Ruthenium Red Ameliorates Arsenic Trioxide Mediated Oxidative Stress and Behavioral Changes in Mice

Priyadarshini Soni[1], Rupesh Kumar Pandey[2*], Surbhi Gupta[3]

[1]Research Scholar, Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh-250005
[2*]Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh-250005.
E-mail: ranu.rupeshpandey@gmail.com
[3]Department of Pharmacology, School of Pharmacy, Bharat Institute of Technology, Meerut Uttar Pradesh - 250005

Abstract
Groundwater poisoning by arsenic (As) is a serious global public health issue, especially in developing nations where access to clean drinking water is still scarce. Severe health problems, including as neurological disorders, vascular diseases, skin lesions, and cancer, have been linked to long-term exposure to arsenic through polluted water, food, inhalation, and skin contact. Hotspots for arsenic contamination include the Mekong Delta, the Terai region of Nepal, the Ganga–Brahmaputra–Meghna plains of Bangladesh and India, and numerous areas of China and Southeast Asia. Arsenic exposure may be a major cause of neurotoxicity, depression, anxiety, and cognitive impairment, according to growing epidemiological and experimental data. Oxidative stress, mitochondrial dysfunction, lipid peroxidation, compromised antioxidant defence systems, and disruptions in neurotransmitter modulation are among the underlying causes. Reactive oxygen species overproduction brought on by arsenic upsets cellular redox equilibrium, lowers glutathione and antioxidant enzyme activity, and encourages neuronal apoptosis and degeneration of the central nervous system. Ryanodine receptors (RyRs) and inositol trisphosphate receptors regulate dysregulation of intracellular calcium signalling, which exacerbates neuronal damage and impairs synaptic plasticity linked to learning and memory impairments. Because it can inhibit mitochondrial-dependent apoptotic signalling and restore calcium homeostasis, ruthenium red (RR), a specific antagonist of RyR-mediated calcium release, has become a promising neuroprotective drug. According to experimental results, RR may enhance cholinergic function in neurodegenerative diseases and lessen oxidative stress-induced neuronal damage. In order to combat arsenic-induced neurotoxicity and related neurological deficits, RyR inhibition may be used to target calcium dysregulation.

Keywords: Arsenic neurotoxicity; Oxidative stress; Mitochondrial dysfunction; Ryanodine receptor; Ruthenium Red

Introduction

Globally, 1.8 billion people drink water tainted with faeces, while about 2.1 billion people lack access to clean water [1-3]. A common environmental contaminant that has seriously contaminated soil and water supplies is arsenic (As) [4]. One term for arsenic is "silent toxin" [5]. It has been discovered that arsenic can be fatal to humans at even very low concentrations [6]. The main ways that arsenic enters the human body are by inhalation, drinking water, skin contact, and the food chain [7 & 8]. Long-term exposure to arsenic has been linked to irreparable harm to a number of physiological systems, including skin conditions, neurological disorders, vascular diseases, and malignancies [9 & 10]. According to epidemiological studies, exposure to arsenic may raise the risk of a number of neurological conditions, including depression, anxiety disorders, and cognitive impairments [11 & 12]. Bangladesh's Ganga-Brahmaputra-Megha deltaic plain [13], Vietnam's Mekong Deltas, Cambodia's Kandal Province, and Hanoi's Red River Basin deltas [14], Nepal's Terai region [15], China's Xinjiang province and Thailand's Chao Phraya basin [16] and the Ganga-Meghna-Brahmaputra (GMB) plain in India are the parts of the world where arsenic (As), a well-known geogenic groundwater pollutant, is found [17-23]. Arsenic's capacity to induce oxidative stress and mitochondrial dysfunction is one among the most significant mechanisms underlying its neurotoxicity [24 & 25]. Additionally, it has been demonstrated that neurodegeneration may result from oxidative stress and mitochondrial malfunction [26]. Numerous clinical conditions, including as inflammation, atherosclerosis, neurological disorders, and cancer, are linked to oxidative stress and the ensuing lipid peroxidation. One fundamental phase of cellular degradation brought on by oxidative stress is lipid peroxidation. Oxidative stress brought on by As exposure causes lipid peroxidation, which causes brain cell death as well as central nervous system (CNS) degeneration [27-29]. One of the numerous vital enzymes required for the healthy operation of the human nervous system is acetylcholinesterase. Serum acetylcholinesterase activity was markedly and dose-dependently reduced in rats by As trioxide. Cholinergic crisis, which may be linked to peripheral neuropathy or CNS injury, was brought on by decreased acetylcholinesterase activity. Cholinergic distress, which may be associated with a peripheral neuropathy or CNS injury, was brought on by decreased acetylcholinesterase activity. There are a number of potential toxicity pathways, and it is still unknown how these processes relate to the symptoms [30]. Impairment of GSH and other antioxidant enzymes resulting from arsenic toxicity, which results in bioenergetic organelles dysfunction, resulting in cellular components being harmed by oxidative processes, is also not well documented [31]. Reduced levels of non-enzymatic antioxidant (GSH) and

enzymatic antioxidants, which function in preventing free radical damage, show that an organism is less able to protect itself from harm caused by ROS [32]. Another study suggested a remarkable increase in thio-barbituric acid reactive substances levels, accompanied by a corresponding reduction in the antioxidant enzymes associated with oxidative stress, including mitochondrial superoxide dismutase (MnSOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST). According to all of these investigations, oxidative stress is linked to arsenic neurotoxicity through elevated ROS production and a disrupted mitochondrial antioxidant enzyme system [33 & 34]. The brain's cells communicate with one another through a number of neurotransmitters. Because it regulates norepinephrine (NE) levels, arsenic-mediated neurotoxicity involving neurotransmitter dysregulation as well as stimulated changes in dopamine & serotonin concentrations [35]. Additionally, arsenic changes the amounts of nitric oxide, amino acid-derived neurotransmitters serotonin, norepinephrine and dopamine, GABA, glutamate & other biogenic amines [36]. A crucial stage in many different biological processes is the release of Ca2+ ions from intracellular membrane-bound storage. Ryanodine receptors and inositol 1,4,5-trisphosphate receptors are two related Ca2+ release channel families that primarily mediate Ca2+ release [37-39]. Both kinds of Ca2+ release channels are found in the SR, a specialized sub-compartment of the ER and muscle. Acidic endosomal structures and mitochondria are two more intracellular organelles that store and release Ca2+. Numerous substances, such as calcium, magnesium, adenosine triphosphate, calmodulin, protein kinases and phosphatases, and redox-active species, regulate these large-conductance channels permeable to monovalent and divalent ions [40]. In order to ensure proper functioning of the pleotropic Ca 2+-dependent activities, such as memory and learning, neural Calcium ion signalling acts as strictly controlled. In neuronal tissue, calcium-induced calcium release (CICR)" drives the stimulation of both RyR channels and intracellular calcium release channels sensitive to IP_3. Learning & memory are usually believed to be largely supported by long-term potentiation (LTP) [41]. Ruthenium red (RR) is recognized as a potent intracellular antagonist of RyR-mediated calcium release that is selective. RR is recognized as a potent intracellular antagonist of RyR-mediated calcium release that is selective. RR is helpful in treating a number of Dementias and related movement disorders, including Alzheimer's and Huntington's disease [42, 43] via reducing the brain's cholinergic dysfunction [44]. It is a rare metal found in the earth's crust, much like other metals in the platinum group. Because it is present as a trace constituent of the earth's crust, with an abundance of 0.0004 parts per million and extremely rare [45]. It comes from platinum ores, just like other metals in the platinum group [46]. RR is used to measure blood calcitonin levels. This information help in the detection and management of thyroid and parathyroid gland disorders. Calcitonin level measurement is crucial in the treatment of medullary thyroid cancer (MTC). The Elecsys cyclosporine test also uses RR to detect cyclosporine. Identifying cyclosporine is crucial for the treatment of patients using cyclosporine therapy who have liver, kidney, heart, lungs, and bone marrow transplants [47]. In the current work, Ruthenium Red is thought to play a part in preventing excessive calcium release from the endoplasmic reticulum, shielding neurons from damage caused by oxidative stress. "By restoring calcium homeostasis and suppressing mitochondrial-dependent apoptotic pathways, ryanodine receptor inhibition confers neuroprotection against toxin-induced neurodegeneration."

