Original Article

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Neurobehavioural and Neurochemical Changes in Arsenic Induced Cerebellar Toxicity in Male Sprague-Dawley Rats: An Experimental Study

Anatomy Section

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ABSTRACT

Introduction: Sodium arsenite, an inorganic arsenic, is naturally present at high level (>50 µg/L) in ground water. Drinking ground water is the biggest threat to public health. Though, there are numerous reports on arsenic neurotoxicity, the arsenic effect on cerebellar neurotoxicity remains vague especially its chronic effect on its neurobehavioural and neurochemical alterations.

Aim: To evaluate the neurobehavioural and neurochemical alterations caused by sodium arsenite in cerebellum of rats.

Materials and Methods: This experimental study was conducted in the Central Animal House at Sri Devaraj Urs Academy of Higher Education and Research (SDUAHER) from November 2019 to February 2020 for a period of 90 days. Total 16 male sprague-dawley rats were randomised into two equal groups. Group I: Control, received normal saline. Group II: Sodium arsenite, doses of 50 Parts per Millions (PPM) for 90 days through oral gavage. Rats were subjected to Open Field Test (OFT) for locomotor and exploratory behaviour and Beam Walking Test (BWT) for motor coordination and balance. Following behavioural tests, rats were anaesthetised. Blood was drawn from a retro-orbital puncture. Brains were dissected and cerebellum was separated. Concentration of Malondialdehyde (MDA), Nitric Oxide (NO) and activity of Glutathione Peroxidase (GPx) were assessed spectrophotometrically in serum and cerebellum of rats. Mean±SD was used for normally distributed data and groups were compared using independent t-test, whereas for non-normally distributed data, Median (25^{th}-75^{th} Percentile) was used and Mann-Whitney U-test was used to compare groups.

Results: Arsenic-treated rats showed a significant increase in arsenic concentration in serum and cerebellum (5.5±1.6 ng/mL, 2.76±0.56 µg/g, respectively) compared to control (1.14±0.43 ng/mL, 0.65±0.29 µg/g, respectively). There was a significant decrease in locomotor and exploratory behaviour and impairment in motor coordination and balance in arsenic treated rats with a p-value <0.001 in comparison with control rats. The arsenic treated rats had significantly enhanced concentration of MDA and NO level and reduced activity of GPx in serum {16.84 (13.84-18.87), 33.79 (30.05-37.17) nmol/mL, and 6.89 (5.24-8.5) mmoles of Reduced glutathione (GSH) oxidised/min/mL, respectively} compared to control {8.81 (8.36-9.48), 17.66 (15.33-21.29) nmol/mL, and 15.16 (12.77-16.59) mmoles of GSH oxidised/min/mL, respectively} and also found increased concentration of MDA and NO level and reduced activity of GPx in tissue {7.98 (7.14-8.92), 24.67 (21.4-28-22) nmol/mg of protein and 2.66 (1.19-3.86) mmoles of GSH oxidised/min/mg protein, respectively} compared to control {3.02 (2.35-3.61), 13.93 (11.0-16.16) nmol/mg of protein and 7.63 (7.08-9.19) mmoles of GSH oxidised/min/mg protein, respectively}.

Conclusion: The oral administration of sodium arsenite at the doses of 50 PPM for 90 days showed interesting alterations in neurobehavioural and neurochemical parameters related to cerebellum of rats.

Keywords: Cerebellum, Glutathione peroxidase, Motor activities, Oxidative stress markers

INTRODUCTION

In 21^{st} century world, industrialisation of society and expansion of factories has vividly expanded the amount of pollutants and environmental pollutions. Environmental pollution due to arsenic is a major toxin and has become a major public health challenge in many countries such as Bangladesh, India, Nepal, Taiwan, Mongolia, Vietnam, Pakistan, China, Afghanistan, Argentina and USA [1]. Arsenic is mainly distributed in the environment through industries using wood preservatives, glass, semiconductors, mining waste, herbicides and pesticides [2]. Due to its increasing production and utilisation, arsenic is ubiquitously present in water, air and soil affecting occupational workers as well as general population [3].

