; Carrizales, L.; Mendoza-Trejo, M. S.; Giordano, M. Chronic exposure to low levels of inorganic arsenic causes alterations in locomotor activity and in the expression of dopaminergic and antioxidant systems in the albino rat. *Neurotoxicol Teratol*, **2010**, 32(6), 640-647.

[5] Chen, C. J. Health hazards and mitigation of chronic poisoning from arsenic in drinking water: Taiwan experiences. *Rev Environ Health*, **2014**, 29(1-2), 13-19.

[6] Ram Kumar, M.; Flora, S. J.; Reddy, G. R. Monoisoamyl 2,3-dimercaptosuccinic acid attenuates arsenic induced toxicity: behavioral and neurochemical approach. *Environ Toxicol Pharmacol*, **2013**, 36(1), 231-242.

[7] Pachauri, V.; Flora, S. J. Effect of nicotine pretreatment on arsenic-induced oxidative stress in male Wistar rats. *Hum Exp Toxicol*, **2013**, 32(9), 972-982.

[8] Zhang, J.; Liu, X.; Zhao, L.; Hu, S.; Li, S.; Piao, F. Subchronic exposure to arsenic disturbed the biogenic amine neurotransmitter level and the mRNA expression of synthetase in mice brains. *Neuroscience*, **2013**, 241, 52-58.

[9] Jing, J.; Zheng, G.; Liu, M.; Shen, X.; Zhao, F.; Wang, J.; Zhang, J.; Huang, G.; Dai, P.; Chen, Y.. Changes in the synaptic structure of hippocampal neurons and impairment of spatial memory in a rat model caused by chronic arsenite exposure. *Neurotoxicology* **2012**, 33(5), 1230-1238.

[10] Tadepalle, N.; Koehler, Y.; Brandmann, M.; Meyer, N.; Dringen, R. Arsenite stimulates glutathione export and glycolytic flux in viable primary rat brain astrocytes. *Neurochem Int*, **2014**, 76, 1-11.

[11] Koehler, Y.; Luther, E. M.; Meyer, S.; Schwerdtle, T.; Dringen, R. Uptake and toxicity of arsenite and arsenate in cultured brain astrocytes. *J Trace Elem Med Biol*, **2014**, 28(3), 328-337.

[12] Ding, X.; Su, Q.; Jiang, M.; Xie, H.; Cong, J.; Wang, L. Arsenic affects on cerebellar development of mice. *Toxicol Mech Methods*, **2013**, 23(9), 672-677.

[13] Rodriguez, V. M.; Carrizales, L.; Mendoza, M. S.; Fajardo, O. R.; Giordano, M. Effects of sodium arsenite exposure on development and behavior in the rat. *Neurotoxicol Teratol*, **2002**, 24(6), 743-750.

[14] Srivastava, P.; Yadav, R. S.; Chandravanshi, L. P.; Shukla, R. K.; Dhuriya, Y. K.; Chauhan, L. K.; Dwivedi, H. N.; Pant, A. B.; Khanna, V. K. Unraveling the mechanism of neuroprotection of curcumin in arsenic induced cholinergic dysfunctions in rats. *Toxicol Appl Pharmacol*, **2014**, 279(3), 428-440.

[15] Yadav, A.; Mathur, R.; Samim, M.; Lomash, V.; Kushwaha, P.; Pathak, U.; Babbar, A. K.; Flora, S. J.; Mishra, A. K.; Kaushik, M. P. Nanoencapsulation of DMSA monoester for better therapeutic efficacy of the chelating agent against arsenic toxicity. *Nanomedicine (Lond)*, **2014**, 9(4), 465-481.

[16] Cheng, Y.; Xue, J.; Jiang, H.; Wang, M.; Gao, L.; Ma, D.; Zhang, Z. Neuroprotective effect of resveratrol on arsenic trioxide-induced oxidative stress in feline brain. *Hum Exp Toxicol*, **2013**, 33(7), 737-747.

[17] Sankar, P.; Telang, A. G.; Kalaivanan, R.; Karunakaran, V.; Suresh, S.; Kesavan, M. Oral nanoparticulate curcumin combating arsenic-induced oxidative damage in kidney and brain of rats. *Toxicology and Industrial Health*, **2016**, 32(3) 410-421.

[18] Bhattacharya, S.; Haldar, P. K. Trichosanthes dioica fruit extract ameliorates arsenic-induced brain toxicity in male albino rats. *J Environ Pathol Toxicol Oncol*, **2013**, 32(2), 141-148.

