Arsenic induced dissolution of neural architecture and signaling are restored through MMPs and AChE inactivation by tea flavonoids in rat, in-silico and in-vitro purified enzyme/theaflavin reaction.

Nandita Medda
Oriental Institute of Science and Technology
Sayantani Maiti
Oriental Institute of Science and Technology
Nirmallya Acharyya
FDA: US Food and Drug Administration
Tanmoy Samanta
IIT Kharagpur: Indian Institute of Technology Kharagpur
Amrita Banerjee
Oriental Institute of Science and Technology
Subrata De
Vidyasagar University
Tamal Ghosh
Government of West Bengal Health & Family Welfare Department
smarajit maiti (maitism@rediffmail.com)
Oriental Institute of Science and Technology https://orcid.org/0000-0002-1354-1303

Research Article

Keywords: Arsenic toxicity, cerebrum and cerebellum, antioxidant system, acetyl cholinesterase, matrix-metalloproteinase, Camellia sinensis

Posted Date: April 20th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1565400/v1

Abstract

Chronic arsenic-exposure causes gastro-intestinal/nephrotoxic/neurodegenerative disorders with keratosis/melanosis and cancers. The reactive-oxygen-species (ROS) cause intrinsic-antioxidant (thiols/uric-acid) depletion eventuating cytoskeletal-extracellular-matrix and DNA damage. Therapeutic chelating-agents have multiple side-effects. The protective potential of tea-extract against arsenic neurotoxicity was studied. Influence of uric-acid in arsenic-toxicity was verified. *Camellia sinensis* (CS dry-leaves) 10mg/ml aqueous), uric-acid (UA) or allopurinol (Allo- urate-depletor) was supplemented to arsenic-intoxicated (0.6 ppm/100g bw/day/4-weeks) female albino-rats and cytotoxicity/biochemistry/histo-architecture/matrix-integrity and DNA-stability were evaluated in their cerebrum-cerebellum. In-vitro purified theaflavin-derivatives were tested on purified-AchE (acetyl-cholinesterase). Higher malondialdehyde, lactate-dehydrogenase and lower uric-acid levels were observed in the arsenic/Allo groups. Degenerations of cerebrum/cerebellum by higher matrix-metalloprotease (MMP2/9)/DNA-damages (comets) are observed. The CS-group restored the adverse-markers, protected tissues/DNA integrity which is supported in the UA-group and opposed in the Allo-group. Impairment of the neural-signaling, evident from the arsenic-exposed animal with unstable-movement/vertigo/tremor accompanied with higher AchE in the cerebellum is significantly restored/protected in the CS-group. This suggests that the neural integrity is maintained by the cellular intrinsic antioxidative-capacity, shown in catalase-SOD1 activities. In-vitro and in silico studies suggest that the theaflavin derivatives (pure, SIGMA) i.e theaflavin-digallate (TFDG) showed the strongest inhibition on purified (SIGMA) AchE (IC_{50} = 2.19 µg/ml) followed by TFMG (IC_{50} = 3.86 µg/ml). The4 CS is protective in arsenic-toxicity. Theaflavin-gallate is a strong AchE inhibitor which can be used in arsenic-induced neuro-muscular depletion. Beside antioxidant/anti-inflammatory/anti-carcinogenic role CS restores intrinsic-antioxidants.

1. Introduction

Several epidemiological studies suggest a strong connection between long term arsenic exposure and detrimental health effects, such as cancer, cardiac diseases, neurological disorders (Calderon et al.,2001; Chou et al.,2001). According to the World Health Organization (WHO), for drinking water, 10 µg/L of arsenic is the maximum acceptable concentration. However, in more than 70 countries across 5 continents, over 200 million people are suffering from the high level of arsenic concentration in groundwater (Huang et al.,2015; Bhattacharjee et al., 2013). Very high arsenic concentrations in groundwater have been documented in Mexico, USA, Chile, China, Argentina, and Hungary (Smedley et al.,2002) as well as in the Indian States (Bhattacharya et al., 2011). Prediction depending on the available data suggest that almost 47 million people are in Pakistan, where over 50% of the groundwater wells contain arsenic concentration, more than the WHO recommended limit for arsenic in drinking water (Sharma et al.,2018). In India, West Bengal was the most affected state, with major registered cases from districts of Nadia and South 24-Parganas. Inorganic arsenic uptake through drinking water usually induces cancer in the skin, urinary bladder, lung and other different parts of our body (National Research Council Subcommittee on Arsenic in Drinking Water, 1999, 2001). ROS-regulated c-reactive protein (CRP)

and NF-κβ activation by arsenic induction can over express certain genes related to the cellular apoptotic degeneration process (Druwe et al., 2012 & Medda et al., 2021). Arsenic exerts a detrimental outcome on general protein metabolism and generates high toxicity by reacting with sulfhydryl groups present in cysteine residues (Rai et al., 2011). Arsenate (AsV) intake in the human body metabolically occurs through membrane transport proteins like aquaporins and inorganic phosphate (Pi) transporters (LeBlanc et al., 2013). If during the time of ATP production, instead of Pi, Asv is used by mitochondrial ATP synthase, then it conjugates ADP with AsV, followed by reduction (by GSH) to arsenite (AsIII) form, which is more cytotoxic (Nemeti et al., 2010). Epidemiological reports suggest that alterations in intelligence measures, depression like mood-disorder, learning-hazards and neural tube defects can also be the outcome of chronic arsenic consumption (Vahter 2009; Ahmed et al., 2011). Impairment in tight Junction's function within the blood brain barrier (BBB) can provide a background for many types of neurological complications and neuro-inflammatory disorders (Ballabh et al.,2005 & Medda et al.,2020). This junction comprises brain capillary epithelial cells and choroid plexus epithelial cells. Arsenic exposure in BBB makes it more permeable and its structural integrity is disrupted by arsenic (Rai et al., 2010). Arsenic can also alter cholinergic signaling, synaptic plasticity, which is responsible for different types of behavioral abnormalities including memory, locomotion, learning etc. (Tyler and Alla, 2014). Tea flavonoids and uric acid are established antioxidants (Maiti et al., 2019 &Fabbrini et al., 2014). There are considerable numbers of references which show the protective effect of tea extract against arsenic toxicity (Acharyya et al., 2015), so, in our experiment we used these against arsenic for determining their efficacy. Allopurinol, which is an analogue of uric acid, has also been used to study against arsenic toxicity.

2. Materials And Methods

Theaflavin (TF), theaflavin monogallate (TFMG) and theaflavin digallate (TFDG),Acetyl cholinesterase (AChE) type VI-S from electric eel was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). Uric acid and allopurinol powder were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.1. Preparation of Solution

2.1.1. Preparation of tea extract

The leaf dusts of Camellia sinensis (CS) were collected locally and dried in incubator for 2 days at 40ºC. Extraction was done by taking 10mg/ml in distilled water. When the color of the aqueous extract became brown, it was collected for the experiment.

2.1.2. Preparation of Arsenic Solution

For this experiment, we used sodium-meta-arsenite as arsenic source by dissolving it in distilled water maintaining 0.6 ppm concentration. We are taking this dose because it is not lethal for the animal but the exposure of arsenic in animal model for a moderate time period like 21 days can generate toxicity in their body.

2.1.3. Preparation of Uric acid- Purchased uric acid was dissolved in deionized water and filtered for having a crystal free solution and the concentration was 8 mg/dL (Hua et al., 2015).

2.1.4. Preparation of allopurinol-Stock allopurinol powder (> 99% pure; Sigma; cat. no. A-8003) was dissolved in sterile 0.9% NaCl solution, pH 10, on the day of use. Typical volumes for oral dosing are around 0.5–0.6 ml of solution in a 150g rat.

2.2. Treatment and Group Distribution of Animal Model

For this experiment, female albino rats weighing 150± 10 were acclimatized for 10 days at 12 hours light-dark cycle, 32± 2ºC temperature, humidity 50-70%. Standards pellet diet (Hindustan Lever, Mumbai, India) was given to them. Rats were purchased from Government accredited rodent firm house (CPCSEA-Committee for the Purpose of Control and Supervision of Experiments on Animals: Reg. no 1A2A/PO/BT/S/15/CPCSEA. <http://cpcsea.nic.in/Auth/index.aspx>) organization under the Dept of Animal Husbandry and Dairy, Ministry of Agriculture and Farmer's Welfare, Govt. of India. Animal experiments were performed in the OIST Animal Resource Facilities. Institutional ethical concerns (Oriental Institute of Science and Technology) Review Board; OIST-IRB, reference no. oist/EC/sm/19_3) were maintained throughout the investigation.