Arsenic primarily enters into the body through direct consumption of drinking water from geological deposits by drilling of tube well [4]. World Health Organization (WHO) and other regulatory bodies have established a Maximum Contaminant Limit (MCL) of 10 µg/L inorganic arsenic (iAs) in drinking water for the safety of human health based on reducing cancer risk, but this limit is not considering endpoints other cancer end points such as digestive effects, reproductive effect [5]. Globally, more than 200 million people are affected by ground-water contaminated with arsenic concentration greater than 10 µg/L [6]. Arsenic affected area in West Bengal and Bihar region of the Ganga Plain in India has extremely high arsenic contaminated water where arsenic concentration in water has been reported upto 1000 µg/L (i.e.,100 PPM) against the safe limit of 10 µg/L recommended by WHO [7,8]. High dosages iAs can cause death but chronic lower levels of arsenic results in serious health problems such as cancers and skin lesions [9]. Numerous experimental and epidemiological data provide evidence that acute and chronic arsenic exposure has been linked with numerous chronic indices in all human and animal organ systems and contributes to wide spectrum of diseases, especially diseases involving central nervous system [10-12].

Cerebellum is an important component of brain which is linked to control of posture, coordination of movements, gait and skilled voluntary movements. Some studies suggest that cerebellum is also involved in cognition, language and executive functioning [13]. Arsenic penetrates the Blood Brain Barrier (BBB) and accumulates in different regions of the brain such as cortex, cerebellum and hippocampus and increases the production of reactive oxygen species in brain which causes an imbalance between the anti-oxidant defense system levels and the production of free radicals to prompt neurotoxicity [14,15].

Arsenic induced oxidative stress causes neuronal injuries leading to neuronal cell death and impairment of brain functions because of its

high energy demand, high oxygen consumption and abundance of polyunsaturated lipid contents and have shown various neurological side-effects such as vertigo, impaired coordination of movements, uncontrolled motor learning, unsteady gait, loss of skilled voluntary movements, impaired learning, memory and concentration [16-18]. Reports in the literature also documented that arsenic-treated rats reduces neuronal viability in primary cultures of rat cerebellar neurons and showed alterations in locomotor behaviour and learning task [19,20].

These literatures indicate that arsenic has an impact on the cerebellum. There are minimal studies with a possible impact of arsenic on neurobehavioural and biochemical changes related with cerebellum [21,22]. Considering the lacunae, the present study aimed to investigate the impact of arsenic in cerebellum associated neurotoxicity by evaluating motor coordination, balance, locomotor and exploratory activities of rats with standard neurobehavioural procedure and also evaluated the biomarkers of oxidative stress.

MATERIALS AND METHODS

This experimental study was conducted in the Central Animal House at Sri Devaraj Urs Academy of Higher Education and Research (SDUAHER) from November 2019 to February 2020 for a period of 90 days. Full details of work plan with experimental animals were approved by Institutional Animal Ethics Committee (IAEC) (IAEC/PHARMA/SDUMC/2018-19/12a from SDUAHER, Kolar). All experiments were made according to guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [23]. All efforts were made to minimise animal suffering and to reduce the number of animals used.

Inclusion criteria: Healthy male Sprague-Dawley rats having 150 to 180 grams in weight were included in the study.

Exclusion criteria: Rats with any signs/symptoms of illness as well as female rats were excluded from this study.

After two weeks of acclimatisation, all 16 rats were randomly divided into two groups of eight rats each.

Group I: Control, received normal saline.

Group II: Arsenic (as sodium arsenite at the doses of 50 PPM once in a day for 90 days through oral gavage).

Study Procedure

The dose of arsenic was chosen according to a previously published study [24]. Because of paucity in behavioural parameters and duration of exposure (90 days), the dose of present study was chosen on previously published report because the experimental animal model start appearing number of clinical and biochemical manifestation after 8-10 weeks of exposure.

Chemical: Sodium arsenite ($NaAsO_2$), purchased from LOBA Chemicals, was used in this study. All other reagents used in this experiment were of analytical grade and obtained from commercial sources.