[19] Komatsu, M.; Hiramatsu, M. The efficacy of an antioxidant cocktail on lipid peroxide level and superoxide dismutase activity in aged rat brain and DNA damage in iron-induced epileptogenic foci. *Toxicology*, **2000**, 148(2-3), 143-148.

[20] Zheng, P.; Zheng, H. M.; Deng, X. M.; Zhang, Y. D. Green tea consumption and risk of esophageal cancer: a meta-analysis of epidemiologic studies. *BMC Gastroenterol*, **2012**, 12, 165.

[21. Gupta, V.K.; Singh, S,; Agrawal A.; Siddiqi N.J.; Sharma B. Phytochemicals Mediated Remediation of Neurotoxicity Induced by Heavy Metals. *Biochem Res Int*, **2015**.

[22] Sharma B.; Singh S.; Siddiqi N.J. Biomedical implications of heavy metals induced imbalances in redox systems. *Biomed Res Int*, **2014**, 640754.

[23] Acharyya, N.; Chattopadhyay, S.; Maiti, S. Chemoprevention against arsenic-induced mutagenic DNA breakage and apoptotic liver damage in rat *via* antioxidant and SOD1 upregulation by green tea (Camellia sinensis) which recovers broken DNA resulted from arsenic-H2O2 related *in vitro* oxidant stress. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*, **2014**, 32(4), 338-361.

[24] Inoue, H.; Akiyama, S.; Maeda-Yamamoto, M.; Nesumi, A.; Tanaka, T.; Murakami, A. High-dose green tea polyphenols induce nephrotoxicity in dextran sulfate sodium-induced colitis mice by down-regulation of antioxidant enzymes and heat-shock protein expressions. *Cell Stress Chaperones*, **2011**, 16(6), 653-662.

[25] Buege, J. A.; Aust, S. D. Microsomal lipid peroxidation. *Methods Enzymol*, **1978**, 52, 302-310.

[26] Garcia, Y. J.; Rodriguez-Malaver, A. J.; Penaloza, N. Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebellar slices. *J Neurosci Methods*, **2005**, 144(1), 127-135.

[27] Sinha, A. K. Colorimetric assay of catalase. *Anal Biochem*, **1972**, 47, 389-394.

[28] Forman, H. J. Critical methods in Free Radical Biology & Medicine. In: Free Radic Biol Med. **2009**, 47, S207.

[29] Chi, H.; Kawano, T.; Tamura, T.; Iwata, H.; Takahashi, Y.; Eguchi, S.; Yamazaki, F.; Kumagai, N.; Yokoyama, M. Postoperative pain impairs subsequent performance on a spatial memory task *via* effects on N-methyl-D-aspartate receptor in aged rats. *Life Sci*, 2013, 93(25-26), 986-993.

[30] Garcia-Martinez, V.; Macias D Fau - Ganan, Y.; Ganan Y Fau - Garcia-Lobo, J. M.; Garcia-Lobo Jm Fau - Francia, M. V.; Francia Mv Fau - Fernandez-Teran, M. A.; Fernandez-Teran Ma Fau - Hurle, J. M.; Hurle, J. M. Internucleosomal DNA fragmentation and programmed cell death (apoptosis) in the interdigital tissue of the embryonic chick leg bud. *J Cell Sci*, **1993**, 106, 201-208.

[31] Kaler, S.; Dhar, P.; Bhattacharya, A.; Mehra, R. D. Preliminary morphological and immunohistochemical changes in rat hippocampus following postnatal exposure to sodium arsenite. *Toxicol Int*, **2013**, 20(2), 160-169.

[32] Rai, N. K.; Ashok, A.; Rai, A.; Tripathi, S.; Nagar, G. K.; Mitra, K.; Bandyopadhyay, S. Exposure to As, Cd and Pb-mixture impairs myelin and axon development in rat brain, optic nerve and retina. *Toxicol Appl Pharmacol*, **2013**, 273(2), 242-258.

[33] Meyer, N.; Koehler, Y.; Tulpule, K.; Dringen, R. Arsenate accumulation and arsenate-induced glutathione export in astrocyte-rich primary cultures. *Neurochem In*, **2013**, 62(7), 1012-1019.

[34] Desagher, S.; Glowinski, J.; Premont, J. Astrocytes protect neurons from hydrogen peroxide toxicity. *J Neurosci*, **1996**, 16(8), 2553-2562.

[35] Makar, T. K.; Nedergaard, M.; Preuss, A.; Gelbard, A. S.; Perumal, A. S.; Cooper, A. J. Vitamin E, ascorbate, glutathione, glutathione disulfide, and enzymes of glutathione metabolism in cultures of chick astrocytes and neurons: evidence that astrocytes play an important role in antioxidative processes in the brain. *J Neurochem*, **1994**, 62(1), 45-53.