Rats were randomly selected for the experiment and they were divided into 5 different groups having four in each group. Control or group-I – animals were fed only drinking water. Arsenic or group-II -0.5 ml of arsenic solution at a concentration of 0.6 ppm was given for 21 days. Arsenic+CS or group-III – 0.5 ml arsenic at a concentration of 0.6 ppm + 0.5 ml lyophilized tea extract for the same time period. Group – IVwas treated with 0.5 ml of arsenic and uric acid 8mg/dL concentration. In group-V,same dose arsenic and 0.5 ml allopurinol from the stock were given.

2.3. Evaluation of Toxicity Level in Brain Sample

By sacrificing the experimental rat, cerebrum and cerebellum were collected. These tissues were homogenized in homogenizer by using ice cold phosphate buffer (pH-4) and cytosols were obtained. Collected cytosols were distributed in two aliquots and were preserved at -20ºC. The cytosol was used for different parameter testing.

2.3.1. Estimation of Non-Protein Thiol Assay (NPSH)

From the prepared cytosol of the brain tissue (cerebrum and cerebellum), NPSH level was measured. This assay was done by a standardized protocol using Ellman's reagent (DTNB) with a slight modification (Forman et al., 2009). At first the sample of different groups were taken and proteins were precipitated by 5% TCA. In the clear cytosol, Di-thio-bis-nitrobenzoic acid (DTNB) was added as a color reagent with Tris HCL buffer. 10 to 15 minutes incubation was maintained in room temperature (RT). Reading of absorbance was taken at 420 nm and the concentration of NPSH was determined against a standard curve of GSH (reduced glutathione).

2.3.2. Malondialdehyde Assay

At first, we took the samples of different groups and treated it with the same amount of 5% TCAand then it was centrifuged at 8-10k rpm. After discarding pellets, supernatant was collected. Collected supernatant was treated with 3% TBA (Thiobarbituric acid) solution. Then it was incubated at 80ºC in water bath for 30 min and finally cooled at RT for 20 min. For the presence of malondialdehyde which is the end product of lipid peroxidation, a slight pink color appeared. By measuring the color intensity in spectrophotometer MDA concentration was calculated in the different groups with the help of molar extinction coefficient of MDA (1.56×10^5 cm^2/mmol) (Maiti and Chatterjee 2001; Maiti et al., 2014).

2.3.3. Acetyl cholinesterase assay

Acetyl cholinesteraseassay was performed by Ellman's method in which DTNB was used as a chromogen and acetylthiocholine iodide as substrate (Govindappa et al., 1987). In the assay, 1 cm quartz cuvette was gradually filled with 0.5 mL of DTNB (3mM), 375 µL of 50 mM Tris-HCl buffer (pH 8.0) and 100 µL of 0.086 U/mL AChE. The reaction was started by addition of ATCl (25 µL). The reaction mixture was monitored at 405 nm for 10 min. Enzyme activity was calculated using the extinction coefficient, ε = 13600 $M^{-1}cm^{-1}$.A standard solution of eserine salicylate was used as positive control for AChE inhibitor.

2.3.4. Determination of pure AChE inhibition by pure TFDG, TFMG and simple TF

Acetyl cholinesterase was assayed using the above method except one change. Here100 µL of 0.086 U/mL AChE (from electric eel) was added in the reaction mixture instead of AChE extract from rat cerebellum. A standard solution of eserine salicylate was used as positive control for AChE inhibitor.

The concentrations of TFDG, TFMG and simple TF were used in the range of 1-8 µg/mLthe percentage of AChE inhibitory activity (% IA) was calculated by using the equation:

$$\% IA = [(A_c - A_t)/A_c] \times 100$$

Where, A_c is the activity of control enzyme (containing all reactants, except the tea components) and $A_t$is the activity of tested compound. All experiments were performed in triplicate.

2.3.5. Estimation of IC_{50} values

The concentrations of the test samples that inhibited the hydrolysis of substrate (acetylthiocholine iodide) by 50% (IC_{50}) were determined by a linear regression analysis between the inhibition percentages against the log concentrations of inhibitors using the OriginPro 8 software and Microsoft office excel 2007 program.

2.3.6. Assay of Catalase in native polyacrylamide gel

Catalase activity assay was done in 8% native polyacrylamide gel electrophoresis (PAGE). 0.003% H_2O_2 (30% solution vol/vol) was given as a substrate. 2% ferric chloride (wt/vol) and 2% potassium ferrocyanide (wt/vol) were used for staining purpose. After staining, transparent bands were developed against dark green-blue background where the enzyme was present because the catalase enzyme removes the hydrogen peroxides from the zone of the gel it occupies. Peroxide is oxidized to O_2; removal of peroxide does not allow the potassium ferricyanide (a yellow substance) to be reduced to potassium ferrocyanide that reacts with ferric chloride to form a Prussian blue precipitate (Maiti et al., 2014).

2.3.7. Superoxide dismutase (SOD) assay in native polyacrylamide gel

Superoxide dismutase (SOD) enzyme was assessed in 12.5% non-denaturing PAGE gel. After completing the electrophoresis, the gel was kept in NBT (nitro bluetetrazolium)solution in shaking condition for incubation. After incubation, the gel was soaked in SOD solution which contained Potassium phosphate, TEMED and riboflavin for approximately 15 min. Then the gel was illuminated in UV trans-illuminator. Gel became purple except the portion containing superoxidedismutase.In purple background, whitish bands of SOD were observed (Acharyya et al., 2014).

2.3.8. Lactate dehydrogenase (LDH) assay in native polyacrylamide gel

For sample running, 8% native gel was electrophoresed at 4˚ C. After completing gel running, the gel was incubated in staining solution in dark condition for 40-45 min. The component of staining solution was Tris HCL, lithium lactate, Nitro blue tetrazolium, phenazine methyl sulfateand NAD+.Band of LDH wasof prominent blue color against the transparent background (Gilmour et al., 1994).

2.3.9. Matrix metalloproteinase (MMP) in native polyacrylamide gel

Samples were run in SDS-polyacrylamide gel electrophoresis. 8% gel was prepared by mixing sterile distilled water, Tris HCL, 20% SDS (sodium dodecyl sulphate), polyacrylamide and gelatin solution (1%) in a tube. After addition of APS (ammonium per sulfate) and TEMED (tetraethyl ethylenediamine), gel solution was poured into the gel chamber. Gel was run at 110V (Bio-Rad apparatus) using pre-cooled running buffer at 4□C.After removing the gel from gel apparatus, the gel was incubated in zymographic development buffer (50mM Tris pH-7.4, 1mM $CaCl_2$,0.02% NaN_3, sterile diH_2O) at 37□C for 42 hours. Then the gel was stained by Coomassie Brilliant Blue R-250 solution at room temperature on a rotary shaker. Staining was followed by using distaining solution (ethanol, acetic acid, dH_2O) until the clear transparent bands were observed in bluish background (Frankowski et al.,2012).

2.3.10. DNA Comet assay

The Comet assay was done with minute modifications of Singh and colleagues' method (Singh et al, 1988). At 37□C, 75 ml of low melting point agarose (0.6%) containing Phosphate buffer saline (PBS) was added to 25 ml of cell suspension (10^5cells). This mixture was then placed onslideswhich were pre-coated with 1% agarose and coverslips were placed. After the solidification of agarose, the coverslip was

removed and the slides were immersed in ice cold lysis buffer (2.5 mM NaCl, 1% Triton X-100, 85 mM EDTA, 10 mM Trizma base,10% DMSO and 1% sodium lauryl sarcosinate, pH -10) for 1 hour at 4◦C. After lysis, the slides were washed for three times in Phosphate buffer saline at room temperature. Next, 50 ml of buffer (control) or T4 endo V (Epicentre) (4 U/slide) in buffer was transferred to the slides. Coverslips were kept and the slides were incubated at 37◦C for 45 min. After removing the coverslip, slides were washed in water twice for removing excess salt. Slides were then placed in a gel electrophoresis chamber (Bio-Rad, USA) filled with alkaline electrophoresis buffer (0.3 M NaOH and 1 mM EDTA) for 25 min. Electrophoresis was run for 30 min at 25 V and the current was adjusted to 300 mA. Slides were then neutralized by PBS and stained with a solution of 10 mg/ml ethidium bromide for 5 min. Excess stain was removed by water wash. Slides were observed by using a fluorescence microscope (Nikon, Eclipse LV100 POL), with the help ofVisComet (ImpulsBildanalyze) software. A total of 100 comets /slide were studied for each experiment (Garcia et al., 1993).