Material and Method

Experimental Design

There were five groups in total for experiments, each consisting of six animals. The group I had 0.9% normal NaCl as normal control group. The drug RR was administered via the route of intraperitoneal injection (i.p.), in Group II RR (Per se) at a dose of 6 mg/kg. At the same time, the Group III received arsenic trioxide (As2 O3) at a dose of 5 mg/kg i.p. Arsenic trioxide (As2 O3) (5 mg/kg i.p.) plus treatment drug RR (3 mg/kg i.p.) was given to Group IV. Arsenic trioxide (5 mg/kg i.p.) plus treatment drug RR (6 mg/kg i.p.) was given to the group V.

Ethical Statement

However, Institutional Animal Ethics Committee (IAEC) approval was sought before implementing the protocol, and guidelines set by the CCSEA of the Ministry of Environment and Forest, Government of India, were followed during the care of animals.

Animal

Swiss Albino Mice (Males, 25-30g) were used and maintained in an animal house under standard laboratory conditions (26+1°C) with ad libitum access to food and water. They were kept under a regular light/dark schedule of 12/12 hours in normal polyethylene cages. Studies were carried out in an enclosure that was partly soundproofed between 9:00 am and 4:00 pm. The subjects were placed in the experimental environment in the laboratory and used until the completion of behavioral tests after seven days of acclimatization to the testing environment. Prior to use to minimize defensive behavior and the stress reaction towards the investigator, each mouse in the experiment was manually handled for five minutes daily for three consecutive days following one day of adaptation to the lab environment.

Drug & Chemicals

All the drug preparations that were used in this study were acquired from a commercial source and were used directly without further manipulation. Analytical-grade chemicals required for biochemical assays and other experiments were provided by the departmental laboratory and the institution's chemical store. All the chemicals employed in this study were prepared using distilled water, and they had an analytical reagent grade. All the reagents and solutions were freshly prepared before being used in the experiments.

Behavioral studies

1. Open Field Test (OFT)- The OFT served as the method in measuring mice's exploratory and anxious behavior. The apparatus used is a 100 x 100 x 40 cm square, black box, containing four quadrants and sixteen equidistant squares. Prior to experimentation, the subjects were allowed three hours to acclimate in the laboratory setting. Individual mice were placed at the center while the entire session was videotaped for five minutes. In addition to eliminating odors, the test arena was washed with 75% ethanol between each session. Some of the behavioral measures included horizontal activity (number of grid crossings), vertical activity (lifting), total distance traveled, and central zone measurements (time spent, distance traveled, and speed) [48].

2. Force Swim Test (FST)- The FST was conducted to identify depression-like behavior. In order to prevent the animals from sinking down into the water, a clear cylindrical water tank of 20 cm diameter and 46 cm height was filled with water at a depth of 30 cm and temperature 23-25 °C. Pre-testing took place 15 minutes prior to testing, and a 6-minute test occurred 24 hours later. The period of immobility, which was characterized as being still except for the purpose of keeping their heads above water, was recorded [50-53].

3. Elevated Plus Maze (EPM)- To assess anxiety-related behavior, the Elevated Plus Maze (EPM) test was used. The apparatus was constructed from acrylic and consisted of two open and two closed arms (50 × 10 cm) that met in the middle at a central platform (10 × 10 cm) elevated 50 cm above the floor. The height of the walls of the closed arms was 40 cm. Prior to the experiment, animals were adapted for five minutes. Based on video observation, animals were placed in the center with their limbs extended and studied for five minutes [54, 55].

4. Morris Water Maze (MWM)- Evaluation of learning and memory ability was done by means of Morris Water Maze test (MWM). Mice had to locate a hidden platform which was located beneath the surface of the water by about 1cm. The pool used was circular (diameter: 150 cm; height: 45 cm) and contained opaque water (temperature: 25 ± 2°C; depth: 30 cm). This pool was divided into four equal quadrants. Each animal received four trials per day for four consecutive days in which two minutes were allotted for each trial and the intervals between trials lasted five to six minutes. Escape Latency Time (ELT) was determined on day four, and Target Quadrant Spending Time (TSTQ) was obtained on day five during probe tests [56-58].

5. Forelimb Support (Hanging wire) Test- Strength and coordination of forelimbs were assessed with the help of the Hanging Wire Test. This test involved observing mice for two minutes while hanging 60 cm off the floor with the help of a 55 cm wire. A score out of five (based on grip and climbing abilities) was noted along with the time of hanging. The best hanging time and the score among three such tests conducted at intervals of five minutes each were analyzed [59, 60].

Biochemical studies

1. Estimation of Glutathione (GSH)-The presence of Glutathione (GSH), an important cell antioxidant, was estimated to evaluate oxidative stress. Tissue homogenate was precipitated by 50% trichloroacetic acid from 10% w/v tissue solution in 0.02 M EDTA and centrifuged at 3000 rpm for 15 minutes. The supernatant obtained was mixed with Ellman's reagent in Tris-HCl buffer, pH 8.9, and absorbance was determined at 412 nm [61-63].

2. Estimation of brain Thio-barbituric acid reactive species (TBARS)- The MDA, which is an end-product of lipid peroxidation and forms a colorful complex when reacted with TBA, was utilized to estimate TBA-reactive substances. The homogenate from the tissues was mixed with 12% TCA, 0.73% TBA, DPTA (0.1 mM), and Tris-HCl buffer (60 mM, pH 7.4). The mixture was heated for one hour at 100°C and then allowed to cool before centrifugation at 10,000 g for five minutes. Calculation of lipid peroxidation was made through the MDA extinction coefficient (153 M^{-1} cm^{-1}) and the determination of absorbance at 535 nm [64].