[36] Rohrdanz, E.; Schmuck, G.; Ohler, S.; Tran-Thi, Q. H.; Kahl, R. Changes in antioxidant enzyme expression in response to hydrogen peroxide in rat astroglial cells. *Arch Toxicol*, 2001, 75(3), 150-158.

[37] Messarah, M.; Saoudi, M.; Boumendjel, A.; Kadeche, L.; Boulakoud, M. S.; El Feki, A. Green tea extract alleviates arsenic-induced biochemical toxicity and lipid peroxidation in rats. *Toxicol Ind Health*, **2013**, 29, 349-359.

[38] Acharyya, N.; Sajed Ali, S.; Deb, B.; Chattopadhyay, S.; Maiti, S. Green tea (Camellia sinensis) alleviates arsenic-induced damages to DNA and intestinal tissues in rat and *in situ* intestinal loop by reinforcing antioxidant system. *Environ Toxicol*, **2015**, 30(9), 1033-1044.

[49] Li, M.; Cai, J. F.; Chiu, J. F. Arsenic induces oxidative stress and activates stress gene expressions in cultured lung epithelial cells. *J Cell Biochem*, **2002**, 87, 29-38.

[40] Maiti, S.; Chatterjee, A. K. Effects on levels of glutathione and some related enzymes in tissues after an acute arsenic exposure in rats and their relationship to dietary protein deficiency. *Arch Toxicol*, **2001**, 75, 531-537.

[41] Shila, S.; Subathra, M.; Devi, M. A.; Panneerselvam, C. Arsenic intoxication-induced reduction of glutathione level and of the activity of related enzymes in rat brain regions: reversal by DL-alpha-lipoic acid. *Arch Toxicol*, **2005**, 79, 140-146.

[42] Chandravanshi, L. P.; Yadav, R. S.; Shukla, R. K.; Singh, A.; Sultana, S.; Pant, A. B.; Parmar, D.; Khanna, V. K. Reversibility of changes in brain cholinergic receptors and acetylcholinesterase activity in rats following early life arsenic exposure. *Int J Dev Neurosci*, **2014**, 34, 60-75.

[43] Zhang, W.; Feng, H.; Gao, Y.; Sun, L.; Wang, J.; Li, Y.; Wang, C.; Zhao, L.; Hu, X.; Sun, H. *et al.* Role of pigment epithelium-derived factor (PEDF) in arsenic-induced cell apoptosis of liver and brain in a rat model. *Biol Trace Elem Res*, **2013**, 151(2), 269-276.

[44] Yen, C. C.; Ho, T. J.; Wu, C. C.; Chang, C. F.; Su, C. C.; Chen, Y. W.; Jinn, T. R.; Lu, T. H.; Cheng, P. W.; Su, Y. C. *et al.* Inorganic arsenic causes cell apoptosis in mouse cerebrum through an oxidative stress-regulated signaling pathway. *Arch Toxicol*, **2011**, 85(6), 565-575.

[45] Singh, V.; Mitra, S.; Sharma, A. K.; Gera, R.; Ghosh, D. Isolation and characterization of microglia from adult mouse brain: selected applications for ex vivo evaluation of immunotoxicological alterations following *in vivo* xenobiotic exposure. *Chem Res Toxicol*, **2014**, 27(5), 895-903.

[46] Druwe, I. L.; Sollome, J. J.; Sanchez-Soria, P.; Hardwick, R. N.; Camenisch, T. D.; Vaillancourt, R. R. Arsenite activates NFkappaB through induction of C-reactive protein. *Toxicol Appl Pharmacol*, **2012**, 261(3), 263-270.

[47] Das, J.; Ghosh, J.; Manna, P.; Sinha, M.; Sil, P. C. Taurine protects rat testes against NaAsO(2)-induced oxidative stress and apoptosis *via* mitochondrial dependent and independent pathways. *Toxicol Lett*, **2009**, 187(3), 201-210.

[48] Mishra, D.; Flora, S. J. Differential oxidative stress and DNA damage in rat brain regions and blood following chronic arsenic exposure. *Toxicol Ind Health*, **2008**, 24(4), 247-256.

[49] Liu, X.; Gao, Y.; Yao, H.; Zhou, L.; Sun, D.; Wang, J. Neuroglobin involvement in the course of arsenic toxicity in rat cerebellar granule neurons. *Biol Trace Elem Res*, **2013**, 155(3), 439-446.