2.3.11. Histo-architecture study of rat brain tissues

Intact and fresh brain tissues (cerebrum and cerebellum) were collected just after sacrifice and these tissues were poured in fixative. Those tissues were embedded in paraffin block, sectioned at 5 microns, stained with eosin and Hematoxylin. For the histo-architectural study, stained slides were observed under light microscope (Nikon, Eclipse LV100, magnification 10X).

2.3.12. Bioinformatics study

Protein and ligand structure retrieval

An X-ray crystallographic structure of human acetylcholine esterase was retrieved from Protein Data Bank (PDB ID: 4M0E). Removal of other molecules complexed with 4M0E was performed using BIOVIA Discovery Studio 2017R2 and the edited 4M0E molecule was saved in PDB format for further use. Again, the three-dimensional structures of substrate; acetylcholine, and different inhibiters like catechingallate (CG), gallocatechingallate (GCG), epicatechin (EC), epicatechingallate (ECG), epigallocatechin (EGC), epigallocatechingallate (EGCG), theaflavin (TF), theaflavinmonogallate (TFMG) and theaflavindigallate (TFDG) were retrieved from PubChem [https://pubchem.ncbi.nlm.nih.gov/] database of bioactive compounds in SDF format. The SDF format was converted to PDB through BIOVIA Discovery Studio 2017R2 and saved for further use.

Ligand and protein structures preparation

The PyMol(https://pymol.org/2/) was used as molecular-visualization tool in this study. The ligand molecules were retrieved as .sdf format and structures were saved in a .pdb format.

Surface-Topology Calculation of Proteins

The factors solvent accessibility is definedby two properties of a protein; pocket, where water enters and cavity, where does not. The CASTp: Computed-Atlas-of-Surface-Topography of Protein

(http://sts.bioe.uic.edu/castp/index.html?j_5e8c7bec25090) was used to define the pockets and cavities.

Molecular docking study

Molecular docking with acetylcholinesterase and acetylcholine was performed and analyzed using Autodock Tools -1.5.6. Different acetylcholine esterase inhibitors interactions were also analyzed by PatchDock server [https://bioinfo3d.cs.tau.ac.il/PatchDock/].

3. Results& Discussion

In this experiment, our aim was to observe how Camellia sinensis (tea extract), uric acid and allopurinol work against arsenic toxicity in brain tissue as a potent antioxidant. Arsenic toxicity generates different types of reactive oxygen species (ROS) and creates oxidative stress. Reactive oxygen species (ROS) contain one or more unpaired electrons. Due to the presence of unpaired electrons, ROS can damage protein, DNA, lipids etc. in our body. When an imbalance happens between the reactive oxygen species generation and availability of antioxidants within the body, this condition can be a major reason for creating oxidative stress (Medda et. 2021). ROS is naturally generated in our body through different metabolic pathways and these ROS are neutralized by various antioxidants by donating electrons. Thus, antioxidants protect the body from oxidative damage.

In the present study, arsenic-exposed animals were observed to be most restless with unstable movements. Those also had a certain degree of vertigo and tremor. Present dose with duration of arsenic ingestion has shown disarrangement of cerebrum and cerebellum tissue (Fig-1). The histo-architecture anomalies like damage, pyknosis, nuclear condensation/de-condensation etc are visualized differentially in different groups. Present histo-pathological data showed vacuolar degeneration in cytoplasm, karyolysis and karyorrhexis in the arsenic group but the CS (tea) supplemented group has shown partial but significant protection, which is evident from the picture (Fig. 1). A similar protection was partially noticed in the uric acid supplemented group. In the arsenic group, the cell body of the neuron has shown to be affected (inset of cortex images). Alteration of cell surface/ membranes and the origin of the dendrites are shown to be degenerated. The sharp edge of the membrane of the cell body which is noticed in the control group was missing in the arsenic-treated group and haziness in the border line suggests a certain degree of neuronal damage (inset box in the cortex images). But in the CS treated group, uric acid and less allopurinol were found to be protected from this degeneration. Myelin disintegration in the central nervous system can damage atrocities. The regulation of myelin basic protein (MBP), myelin-associated glycoprotein and neurofilament (NF) are reported to be associated with arsenite toxicity. It also has the ability to upregulate vacuolated axons, especially those that are present in the corpus-callosum region (Rai et al., 2013). It has previously been reported that arsenic linked to oxidative stress has a relationship with neuro-degeneration.

The GPx, superoxide dismutase and catalase, like antioxidant enzymes, serve as the first line of defense against reactive oxygen species (ROS). SOD catalyses the superoxide (·O2 -) radicals to form hydrogen peroxide (H2O2) (Wang et al., 2013). In arsenic metabolism, superoxide dismutase enzyme activity may

decrease due to an increase in superoxide production (Maiti et al., 2017). The zymogram picture of a native polyacrylamide gel of superoxide dismutase (SOD) activity assay, arsenic group, exhibited less prominent band signifying more toxicity [Fig. 2-C, G]. But the other groups treated with uric acid, CS and allopurinol showed more prominent bands of SOD. Hydrogen peroxide, which is formed by superoxide dismutase enzyme, is further processed by glutathione peroxidase (GPx) and catalase. GPxcatalyses the reduction of H2O2 and converts it into H2O with GSH as a substrate (Binuet al., 2017). In native PAGE, catalase activity indicates toxicity in the arsenic-treated group by showing decreasing band strength than the other groups [Fig. 2-B, F].

Lactate dehydrogenase (LDH) enzyme is a toxicity marker. Significant increase of lactate dehydrogenase (LDH) level has been documented along with arsenic exposure (Karimet al., 2010). In our study, lactate dehydrogenase assay in gel zymogram has shown a less prominent band in tea than arsenic in the cerebellum [Fig. 2, E]. Although in the cerebrum, prominent bands are present in tea and the allopurinol group in the gel zymogram. But less amount of LDH in the arsenic group was seen than tea flavonoid, uric acid and allopurinol [Fig. 2, A]. If a hypoxic environment, low oxygen and ischemic conditions occur, then lactate can be formed. Lactate to pyruvate conversion is catalysed by lactate dehydrogenase. If a greater amount of LDH is generated then it would be able to restore its pyruvate level. Thus, LDH has the capacity to reduce oxygen related hypoxic conditions. Arsenic has the capacity to influence LDH. Arsenic also holds the potential to affect brain tissues (cerebrum, cerebellum) when it crosses the blood-brain barrier (BBB). For this reason, blood-brain-barrier (BBB) integrity is very essential to fighting against arsenic-associated neurotoxicity. Studies reveal that arsenic exposure can alter the BBBs, functional integrity as well as impairment in Tight Junction's (TJs) function, which are the most possible threat to ischemic damage (Kim et al., 2013). TJs alteration is the resulting effect of two enzymes' activation; Matrix metalloproteinase 2 and 9. MMP2 and MMP 9 are responsible for the BBB leakage by disrupting basal lamina protein and by altering the function of TJs (Ramos-Fernande et al., 2011). In our experiment, matrix metalloproteinase 2 and 9 expressions were prominently found in the arsenic group but it was not significant in control and arsenic + tea group [Fig. 2, D]. Here, tea extract reveals its protective effect against arsenic by suppressing these metalloproteinase activities. The naive brain cells, mainly neurons and glial cells express low levels of MMP9, which is predominantly found in the cerebellum, hippocampus and cerebral cortex region (Dzwonek et al., 2004; &Bednarek et al., 2009). Upregulation of MMP9 indicates neuronal plasticity (Vafadari et al., 2016). Enhanced plasticity may produce silent synapses (Liao et al., 1995). It has been reported that increased levels of MMP-9 enhance silent synapse formation (Magnowska et al., 2016) while genetic removal of MMP-9 prevents occurrence (Stefaniuk et al., 2017).

In vivo, there are many antioxidants to struggle against toxic substances and make them less toxic. Non-protein thiol (NPSH) acts as an antioxidant which can scavenge reactive oxygen species and reduce oxidative stress and it also has a great role in the DNA repair and synthesis process. Different concentrations of arsenic in the biological system attack free thiols and diminish its level (Patel et al., 2010). Our experiment in cerebrum, non-protein thiol (NPSH) content was at increased level in the group where tea, uric acid and allopurinol were used with arsenic than in only the arsenic treated group [Fig. 3,

A]. But in the cerebellum region, there was a significant increase of thiol in the arsenic group [Fig. 3, D]. When ROS generation starts, the body boosts its antioxidants to fight against it. Antioxidant boosting may be a possible reason behind this increasing level of NPSH.