3. Estimation of brain myeloperoxidase (MPO) level- Quantification of the activity of MPO was done using spectrophotometry (UV-2700, Thermo Scientific). Brain tissue was first homogenized in 50 mM cold potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl trimethyl ammonium bromide, before being centrifuged at 11,000 g at 4ºC for 20 minutes. Then, 986 µL of phosphate buffer with hydrogen peroxide (0.0005%) and 0.167 mg/mL of o-dianisidine dihydrochloride was mixed with 34 µL of supernatant. Absorbance reading was taken at 460 nm. One unit of MPO was regarded as one nM peroxide usage per minute at 2 nm [65].

4. Estimation of Acetylcholinesterase in brain- Brain homogenate was added to 1% Triton X-100 solution (in 0.03M NaPO4, pH-7) with a volume of 500 µl and centrifuged for 30 minutes. The supernatant was collected and kept at 4°C for determination of acetylcholinesterase activity through. Kinetic curve for the enzyme activity was recorded using a spectrophotometer at 412 nm in every 15 seconds. One unit of acetylcholinesterase enzyme was defined as micromoles (µmol) of acetylthiocholine iodide hydrolyzed per min per mg of protein. Specific activity was determined as µmol/min/mg protein [66].

5. Hematoxylin and eosin (H&E) staining- Hematoxylin and Eosin (H&E) Stain is one of the most common techniques of histology. The sections are first stained with the Harris Hematoxylin stain, which involves oxidation of hematoxylin to hematein, which is capable of forming hemalums that are bound to chromatin and staining the nuclei and other organelles

purple. These will be converted to a blue color using “blueing” through an alkaline solution. Lastly, the sections are counterstained with eosin Y, giving a pink color.

Statistical Analysis:
The data were statistically using Graph prism pad 5 and represented using the mean ± standard error mean (SEM). Statistical analysis was performed using Analysis of Variance (ANOVA) to determine Statistical significance. Following ANOVA, a post hoc test, especially the Tukey test was applied to further analyze and compare the data for significance differences among different groups. P value (0.05, 0.01, 0.001) is elaborated for each obtain result.

Results

ASSESSMENT OF BEHAVIOR PARAMETERS

Effect of Ruthenium Red on Locomotor Behaviors (OFT)

In comparison to the control group, **figure 1** illustrates the As2O3-treated group did not show any significant changes in locomotor activity. This suggests that there was no noticeable motor impairment in the animals. Administration of ruthenium red at doses (3 and 6 mg/kg, i.p.) with As2O3 did not show any significant change in locomotor activity as compared to the As2O3 group.

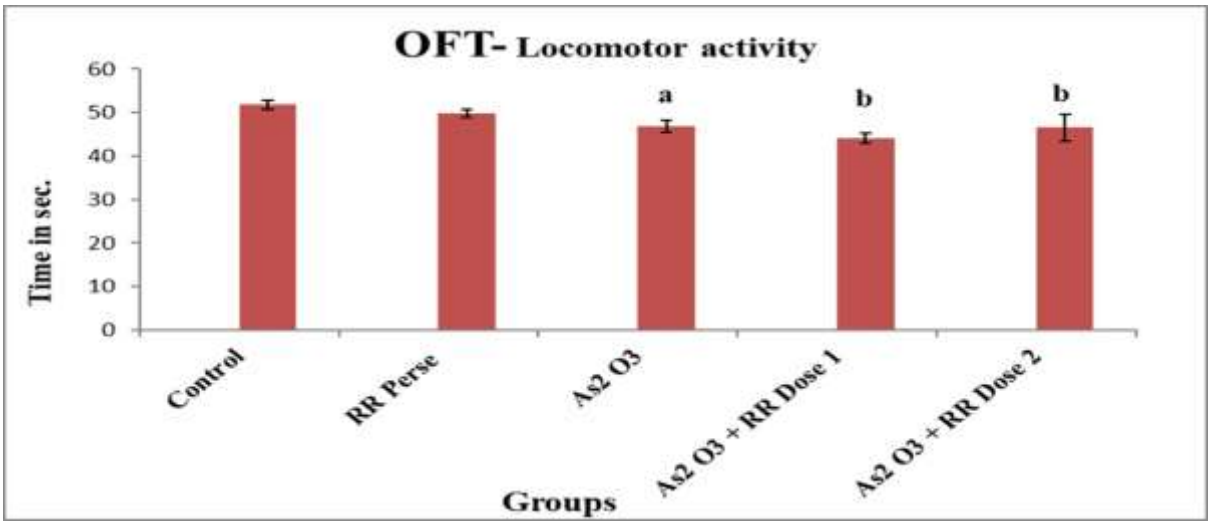


Figure 1

Fig.1 Impact of RR on locomotor activity. Results were expressed as mean ± SEM (n=6), one way ANOVA followed by tukey’s multiple comparison test. Locomotor activity = [F (8,53) =1.90, P<0.05]; remarkable difference at $^{a}P<0.05$ vs control group; difference $^{b}P<0.05$ vs As2O3. There was no significant difference in all the groups. OFT (Open Field test), RR (Ruthenium Red) As2O3 (Arsenic Trioxide)

Effect of Ruthenium Red on Immobility Time (FST)

On comparing of the control group, **figure 2** presented the As2O3 group raised the immobility time which indicates depression-like behaviour in the animals. While animals treated with ruthenium red at 3 and 6 mg/kg i.p. with the As2O3 respectively showed a reduction in the immobility time that indicated the reduced depressive behaviour in the animals. Ruthenium red at dose 6 mg/kg i.p has markable reduction in immobility time thus indicating improved depression-like behaviors.