[50] Chen, G.; Goeddel D.V.; TNF-R1 signaling: a beautiful pathway. *Science*, **2002**, 296(5573), 1634-1635.

[51] Mogi M.; Harada M.; Riederer P.; Narabayashi H.; Fujita K.; Nagatsu T. Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neurosci Lett*, **1994**, 165(1-2):208-10.

[52] Chokor, R.; Lamy, S.; Annabi, B. Transcriptional targeting of sphingosine-1-phosphate receptor S1P2 by epigallocatechin-3-gallate prevents sphingosine-1-phosphate-mediated signaling in macrophage-differentiated HL-60 promyelomonocytic leukemia cells. *Onco Targets Ther*, **2014**, 7, 667-677.

[53] Huo, T. G.; Li, W. K.; Zhang, Y. H.; Yuan, J.; Gao, L. Y.; Yuan, Y.; Yang, H. L.; Jiang, H.; Sun, G. F. Excitotoxicity Induced by Realgar in the Rat Hippocampus: the Involvement of Learning Memory Injury, Dysfunction of Glutamate Metabolism and NMDA Receptors. *Mol Neurobiol*, **2015**, 51(3), 980-994.

[54] Olney, J. W.; McGeer, E. G.; McGeer, P. L. Neurotoxicity of excitatory amino acids, in Kainic Acid as a Tool in Neurobiology. *Raven Press, New York*, **1978**, 95-112,

[55] Doble, A. The role of excitotoxicity in neurodegenerative disease: implications for therapy. *Pharmacology and Therapeutics*, **1999**, 81(3), 163-221,

[56] Gelbard, H. A; Dzenko, K. A.; DiLoreto, D. ; Del Cerro, C.; Del Cerro, M.; Epstein, L. G. Neurotoxic effects of tumor necrosis factor alpha in primary human neuronal cultures are mediated by activation of the glutamate AMPA receptor subtype: implications for AIDS neuropathogenesis. *Developmental Neuroscience*, **1993**, 15(6), 417-422,

[57] Chao, C. C.; Hu, S. Tumor necrosis factor-alpha potentiates glutamate neurotoxicity in human fetal brain cell cultures. *Developmental Neuroscience*, **1994**, 16(3-4),172-179.

[58] Huo, T.; Chang, B.; Zhang, Y.; Chen, Z.; Li, W.; Jiang, H. Alteration of amino acid neurotransmitters in brain tissues of immature rats treated with realgar. *J Pharm Biomed Anal*, **2012**, 57, 120-124.

[59] Maiti, S.; Chattopadhyay, S.; Deb, B.; Samanta, T.; Maji, G.; Pan, B.; Ghosh, A.; Ghosh, D. Antioxidant and metabolic impairment result in DNA damage in arsenic-exposed individuals with severe dermatological manifestations in Eastern India. *Environ Toxicol*, **2012**, 27(6), 342-350.

[60] Acharyya, N.; Deb, B.; Chattopadhyay, S.; Maiti, S. Arsenic-Induced Antioxidant Depletion, Oxidative DNA Breakage, and Tissue Damages are Prevented by the Combined Action of Folate and Vitamin B12. *Biol Trace Elem Res*, **2015**, 1-11.

[61] Cutler, R. G. Urate and ascorbate: their possible roles as antioxidants in determining longevity of mammalian species. *Arch Gerontol Geriatr*, **1984**, 3(4), 321-348.

[62] Toncev, G. Therapeutic value of serum uric acid levels increasing in the treatment of multiple sclerosis. *Vojnosanit Pregl*, **2006**, 63(10), 879-882.

[63] Ninomiya, Y.; Cui, X.; Yasuda, T.; Wang, B.; Yu, D.; Sekine-Suzuki, E.; Nenoi, M. Arsenite induces premature senescence *via* p53/p21 pathway as a result of DNA damage in human malignant glioblastoma cells. *BMB Rep*, **2014**, 47(10), 575-580.

[64] Aposhian, H. V.; Zakharyan, R. A.; Avram, M. D.; Kopplin, M. J.; Wollenberg, M. L. Oxidation and detoxification of trivalent arsenic species. *Toxicol Appl Pharmacol*, **2003**, 193(1), 1-8.

[65] Mates, J. M. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*, **2000**, 153(1-3), 83-104.

[66] Tolmasoff, J. M.; Ono, T.; Cutler, R. G. Superoxide dismutase: correlation with life-span and specific metabolic rate in primate species. *Proc Natl Acad Sci U S A*, **1980**, 77(5), 2777-2781.