According to several mechanistic studies, vascular tissue inflammation can take place due to arsenic toxicity. Arsenic disrupts lipid metabolism by activating the oxidative signalling pathway (States et al., 2009) and by increasing the lipid-peroxidation products, MDA. As a result, cell membrane integrity is disrupted. In the brain, this disruption can lead to Alzheimer's disease in the long run (Romero et al., 1998). Here a certain amount of MDA increase in the tea group [Fig. 3, B] suggests tea has shown its pro-oxidant properties. The arsenic group showed more MDA than control in the cerebrum. But in the cerebellum, tea (CS) and uric acid exhibit protective roles by decreasing malondialdehyde, the lipid damaging end products [Fig. 3, E].

Acetylcholine is a crucial neuro-modulator that plays an important role in muscle cells and the brain. Imbalances of this neural messenger can manifest in different types of neurological disorders. In nerve impulse transmission, acetylcholine esterase (AChE) is involved in the rapid hydrolysis of acetylcholine into acetate and choline in different pathways of the nervous system (Paul & Borah., 2017). This hydrolysis is critical for the cholinergic neuron to return to its resting state. This enzyme inhibition leads to acetylcholine accumulation. At the time of nerve impulse transmission, presynaptic neurons release acetylcholine into the synaptic cleft for binding to their receptors (nicotinic & muscarinic) present on the post-synaptic neuron which helps in transmitting the signal. AChE may also be present in post-synaptic neurons and hydrolyze the acetylcholine to terminate the signal relaying. Then, the resulting choline is received by the pre-synaptic nerve and combines acetyl CoA with liberated choline and recycles acetylcholine (Martorana et al., 2010). In spectrophotometric assay, tea exhibited increasing acetyl choline esterase activity in a cerebrum. Arsenic has displayed a certain increase in AChE activity with higher inter- individual variations, uric acid and allopurinol have shown almost similar to that of the control [Fig. 3, C]. Basically, no significant alterations were noticed in the cerebrum tissues. But the cerebellum pointed to a significantly higher AChE activity in the arsenic exposed group, which is completely restored in the CS group and this protection, unlike in the Allopurinol group, was also noticed in the Uric acid group [Fig. 3, F]. Studies have found that low levels of acetylcholine remain available in Alzheimer's patients (Franis et al., 2005). Medications which are used against this type of neuronal disorder should have the efficiency to enhance acetylcholine by blocking acetylcholine esterase. So, from this aspect we can suggest that tea and uric acid have shown a suppressing effect of AChE, which is beneficial for maintaining the integrity of neuronal communication.

Damage to DNA is mostly assessed by performing DNA comet assay or single cell gel electrophoresis. This process is observed under fluorescence microscopy. In our study, the arsenic group has shown more DNA breakage patterns than any other groups (Acharyya et al., 2015&Maiti et al., 2010). In the tea and uric acid group, no such damage was noticed. The allopurinol group has shown slight DNA breakage in this assay [Fig. 4]. So, as a therapeutic, tea and uric acid are both potent to stabilize the double stranded DNA and can prevent arsenic-induced DNA damage.

According to the literature, acetylcholine interacts with the catalytic site of acetyl cholinesterase, located at the base of a 20 Å long internal grove, containing specific amino acid composition of SER 203, GLU334 & HIS 447 [Fig. 5]. Among them, SER 203 basically initiates the hydrolysis of acetylcholine by a nucleophilic attack. HIS 447 acts like an acid-base element both in the case of acetylation and deacetylation. But the function of GLU 334 is somehow controversial. Three different hypotheses have been made about the proper function of GLU 334. According to the charge-relay hypothesis, GLU 334 acts as a base and it accepts a proton from HIS 447 and accelerates the acetylcholine hydrolysis phenomenon. According to the low barrier hydrogen bond (LBHB) or short strong hydrogen bond (SSHB) hypothesis, GLU 334 supplies some sort of energy to stabilize the reaction. Whereas, the NMR experiment revealed that a short hydrogen bond was formed between HIS 447and GLU 334, and no such direct connection was reported between GLU 334 and acetylcholine.

According to a present study on molecular docking between acetylcholine and acetyl cholinesterase (PDB ID: 4M0E), two consecutive sites were found which demonstrated the entry of acetylcholine within 20 Å long internal grove and movement towards the catalytic site respectively (Fig. 1A and 1B). At the docking site 1, acetylcholine initially forms a hydrogen bond with HIS 405: HD1 (H bond length: 1.861, binding energy – 3.39), a surface amino acid present in the enzyme (Fig. 1C), then it moves towards the docking sub-site at the interior position of the enzyme (Fig. 1B). Hydrogen bond was observed between acetylcholine and SER203 (H bond length: 2.05, binding energy − 3.99) at that position. The presence of two sides of amino acid residues, HIS447 and GLU202, confirmed the location as a catalytic site. The above result represents the interaction pattern between acetylcholine and acetyl cholinesterase (PDB ID: 4M0E).

In this experiment, we used tea flavonoid, uric acid and allopurinol against arsenic toxicity. Allopurinol is an antagonist of uric acid and inhibits xanthine oxidase in a dose dependent way and acts as a free radical scavenger and hypochlorous acid scavenger (Augustin et al., 1994). There are several references concluding that allopurinol helps to suppress the level of uric acid. In our experiment, uric acid level in serum was measured by rats distributed in different groups where it showed the reduction of serum uric acid level in the arsenic group but increased where tea and uric acid were given along with arsenic [Fig. 3, G]. From this observation, we can state that arsenic can reduce the uric acid level (Maiti et al.,2017). Uric acid is a known antioxidant when it remains at the limit of physiological concentration or normal level. Arsenic induced toxicity can happen in various ways involving different signalling pathways. Among them, arsenic mediated uric acid depletion may contribute to a path to antioxidant depletion and toxicity generation within the body. This is the reason why we have chosen the uric acid dose slightly higher so that the required uric acid level is maintained even after being suppressed by arsenic and can show the antioxidant phenomena. Here, we have also seen that tea extract gave a supporting influence for restoring arsenic mediated UA reduction [Fig. 3, G]. In our experiment, uric acid has shown its antioxidant properties by restoring the non-protein thiol, catalase, SOD and by decreasing the level of malondialdehyde (MDA) [Fig. 3]. So, it was expected that if we use allopurinol in a group, it will suppress the antioxidant properties as it suppresses uric acid. But surprisingly, this allopurinol group has also displayed antioxidant properties very efficiently. From here we can conclude that allopurinol itself acts as

an antioxidant against ROS and is very effective for protecting the damage caused by toxicity (Fig. 6). Allopurinol, holding an analogous structure like uric acid, is responsible for its antioxidant properties. Uric acid is an endogenous molecule, so it has some biological antioxidant impact as well as it can involve other physiological interactions. Allopurinol is a xenobiotic which is used as a therapeutic against uric acid. Due to the structural features of allopurinol being closely similar to uric acid, it can block the uric acid level. When uric acid is present in large amounts it can show toxic effects. Because allopurinol is not an endogenous molecule, no other physiological association is present, like uric acid. For this reason, in some situations, allopurinol is more effective against arsenic toxicity than uric acid.

4. Conclusions

Our experiment in the cerebrum and cerebellum has shown a clear idea that our given dose of arsenic is efficient to produce toxicity. Tea having antioxidant and anti-apoptotic properties helps in detoxification against arsenic toxicity, but at some points, it has also indicated its pro-oxidant properties. Although uric acid and allopurinol are antagonists of each other, they both still have roles in reducing toxicity. Further research on this aspect would be able to represent the conclusive remarks more strongly.

Abbreviations

AChE -Acetyl Cholinesterase, ADP-Adenosine di-phosphate, Allo-Allopurinol, APS-Ammonium per sulfate, ATCI- acetylthiocholine iodide, ATP-Adenosine tri-phosphate, As^{III} – Arsenite, As^{V}-Arsenate, BBB-Blood brain barrier, $CaCl_2$-Calcium chloride, CASTp-Computed-Atlas-of-Surface-Topography of Protein, CG-Catechin gallate , CRP- c-reactive protein CS-Camellia sinensis, DMSO-Dimethylsulfoxide, DNA-Deoxyribonucleic acid, DTNB- Di-thio-bis-nitrobenzoic acid, EC-Epicatechin, ECG-Epicatechin gallate, EDTA- Ethylenediamine tetraacetic acid, EGC-Epigallocatechin, EGCG-Epigallocatechin gallate, GCG-Gallocatechingallate GPx-Glutathione peroxidase, GSH-Reduced glutathione, HCL-Hydrochloric acid,H_2O_2-Hydrogen peroxide, LBHB-Low barrier hydrogen bond, LDH-Lactate dehydrogenase, MBP-Myelin basic protein, MDA-Malondialdehyde, MMP 2&9-Matrix metalloproteinase 2 and 9,NaCl- Sodium chloride, NAD^+-Nicotinamide adenine dinucleotide , NaN_3- Sodium Azide, NBT -Nitro blue tetrazolium,NF-Neurofilament,NF-κβ -Nuclear factor kappa β, NPSH-nonprotein Thiol,PAGE - Polyacrylamide gel electrophoresis, PBS-Phosphate buffer saline, PDB-Protein Data Bank, ROS-Reactive oxygen species, RT-Room Temperature, SDF- Structure data *file*, SDS-Sodium dodecyl sulphate, SOD-Superoxide dismutase, TBA -Thiobarbituric acid, TEMED-tetraethyl ethylenediamine, TF-Theaflavin, TFDG-Theaflavin digallate, TFMG-Theaflavin monogallate, TJs -Tight Junctions, UA-Uric Acid, WHO-World Health Organization.