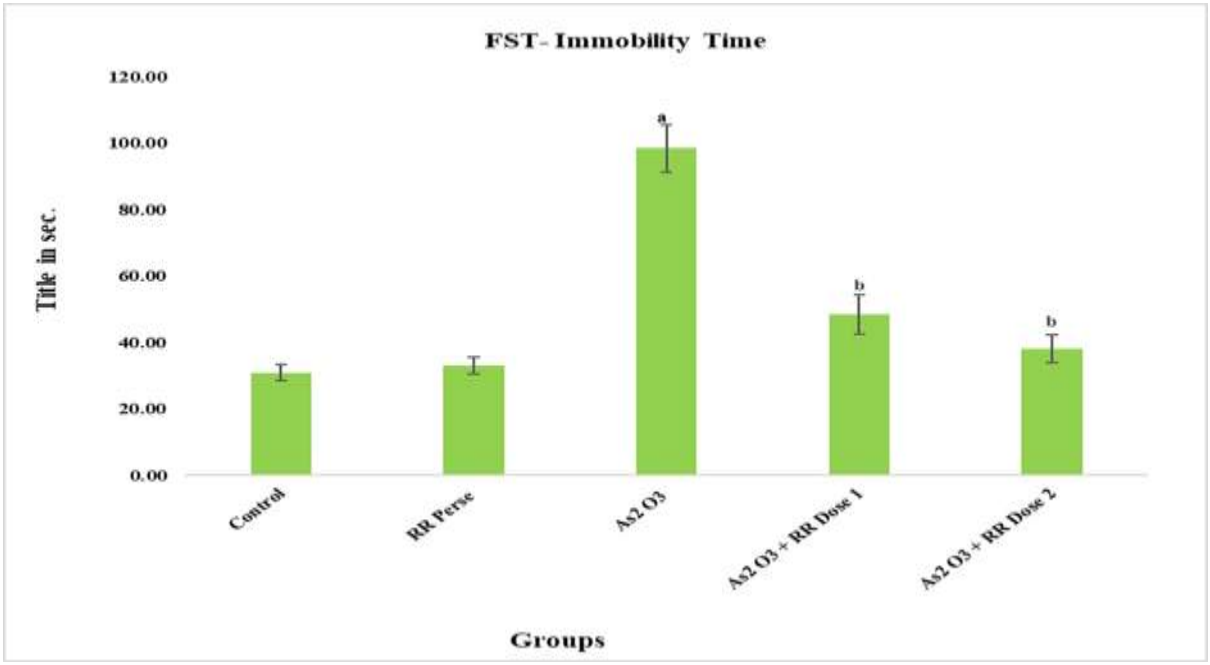


Figure 2

Fig.2 Impact of RR on immobility time. Results were expressed as mean ± SEM (n=6), one way ANOVA followed by tukey's multiple comparison test. Immobility time = [F (8,53) =24.93, P<0.05]; remarkable difference at [a]P<0.05 vs control group; difference [b]P<0.05 vs As2O3. FST (Force Swim Test), RR (Ruthenium Red) As2O3 (Arsenic Trioxide)

Effect of Ruthenium Red on Anxiety-Like Behaviours (EPM)

The impact of ruthenium red on the amount of time that animal treated with As2O3 spent in the open arm during EPM is shown in **figure 3.** The animals in the As2O3 group exhibited anxious behaviour, as evidenced by their significantly reduced time spent in the open arms of the EPM compared to the control group. While animals treated with ruthenium red at 3 and 6 mg/kg i.p. dose with the As2O3 respectively remains in the open arm for a much longer period of time in EPM indicating less anxiety in the animals. Ruthenium red at dose 6 mg/kg i.p has significantly improved anxiety in animals.

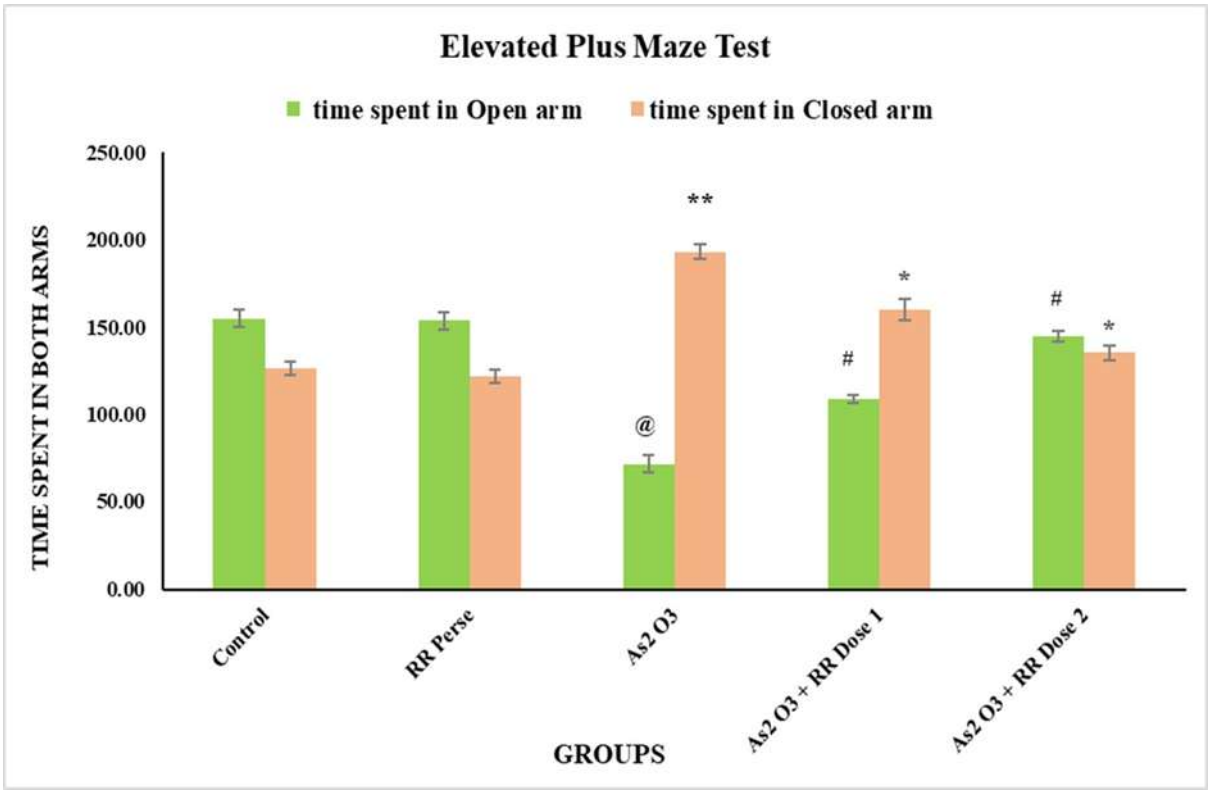


Figure 3

Fig.3 Impact of RR on time spent in **open arm**. Results were expressed as mean ± SEM (n=6), one way ANOVA followed by tukey's multiple comparison test. Time spent (Open Arm) = [F (8,53) =12.73, P<0.05]; remarkable difference at @ P<0.05 vs control group; difference # P<0.05 vs As2O3. EPM (Elevated Plus Maze), RR (Ruthenium Red) As2O3 (Arsenic Trioxide).
Impact of RR on time spent in **close arm**. Results were expressed as mean ± SEM (n=6), one way ANOVA followed by tukey's multiple comparison test. Time spent (close arm) = [F (8,53) =6.71, P<0.05]; remarkable difference at @P<0.05 vs control group; difference *P<0.05 vs As2O3. EPM (Elevated Plus Maze), RR (Ruthenium Red) As2O3 (Arsenic Trioxide)

Effects of Ruthenium Red on Escape Latency (ELT) and Time Spent in The Target Quadrant (TSTQ) (MWM)
The effectiveness of ruthenium red on ELT in animals treated with As2O3 was shown in
Figure 4 a on day four of the trial, the As2O3-treated animal exhibited far higher ELT than the control animal, suggesting learning impairment. ruthenium red at (3 & 6 mg/kg i.p.) with the As2O3, reduce ELT on the fourth day of the trial when compared to animals As2O3 group. Ruthenium red at dose 6 mg/kg i.p has significantly reduced ELT on the fourth day of the trial indicating learning restoration.
Figure 4 b illustrates how ruthenium red affect TSTQ in animals treated with As2O3. When compared to the control animal, As2O3 had a considerably lower TSTQ on the fifth day of the test, indicating memory impairment. On the fifth test day, however, ruthenium red (3 & 6 mg/kg i.p.) with the As2O3 respectively, increased TSTQ on the fifth day of the test. Ruthenium red at dose 6 mg/kg i.p has significantly increased TSTQ on the fifth day of the test indicating memory impairment.

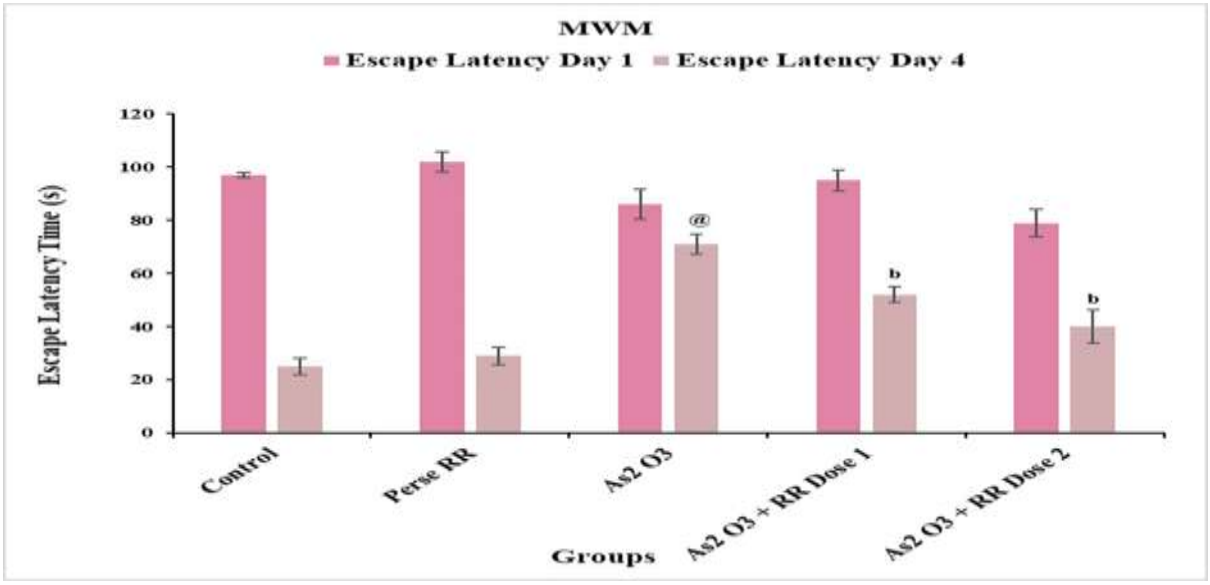


Figure 4a

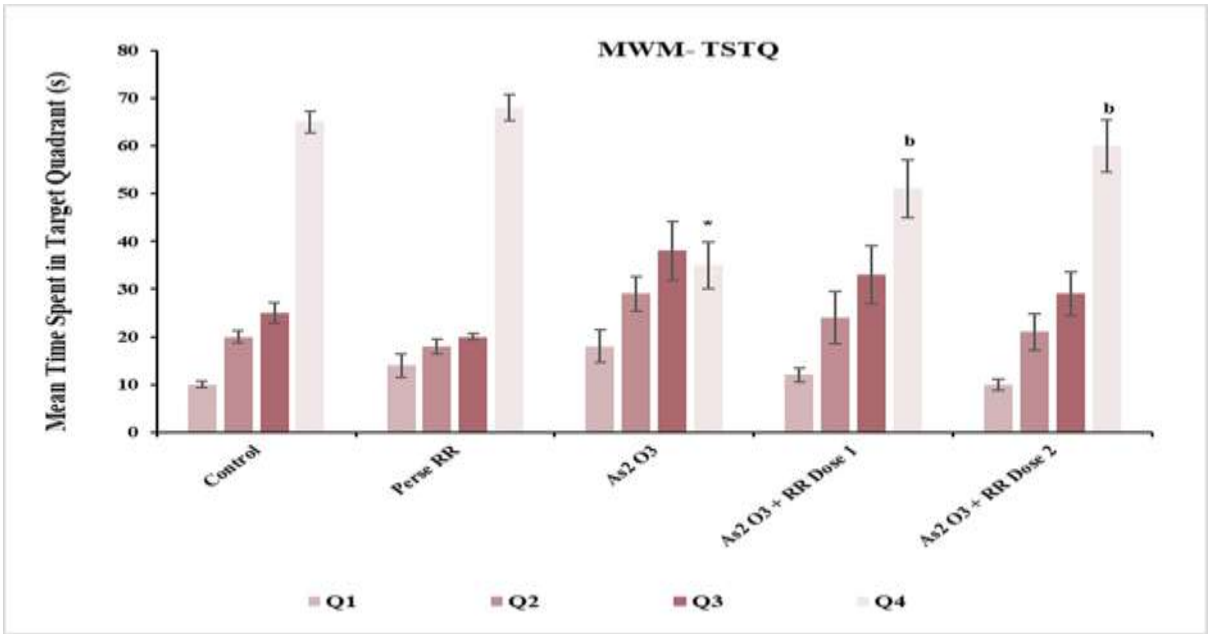


Figure 4b

Fig. 4 a & b Effect of RR on escape latency time (ELT) and time spent in the target quadrant (TSTQ). Results were expressed as mean ± SEM (n=6), one way ANOVA followed by tukey's multiple comparison test.
4a. Animal exhibited reduced day 4 ELT throughout the acquisition test and a noticeable decline on the 4th day ELT= [F (8,53) =13.5, P<0.05]; [a]P<0.05 vs day 1 in respective group; [@]P<0.05 vs day 4 in control; [b]P<0.05 vs day 4 in As2O3 group, signifying regular learning capabilities.
4b. TSTQ =4.85, P<0.05]; [a]P<0.05 vs mean time spent in another quadrant in respective group; [*]P<0.05 vs time spent in target quadrant by control; [b] P<0.05 vs time spent in target quadrant by As2O3, signifying memory impairment.
MWM (Moris Water Maze), ELT (Escape latency Time), TSTQ (time spent in target quadrant), RR (Ruthenium Red) As2O3 (Arsenic Trioxide)

Effect of Ruthenium Red on Latency to Fall (Hanging Wire)
When compared to the control group, **Figure 5** shows the As2O3 group has shorter fall latency time this shows the motor muscle weakness. The latency time to fall was increased by ruthenium red (3 & 6 mg/kg i.p.) with the As2O3 respectively which affects the latency to fall time. Ruthenium red at dose 6 mg/kg i.p has distinctive reduction in latency to fall time thus indicating improved motor muscle performance in animals.