Declarations

Conflict of interests: We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced

its outcome

Authors contribution: First draft-NM, Experimentation-NM, SaM, NA, TS, AM, Hypothesis- SM, Study design-SM, NM, AB, NA and TS, Analysis and data interpretation, critical review, final drafting - SM, SD, TG

Data Availability statement: All data are available upon request

ACKNOWLEDGEMENT

We are indebted to WB State DST (Dept. of Science & Technology) for providing us funding (930/Sanc. /ST/P/S&T/1G-5/2016).

References

1. Acharyya, N., Ali, S.S., Deb, B., Chattopadhyay, S., Maiti, S., 2015. 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. 30(9), 1033-44.
2. Acharyya, N., Chattopadhyay, S., Maiti, S., 2014. Chemoprevention against arsenic-induced mutagenic DNA breakage and apoptotic liver damage in rat via antioxidant and SOD1 upregulation by 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. 32(4),338 – 61.
3. Ahmed, S., Mahabbat-e Khoda, S., Rekha, R.S., Gardner, R.M., Ameer, S.S., Moore, S., Ekström, E.C., Vahter, M., Raqib, R., 2011. Arsenic-associated oxidative stress, inflammation, and immune disruption in human placenta and cord blood. Environ Health Perspect.119(2),258
4. Augustin, A.J., Boker, J., Blumenroder, S.H., Lutz, J., Spitznas, M., 1994. Free radical scavenging and antioxidant activity of allopurinol and oxypurinol in experimental lens induced uveitis. Invest.Opthalmol. Vis. Sci.35(110), 3897 – 904.
5. Ballabh, P., Hu, F., Kumarasiri, M., Braun A, Nedergaard M., 2005. Development of tight junction molecules in blood vessels germinal matrix, cerebral cortex and white matter. Pediatr. Res.58,791–798.
6. Bednarek, N., Clement, Y., Lelievre, V., Olivier, P., Loron, G., Garnotel, R., Gressens, P., 2009. Ontogeny of MMPs and TIMPs in the murine neocortex. Pediatr. Res. 65(3), 296–300.
7. Bhattacharjee, P., Chatterjee, D., Singh, K.K., Giri, A.K., 2013. Systems biology approaches to evaluate arsenic toxicity and carcinogenicity: an overview. Int. J. Hyg. Environ. Health. 216, 574–586.
8. Bhattacharya, P., Hossain, M., Rahman, S.N., Robinson, C., Nath, B., Rahman, M., Islam, M.M., Von Brömssen, M., Ahmed, K.M., Jacks, G., Chowdhury, D., Jakariya, M., Persson, L.Å., Vahter, M., 2011. Temporal and seasonal variability of arsenic in drinking water wells In Matlab, southeastern Bangladesh: a preliminary evaluation on the basis of a 4-year study. J. Environ. Sci. Health A Tox Hazard Subst. Environ. Eng. 46(11), 1177–1184.

9. Binu, P., Priya, N., Abhilash, S., Vineeta, K.C., Nair, R.H., 2017. Studies on curative efficacy of mono-terpene-eugenol on anti-leukemic drug arsenic trioxide induced cardiotoxicity. Biomed.Pharmacother. 91,559–566.
10. Calderon, J., Navarro, M.E., Jimenez-Capdeville M.E., 2001. Exposure to arsenic and lead and neuropsychological development in Mexican children. Environ. Res. 85, 69–76.
11. Chiou, H.Y., Chiou, S.T., Hsu, Y.H., 2001. Incidence of transitional cell carcinoma and arsenic in drinking water: a follow up study of 8102 residents in an arseniasis-endemic area in north-eastern Taiwan. Am. J. Epidemiol.153, 411–418.
12. Druwe, I.L., Sollome, J.J., Sanchez-Soria, P., Hardwick, R.N., Camenisch, T.D., Vaillancourt, R.R., 2012. Arsenite activates NFκB through induction of C-reactive protein. Toxicol. Appl. Pharmacol. 261, 263–270.
13. Dzwonek, J., Rylski, M., Kaczmarek, L., 2004. Matrix metalloproteinases and their endogenous inhibitors in neuronal physiology of the adult brain. FEBS Lett. 567(1),129–235.
14. Fabbrini, E., Serafini, M., Baric, I.C., Hazen, S.L., Klein, S., 2014. Effect of plasma uric acid on antioxidant capacity, oxidative stress, and insulin sensitivity in obese subjects. Diabetes. 63 (3), 976–981.
15. Forman, H.J., 2009. Critical methods in free radical biology &medicine. Free Radic Biol. Med. 47, Suppl 2,S207.
16. Francis, P.T., 2005. The interplay of neurotransmitters in Alzheimer's disease. CNS Spectr.10(11suppl 18),6–9.
17. Frankowski, H., Gu, Y.H., Heo, J.H., Milner, R., Del Zoppo, G.J. 2012. Use of gel zymography to examine matrix metalloproteinase (gelatinase) expression in brain tissue or in primary glial cultures. Methods Mol. Biol. 814, 221 – 33.
18. Garcia, M.V., Macias, D., Fau, GY., Ganan, Y., Garcia-Lobo J.M., Francia, M.V., Fernandez-Teran, M.A., Hurle, J.M., 1993. Internucleosomal DNA fragmentation and programmed cell death (apoptosis) in the interdigital tissue of the embryonic chick leg bud. J. of Cell Sci.106, 201–208.
19. Gilmour, M., Flint, H.J., Mitchel, W.J., 1994. Multiple lactate dehydrogenase activities of the rumen bacterium Selenomonousruminatium, Microbio. 140,2077–2084.
20. Govindappa, T., Govardhan, L., Jyothy, P.S., Veerabhadrappa, P.S., 1987. Purification and characterisation of acetylcholinesterase isozymes from the latex of Synadeniumgrantii Hook, 'f'. Indian J Biochem. Biophys.24(4), 209 – 17.
21. Hua, X.,, Jianqin, S., Yanqiu, C., Min, Z., Shijie, Li., Yan, Wang., 2015.EGCG Attenuates Uric Acid-Induced Inflammatory and Oxidative Stress Responses by Medicating the NOTCH Pathway. Oxid. Med. Cell. Longev. 2015,214836.
22. Huang, L., Wu, H., Vander Kuijp T.J., 2015. The health effects of exposure to arsenic-contaminated drinking water: a review by global geographical distribution. Int. J. Health Res. 25, 432–452.
23. Karim, MR.., Salam, K.A., Hossain, E., Islam, K., Ali N., Haque, A., Saud Z.A., Yeasmin, T., Hossain, M., Miyataka, H., Himeno, S., Hossain, K., 2010. Interaction between chronic arsenic exposure via

drinking water and plasma lactate dehydrogenase activity. Sci. Total Environ,409(2),278 – 83.