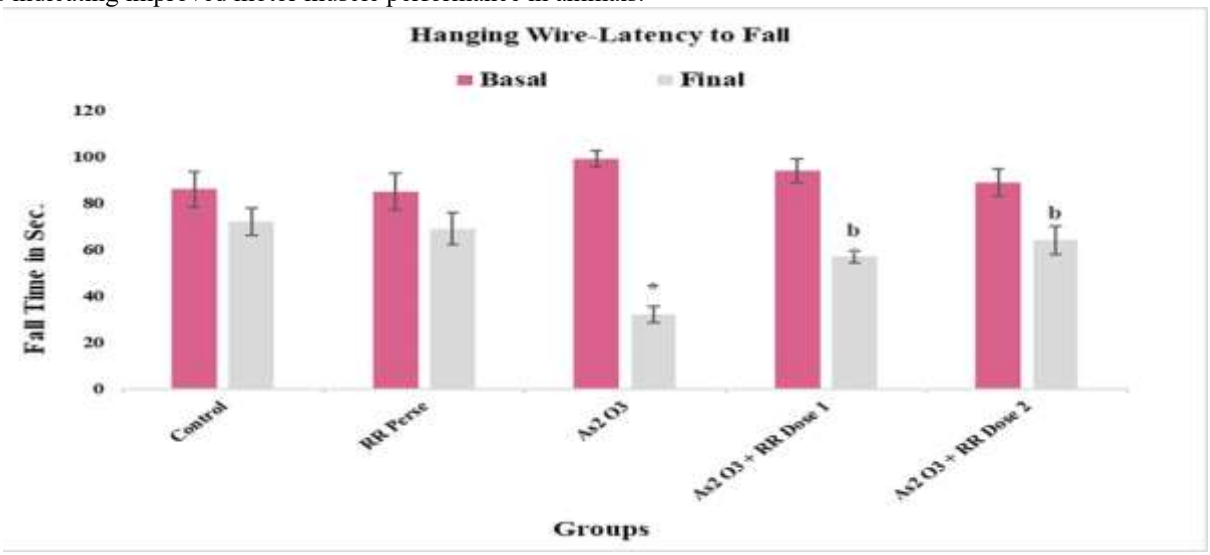


Figure 5

Fig.5 Impact of RR on Hanging wire. Results were expressed as mean ± SEM (n=6), one way ANOVA followed by tukey's multiple comparison test. Latency to fall time = [F (8,53) =14.78, P<0.05]; remarkable difference at [*] P<0.05 vs control group; difference [b] P<0.05 vs As2O3. RR (Ruthenium Red) As2O3 (Arsenic Trioxide)

ESTIMATION OF BIOCHEMICAL PARAMETERS
Effect of Ruthenium Red on Reduced Glutathione (GSH)
GSH is an essential antioxidant that plays a major role in protecting cells from oxidative damage. The impact of ruthenium red on GSH levels in animals treated with As2O3 is shown in **figure 6**. Animals treated with As2O3 continued to have much lower levels than the control group, indicating oxidative stress. The GSH level was considerably raised by ruthenium red (3 & 6 mg/kg i.p.) with the As2O3 indicating improvement in oxidative stress. Ruthenium red at dose 6 mg/kg i.p has noticeable increase in the GSH level which indicates reduction in oxidative stress.

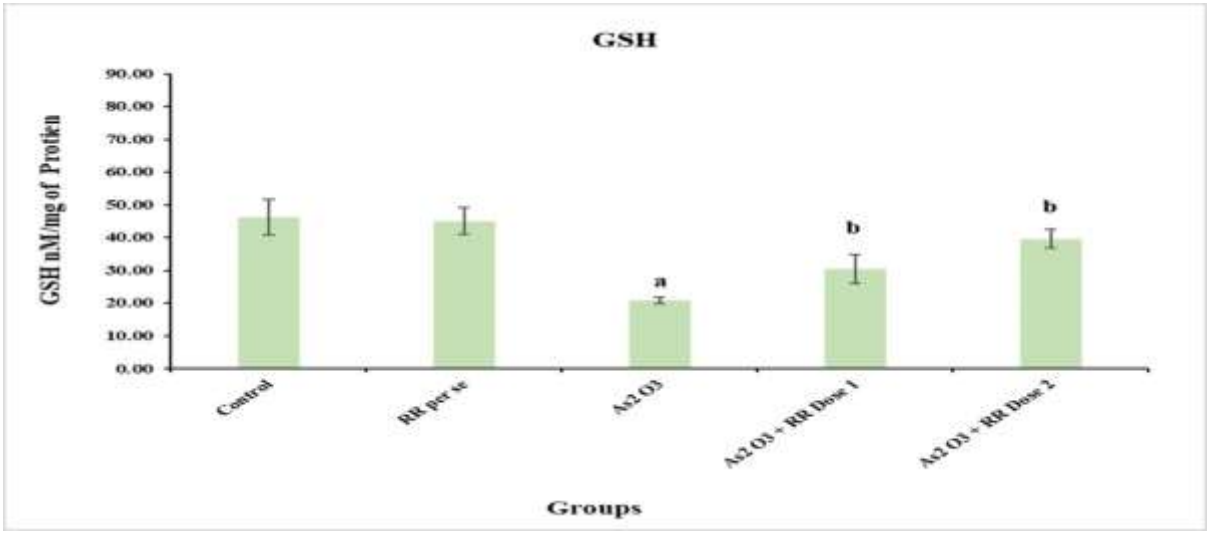


Figure 6

Fig.6 Impact of RR on GSH level. Results were expressed as mean ± SEM (n=6), one way ANOVA followed by tukey's multiple comparison test. GSH = [F (8,53) =2.59, P<0.05]; remarkable difference at [a]P<0.05 vs control group; difference [b]P<0.05 vs As2O3.
GSH (Reduced Glutathione), RR (Ruthenium Red) As2O3 (Arsenic Trioxide)

Effect of Ruthenium Red on Thiobarbituric Acid Reactive Species (TBARS)
Figure 7 illustrates oxidative stress by showing a rise in TBARS content in As2O3. The concentration of TBARS was considerably lowered by ruthenium red-treated animals at dose 3 and 6 mg/kg i.p. with the As2O3. Ruthenium red at dose 6 mg/kg i.p significantly raised the concentration of TBARS which indicates improved oxidative stress in the animals.

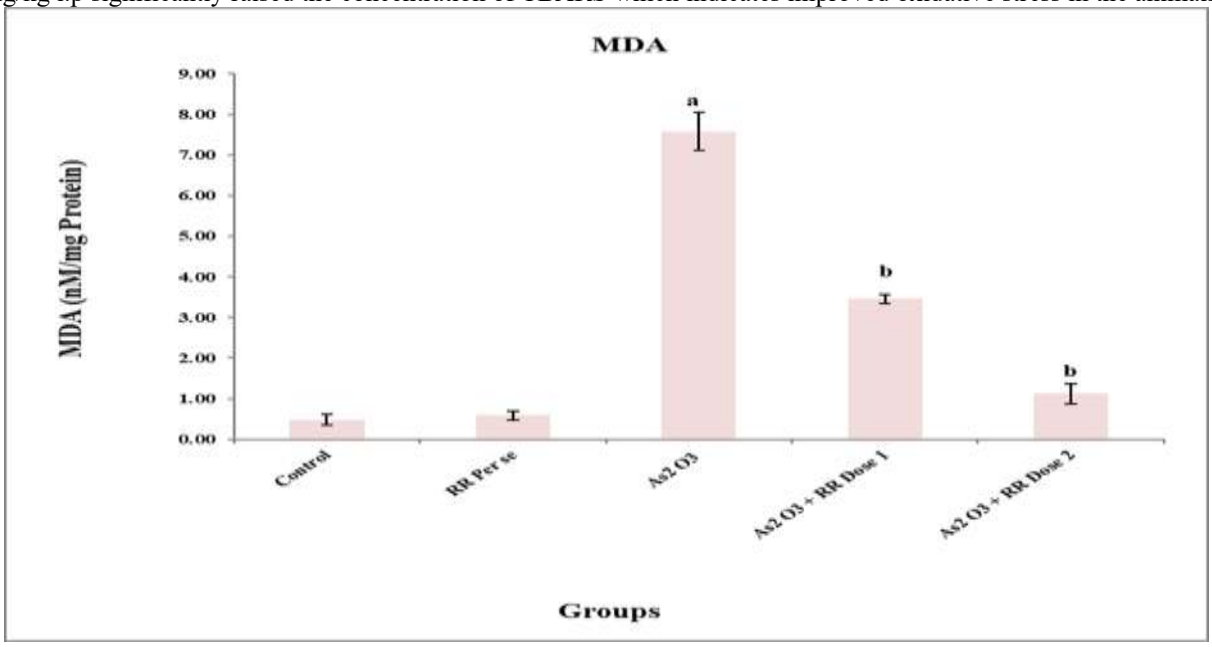


Figure 7

Fig.7 presents impact of RR on MDA. Results were expressed as mean ± SEM (n=6), one way ANOVA followed by tukey's multiple comparison test. TBARS = [F (8,53) =109.2, P<0.05]; a remarkable difference at [a] P<0.05 vs control group; difference [b] P<0.05 vs As2O3. TBARS (Thiobarbituric Acid Reactive Species), RR (Ruthenium Red) As2O3 (Arsenic Trioxide)

Effect of Ruthenium Red on Myeloperoxidase (MPO)
When compared to the control group, **figure 8** shows a discernible rise in myeloperoxidase levels in the As2O3 group, indicating inflammation. When compared to As2O3, the concentrations of myeloperoxidase were considerably lower in animals treated with ruthenium red at 3 and 6 mg/kg i.p. with the As2O3 indicating the decreased inflammation. Ruthenium red at dose 6 mg/kg i.p significantly lowers the myeloperoxidase levels, shows reduction in inflammation in the animals.

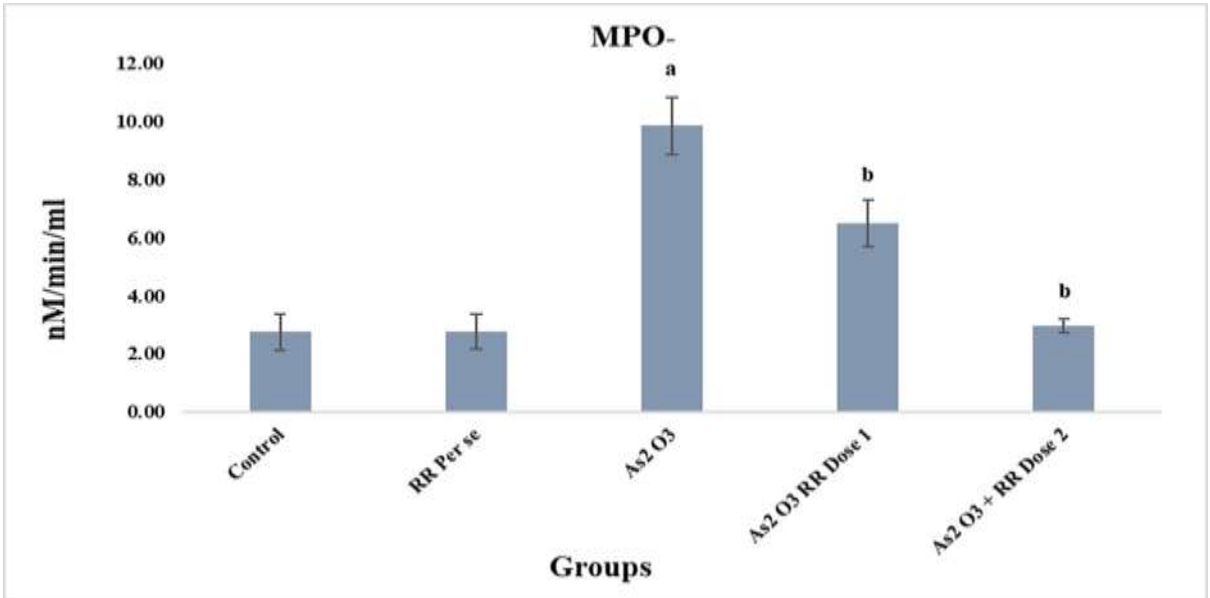


Figure 8

Fig.8 presents impact of RR on MPO. Results were expressed as mean ± SEM (n=6), one way ANOVA followed by tukey's multiple comparison test. MPO = [F (8,53) =12.29, P<0.05]; a remarkable difference at [a]P<0.05 vs control group; remarkable difference [b]P<0.05 vs As2O3. MPO (myeloperoxidase), RR (Ruthenium Red) As2O3 (Arsenic Trioxide)

Effect of ruthenium Red on AChE activity

When compared to the control group, **figure 9** shows a discernible rise in AChE levels in the As2O3 group, indicating the effects on memory and learning of animals. In contrast to As2O3, ruthenium red treated animals at 3 and 6 mg/kg i.p. with the As2O3 decreased the concentration of AChE which indicating an improvement in memory and learning of the animals. Ruthenium red at dose 6 mg/kg i.p significantly lowers the AChE level shows memory restoration in the animals.