24. Kim, J.H., Byun, H.M., Chung, E.C., Chung, H.Y., Bae, O.N., 2013. Loss of integrity: impairment of blood-brain barrier in heavy metal associated ischemic stroke. Toxicol. Res.29(3),157–164.
25. LeBlanc, M.S., McKinney, E.C., Meagher, R.B., Smith, A.P., 2013. Hijacking membrane transporters for arsenic phytoextraction. J. Biotechnol.163,1–9.
26. Liao, D., Hessler, N.A., Malinow, R., 1995. Activation of postsynaptically silent synapses during pairing- induced LTP in CA1 region of hippocampal slice. Nature. 375(6530),400–404.
27. Maiti,S., Acharyya, N., Ghosh, T.K., Ali, S.S., Manna, E., Nazmeen, A., Sinha, N.K. 2017. Green tea (Camellia sinensis) protects against arsenic neurotoxicity via antioxidative mechanism and activation of superoxide dismutase activity. Cent. Nerv. Syst. Agents Med. Chem.17(3),187–195.
28. Maiti, S., Chatterjee, A.K., 2001. 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.75, 531–537.
29. Maiti, S., Chattopadhyay, S., Acharyya, N., Deb, B., Hati, A.K., 2014. Emblica officinalis (amla) ameliorates arsenic-induced liver damage via DNA protection by antioxidant systems. Mol. Cell. Toxicol.10, 75–82.
30. Maiti, S., Chattopadhyay, S., Deb, B., Samanta, T., Maji, G., Pan, B., Ghosh, A., Ghosh, D., 2012. Antioxidant and metabolic impairment result in DNA damage in arsenic-exposed individuals with severe dermatological manifestations in Eastern India. Environ.Toxicol.27(6),342 – 50.
31. Maiti, S., Nazmeen, A., Medda, N., Patra, R., Ghosh, T.K., 2019. Flavonoids green tea against oxidant stress and inflammation with related human diseases,
32. Clin. Nutri. Exp.24, 1–14.
33. Magnowska, M., Gorkiewicz, T., Suska, A., Wawrzyniak, M., Rutkowska-Wlodarczyk, I., Kaczmarek, L., Wlodarczyk, J., 2016. Transient ECM protease activity promotes synaptic plasticity.Sci. Rep.10(6),27757.
34. Martorana, A., Esposito, Z., Koch, G., 2010. Beyond the cholinergic hypothesis: Do current drugs work in Alzheimer's disease? CNS Neurosci. Ther. 16(4),235–245.
35. Medda, N., De, S.K., Maiti, S., 2021. Different mechanisms of arsenic related signaling in cellular proliferation, apoptosis and neo-plastic transformation. Ecotoxico.l Environ. Saf. 208, 111752.
36. Medda, N., Patra, R., Ghosh, T.K., Maiti, S., 2020. Neurotoxic mechanism of arsenic: synergistic effect of mitochondrial instability, oxidative stress, and hormonal-neurotransmitter impairment. Biol. Trace Elem. Res.198(1), 8–15.
37. National Research Council Subcommittee on Arsenic in Drinking Water. 1999. Arsenic in Drinking Water. National Academy Press, Washington, DC.
38. National Research Council Subcommittee on Arsenic in Drinking Water. 2001. Arsenic in Drinking Water. 2001 Update. National Academy Press, Washington, DC.

39. Nemeti, B., Regonesi, M.E., Tortora, P., Gregus, Z., 2010. Polynucleotide phosphorylase and mitochondrial ATP synthase mediate reduction of arsenate to the more toxic arsenite by forming arsenylated analogues of ADP and ATP. Toxicol.Sci.117, 270–281.
40. Patel, H.V., Kalia, K., 2010. Subchronic arsenic exposure aggravates nephrotoxicity in experimental diabetic rats. Ind.J. Exper.Biol.48,762–768.
41. Paul, R., Borah, A., 2017. Global loss of acetylcholinesterase activity with mitochondrial complexes inhibition and inflammation in brain of hypercholesterolemic mice. Sci. Rep. 7(1),17922.
42. Rai, A., Maurya, S., Khare, P., Srivastava, S.A., Bandapadhayay, S.,2010. Characterization of developmental neurotoxicity of As, Cd and Pb mixture: synergistic action of metal mixture in glial and neuronal functions, Toxicol. Sci.118(2010), 586–601.
43. Rai, A., Tripathi, P., Dwivedi, S., Dubey, S., Shri, M., Kumar, S., Tripathi, PK., Dave, R., Kumar, A., Singh, R., Adhikari, B., Bag, M., Tripathi, R.D., Trivedi, P.K., Chakrabarty, D., Tuli, R., 2011. Arsenic tolerances in rice (Oryza sativa) have a predominant role in transcriptional regulation of a set of genes including Sulphur assimilation pathway and antioxidant system. Chemosphere.82(7), 986–995.
44. Rai, N.K., Ashok, A., Rai, A., Tripathi, S., Nagar, G.K., Mitra, K., Bandyopadhyay, S., 2013. Exposure to As, Cd and Pb-mixture impairs myelin and axon development in rat brain, optic nerve and retina. Toxicol. Appl. Pharmacol.1;273(2),242 – 58.
45. Ramos-Fernandez, M., Bellolio, M.F., Stead, L.G., 2011. Matrix metalloproteinase-9 as a marker for acute ischemic stroke: a systematic review. J. Stroke Cerebrovasc.Dis.20(1),47–54.
46. Romero, F.J., Bosch-Morell, F., Romero, M.J., Jareno E.J, Romero, B., Marin, N., Roma, J. 1998. Lipid peroxi,dation products and antioxidants in human disease, Environ. Health Prospect.106(suppl 5),1229-34.
47. Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell. Res.175(1),184–191.
48. States, J.C., Srivastava, S., Chen Y., Barchowsky, A., 2009. Arsenic and Cardiovascular Disease. Toxicol. Sci.107,312–323.
49. Stefaniuk, M., Beroun, A., Lebitko, T., Markina, O., Leski, S., Meyza, K., Grzywacz, A., Samochowiec, A., Radwanska, K., Kaczmarek, L., 2017. Matrix metalloproteinase-9 and synaptic plasticity in the central amygdala in control of alcohol seeking behavior. Biol. psychiatry. 81(11),90–91.
50. Tyler, CR.,Alla, A.M., 2014. The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review. Curr. Environ. Health Rep.1(2), 132–147.
51. Vafadari, B., Salamian, A., Kaczmarek, L., 2016. MMP-9 in translation: from molecule to brain physiology, pathology and therapy. J. Neurochem. 139(suppl 2),91–114.
52. Vahter, M., 2009. Effects of arsenic on maternal and fetal health. Annu. Rev. Nutr.29, 381 – 99.
53. Wang, X., Fang, H., Huang, Z., Shang, W., Hou, T., Cheng, A., Cheng, H., 2013. Imaging ROS signaling in cells and animals. J. Mol. Med. (Berl.),91(8),917–927.

54. Xie, H., Sun, J., Chen, Y., Zong, M., Li, S., Wang, Y., 2015. EGCG Attenuates Uric Acid-Induced Inflammatory and Oxidative Stress Responses by Medicating the NOTCH Pathway. Oxid. Med. Cell. Longev.2015,214836.

Tables

Table 1 Analysis of predicted ACE value of different Ache enzyme-inhibitor interactions at docking site 1 and 2.

Sl. No.	Component	Docking Site S_1	Docking Site S_2
		ACE Value (No. of docking in site S1 within 20)	ACE Value (No. of docking in site S2 within 20)
1	TFDG	-344.71 (5)	-369.87 (5)
2	TFMG	-287.03 (5)	-347.06 (3)
3	TF	-284.66 (2)	-300.44 (4)

Figures

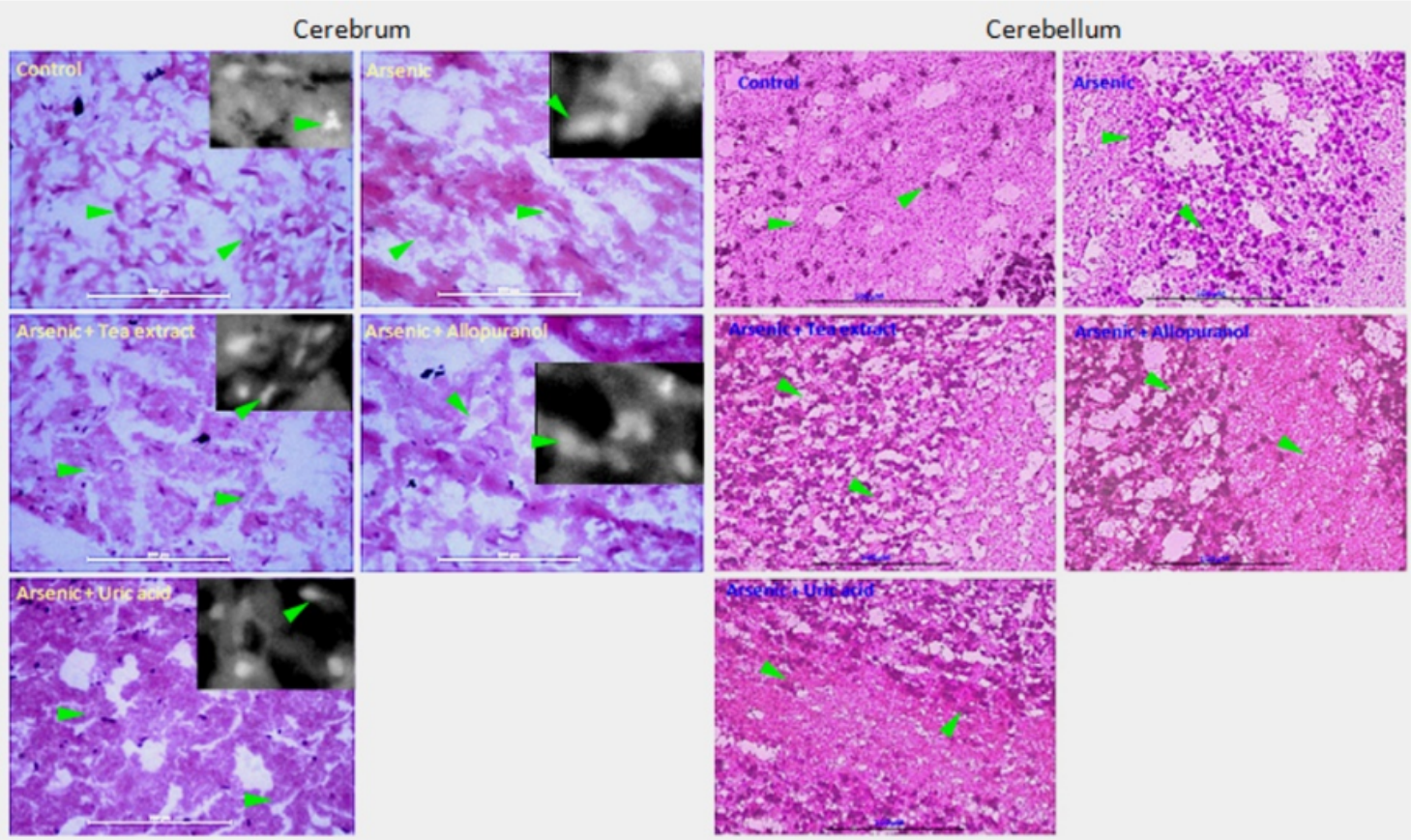


Figure 1

Cerebrum and cerebellum tissue were sectioned by cryostat microtome, serially sectioned at 5 µM, stained with eosin and hematoxylin (Harris) and observed under a light microscope (20X) to study the histoarchitechture of the tissues. The representative images are presented from the different study-groups as mentioned above.

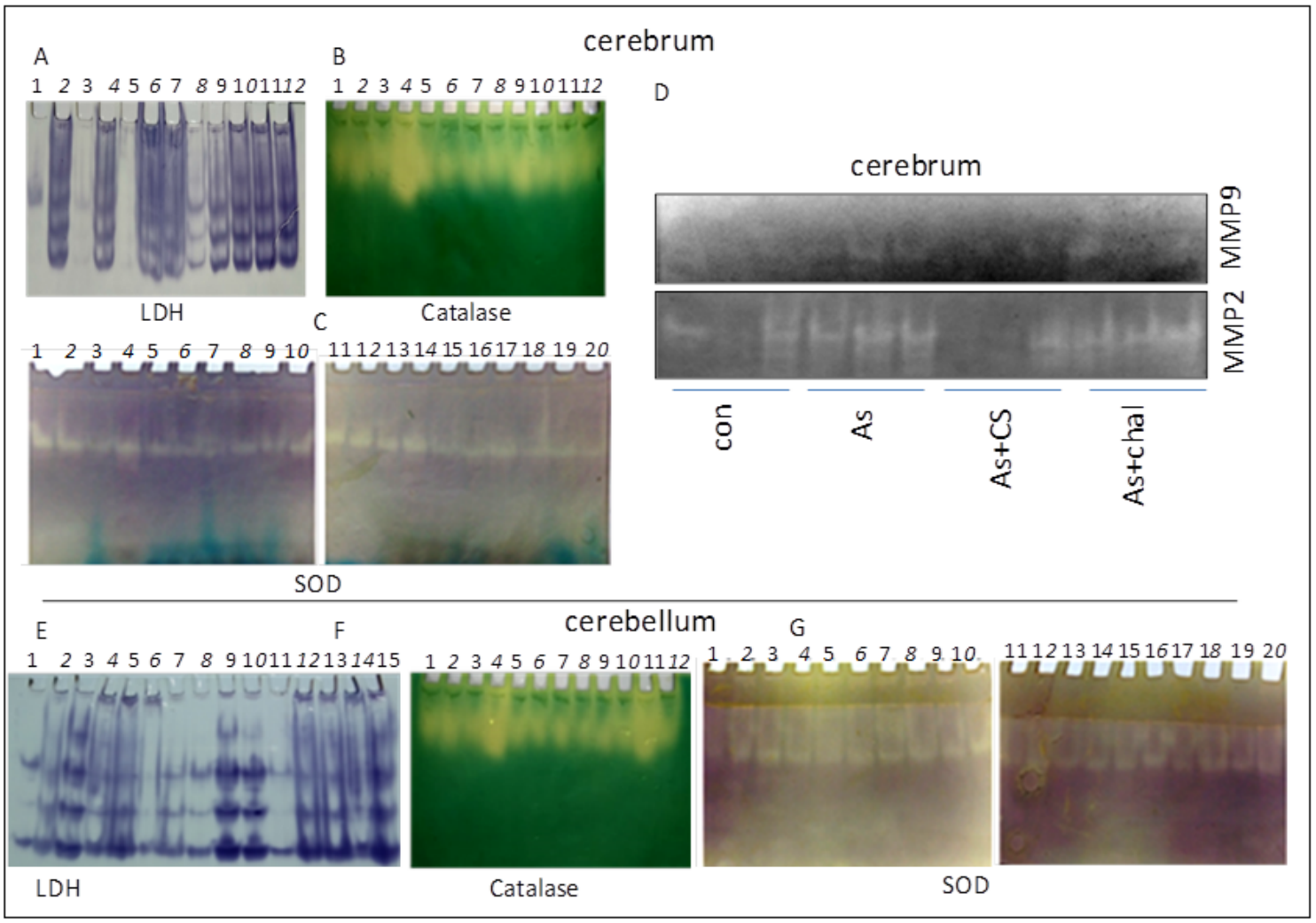


Figure 2

Effects of tea extract, uric acid and uric acid analog allopurinol on arsenic induced alterations of antioxidant enzymes in rat brain tissues. The effects are tested on matrix degenerating enzymes MMPs.

[A]-Lactate dehydrogenase assay-1-2-con, 3-5-As,6-7-As+CS, 8-10-As+UA,11-12-As+Allo.

[B]-Catalase assay in cerebrum-1-3-con,4-5-As+CS, 6-8-As, 9-10-As+UA, 11-12-As+Allo.

[C]-Superoxide dismutase assay-1-4-con, 5-8-As, 9-12-As+CS, 13-16-As+UA, 17-20-Allo.

[D]-MMP2 and MMP9 Zymogran. Group distribution is shown in figure. In fourth group chalcone (α,β-unsaturated ketone, 4 mg/100gm b.w. for 7 days) an oxidative-stress modifier was supplemented with arsenic. No significant alteration was noticed with comparison to the only arsenic treated group.

[E]-LDH assay in cerebellum-1-3-con, 4-6-As, 7-9-As+CS, 10-12-As+UA, 13-15-As+Allo.