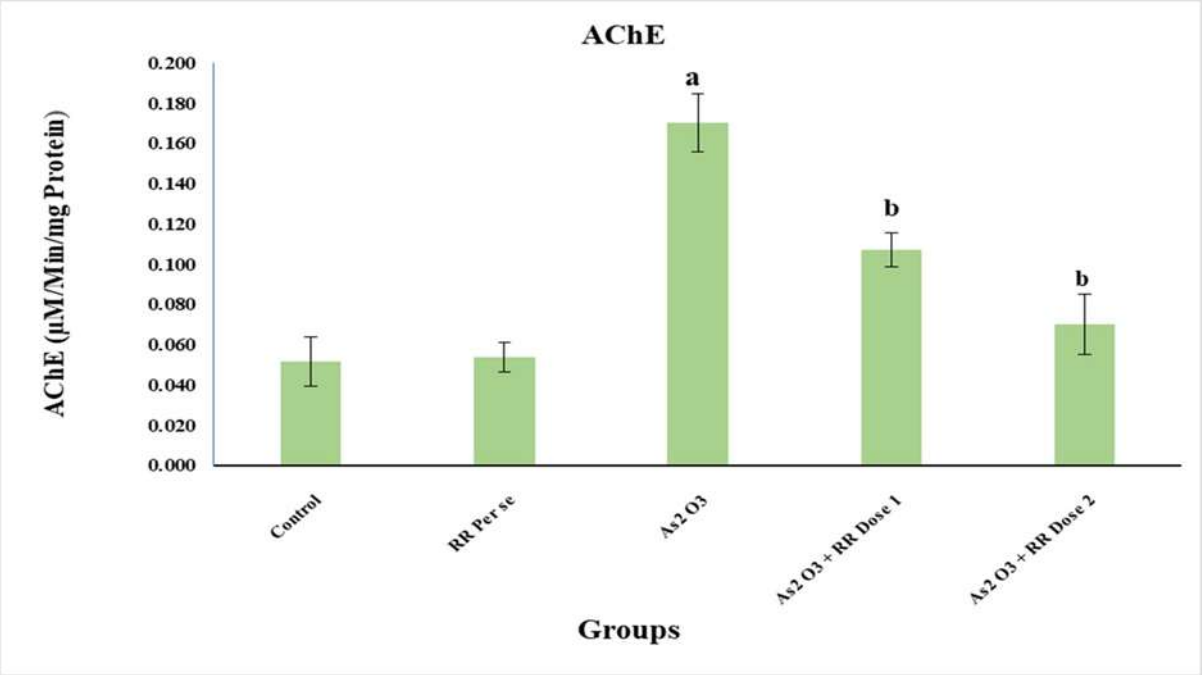
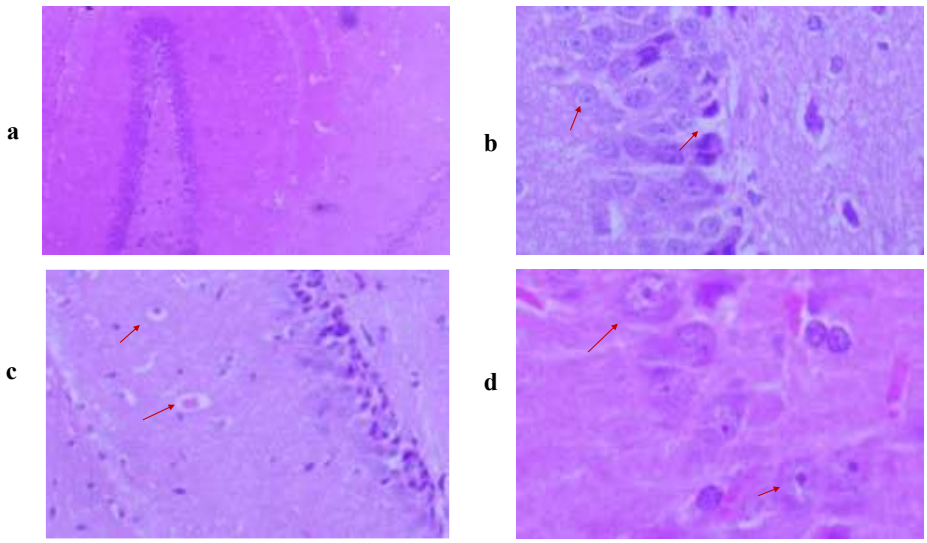


Figure 9

Fig.9 presents impact of RR on Ach. Results were expressed as mean ± SEM (n=6), one way ANOVA followed by tukey's multiple comparison test. AchE = [F (8,53) =6.70, P<0.05]; a remarkable difference at [a] P<0.05 vs control group; remarkable difference [b]P<0.05 vs As2O3.
AchE (Acetylcholine Esterase), RR (Ruthenium Red) As2O3 (Arsenic Trioxide)

Effect of Ruthenium Red on Hematoxylin and Eosin (H&E) Staining



(a) Control group- there was normal histological structure observed, neurons had complete cell bodies and nuclei and seemed to be well-organized, no vacuolization, pyknosis or neurodegeneration observed in the hippocampus region.

(b) Arsenic Trioxide group- significant neurotoxic alterations were noted-degeneration and shrinking of neurons, nuclear deposition and significant pyknosis, vacuolization of the cytoplasm, neuronal layer disturbance, significant neuronal death and structural deformation were observed in the hippocampal regions, suggesting arsenic-induced cognitive impairment.
(c) Ruthenium Red dose 1 with the arsenic trioxide shows marked decrease in neuronal integrity, decreased pyknosis.
(d) Ruthenium Red dose 2 with the arsenic trioxide moderate improved neuronal integrity, decreased pyknosis and vacuolization, preservation of hippocampal architecture, and decreased neuronal death.

Conclusion

In conclusion, long-term exposure to arsenic through tainted groundwater poses a major global threat to the environment and public health, especially in densely populated areas that rely on untreated water supplies. A growing body of research indicates that oxidative stress, mitochondrial malfunction, lipid peroxidation, compromised antioxidant defense mechanisms, neurotransmitter imbalance, and dysregulated intracellular calcium signalling are all closely linked to arsenic-induced neurotoxicity. Neuronal apoptosis, cognitive decline, and neurodegenerative changes are largely caused by excessive production of reactive oxygen species and disruption of calcium homeostasis. Ryanodine receptor-mediated calcium release is a prospective therapeutic target because it is essential to these pathogenic processes. By stabilizing intracellular calcium levels, lowering oxidative stress, maintaining mitochondrial integrity, and averting apoptotic neuronal damage, ruthenium red, a specific inhibitor of ryanodine receptor-mediated calcium release, may have neuroprotective effects. Therefore, altering calcium signalling pathways could be a useful tactic for reducing brain deficits brought on by arsenic. To fully comprehend the molecular mechanisms at play and to determine the therapeutic efficacy and safety of Ruthenium Red in arsenic-associated neurodegenerative diseases, more experimental and clinical research is needed.

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