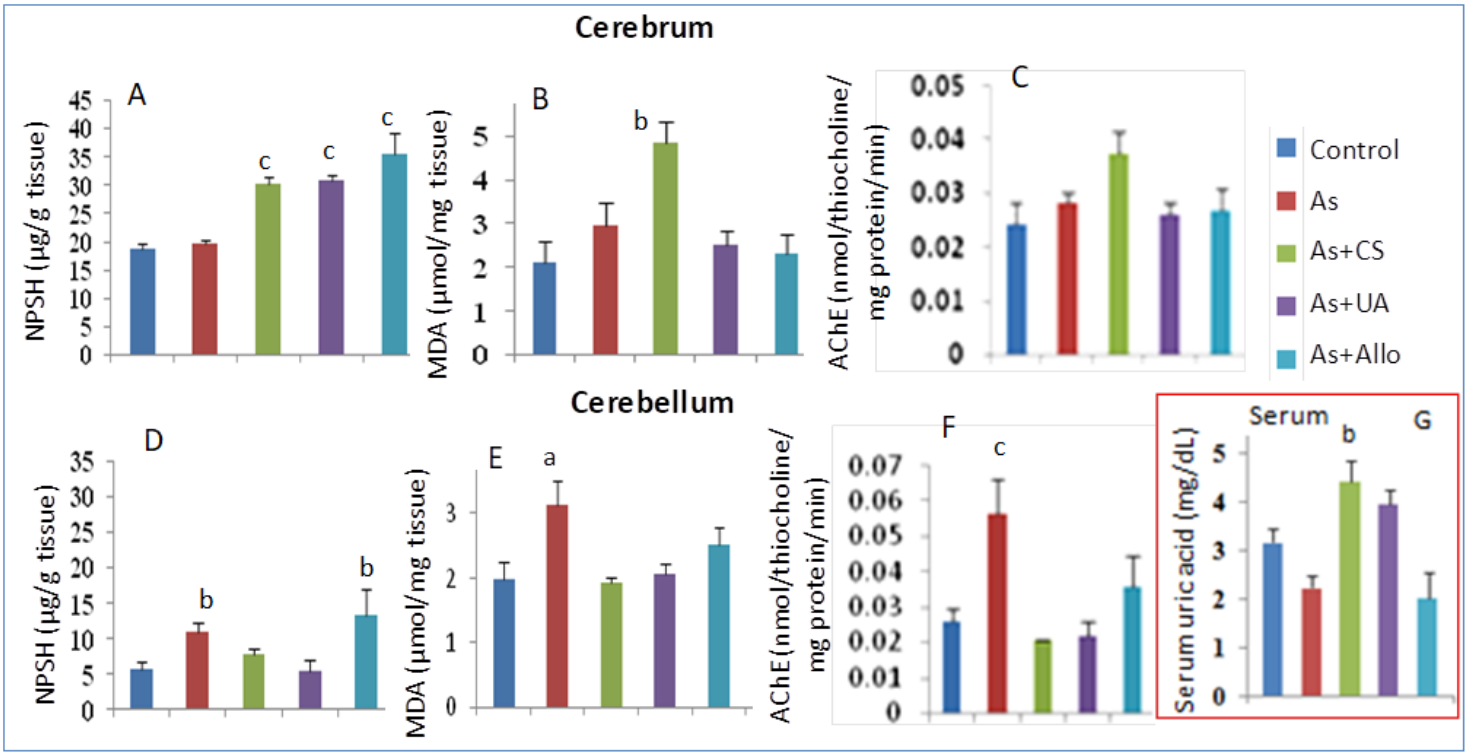
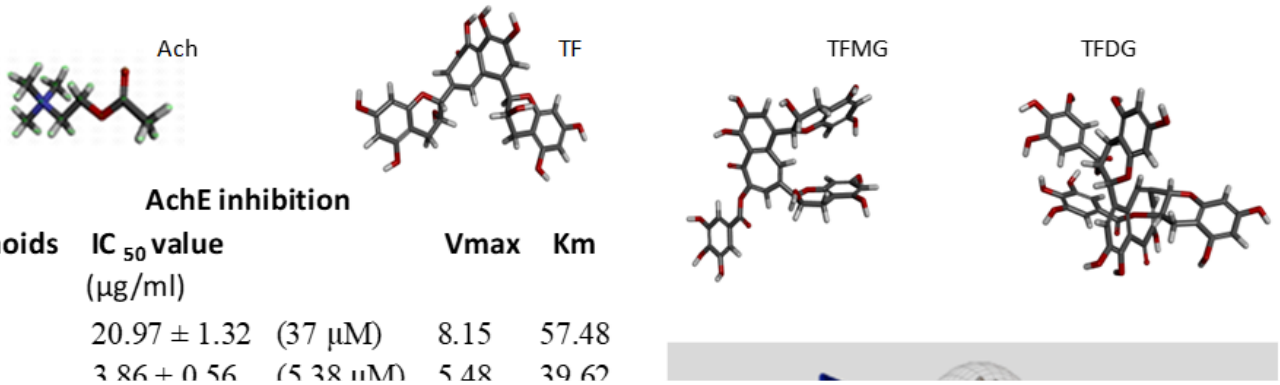


Figure 3

Effects of tea extract, uric acid and uric acid analog; allopurinol on arsenic induced alterations of different oxidant stress parameters and brain signaling molecule acetyl choline esterase in rat brain tissues.

Figure 4

Effects of tea extract, uric acid and allopurinol on arsenic induced alterations of cellular DNA stability in rat brain tissues. Single cell DNA-status is analyzed by the Comet assay.



AchE inhibition

Flavonoids	IC $_{50}$ value (µg/ml)		Vmax	Km
TF	20.97 ± 1.32	(37 µM)	8.15	57.48
TFMG	3.86 ± 0.56	(5.38 µM)	5.48	39.62

Figure 5

Effects of pure flavonoids like TF (theaflavin), TFMG (theaflavin monogallate) and TFDG (Theaflavin digallate) on partially purified AchE from rat brain tissues. Bioinformatics and molecular modeling data strongly support our present finding of in vivo and in vitro results.

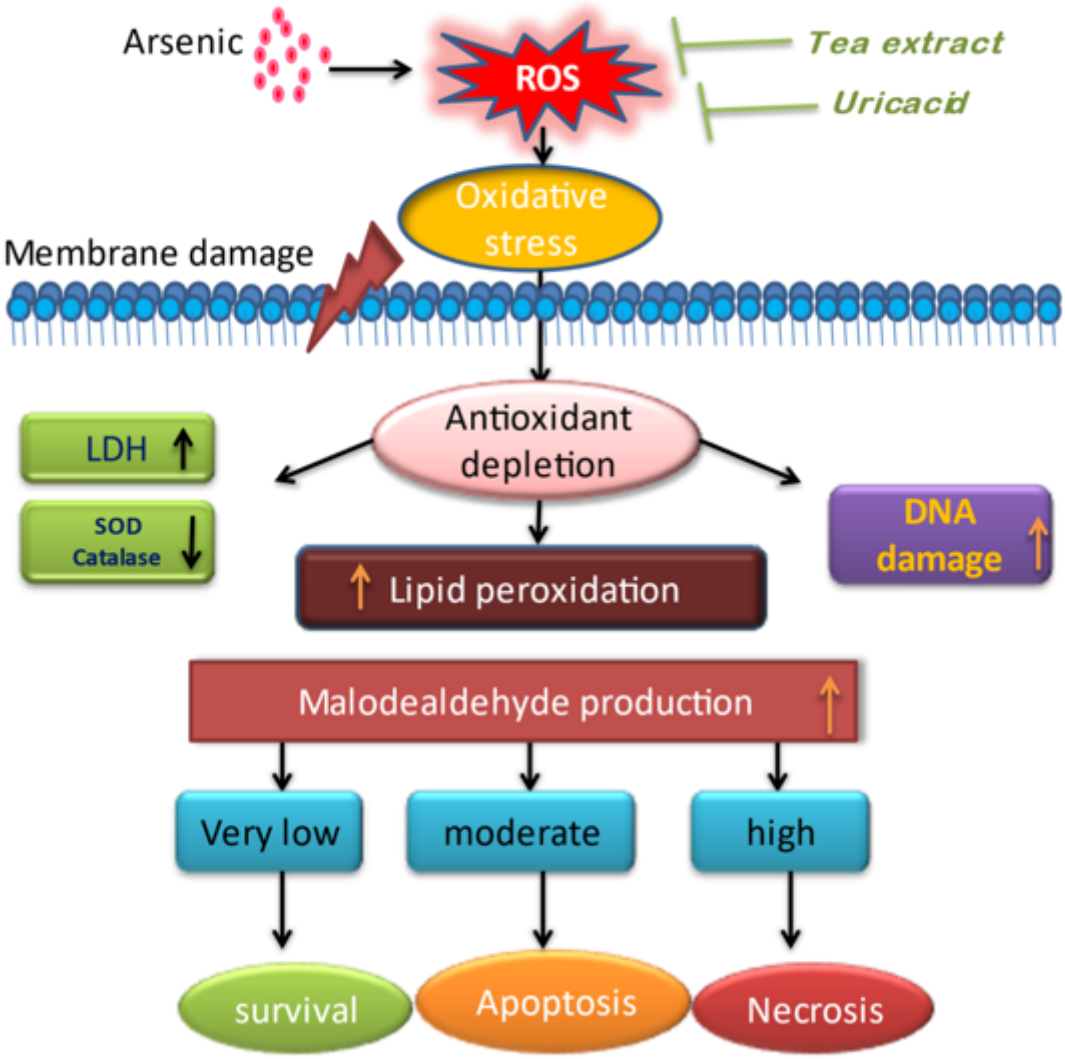


Figure 6

Schematic diagram is shown on possible arsenic toxicity mechanism and its prospective therapeutic targets.