Experimental animals: Male Sprague-Dawley rats free of specific pathogens were purchased from Biogen Attibele, Bangalore, India and kept in the central animal house of Sri Devaraj Urs Medical College, Kolar Karnataka, India. Rats were 10 to 12 weeks of age with a body weight varying from 150 to 180 grams and procured in well-maintained temperature ranging from 20°C-26°C with a 12 hours dark/light cycles. They were housed in polypropylene home cages confirming two rats per cage. Throughout the experimentation, rats were given free access to rodent feed pellets and water Ad-libitum.

Neurobehavioural Studies

After 90 days of treatment, all rats underwent behavioural tests. The test was performed in a separate room maintaining 20°C-26°C with noise and dust free room. Rats were acclimatised to the test room for 30 minutes prior to the testing. The tests were performed between 09:00 AM to 03:00 PM.

1. Open Field Test (OFT): OFT was used to assess locomotion and exploratory behaviours [25].

Equipment: Briefly, OFT consisted of an open-top box (90×90 cm) with 45 cm high walls to prevent the rats from escaping. The floor of arena was painted black. White lines were drawn on arena floor and divided into 36 square blocks and each block was 15 cm^2 long. The central part consisted of four squares in the center of the apparatus and marked with a red line. Video camera and a 60 W white light bulb were fixed above the arena.

Procedure: Each rat was gently placed in the center of arena and allowed freely to explore the arena for 300 seconds. At the end of exploration, rats were transferred to their respective home cages. After each trails, arena was cleaned with 70% ethanol.

The following parameters were assessed [26]:

a. Number of square line crossed
b. Number of rearing
c. Number of wall-rearing
d. Time spent in center
e. Freezing time
f. Number of grooming

Note: Line crossed was only considered if the rats cross one of the lines with all four paws. Freezing time was only considered if the rats remain static for more than six seconds.

2. Beam Walking Test (BWT): Motor coordination and balance of rats were assessed by measuring the rat's ability to traverse narrow beams to reach an enclosed safety platform [27].

Equipment: The Beam consisted of 150 cm long strips of wood, stretched between a blind and home end. It was 5 cm and 2.5 cm square thick, placed 50 cm above the floor. Just below the beam, a soft cloth was placed to prevent rats from injury in case of fall from the beam during experiment. An aversive stimulus (60 W light) was positioned near the blind end of the beam [27].

Procedure: Rats were individually kept at the blind end and allowed to walk on the beam. The animals' performances were recorded with a video-camera. After each trails, test apparatus was cleaned with 70% alcohol to remove any smell and residue.

The following parameters were assessed:

a. Time taken to traverse the beam
b. Number of foot slips
c. Number of near fall
d. Actual fall

Note: All neurobehavioural tests were assessed three times and mean of these values was taken.

Sample collection: Following the behavioural tests, all rats were fasted over-night except water. Rats were deeply anaesthetised by injecting Ketamine (92 mg/kg Body-weight) and Xylocaine (10 mg/kg Body-weight) intraperitoneally and sacrificed by cervical decapitation. Blood was drawn from retro-orbital plexus and collected in heparinised vials, centrifuged to collect serum and stored in -80°C until use. Brain was removed, rinsed in cold saline and cerebellum was separated quickly. Half of the cerebellum was quickly frozen in liquid nitrogen and stored at -80°C and used for biochemical analysis. Remaining half was stored at -80°C and used for arsenic estimation.

Estimation of Arsenic Concentration in Serum and Cerebellum of Rat

Arsenic concentration in serum and cerebellum were assessed according to the procedure reported by Ballentine R et al., [28]. A 200 mg of cerebellum was added in di-acid mixture which contains HNO_3 and $HClO_4$ in the ratio of 10:4, respectively. The sample was digested over a sand bath fume-hood chamber until a white gelatinous residue was formed. It was cooled and brought

to 20 mL of volume by adding distilled water. Aliquots of digested and undigested samples such as cerebellum and serum were used to estimate arsenic using Atomic Absorption Spectrophotometer (Thermo-Scientific iCE 3000 series AA Spectrophotometer). The values are expressed in µg/g for tissue and ng/mL in serum.

Preparation of tissue homogenate for biochemical parameters: Each cerebellar tissue was homogenised individually in phosphate buffer Solution (pH 7.4) by using Glass-Teflon homogeniser. The cerebellar tissue segments were cut and fabricated in a 1:10 W/V (i.e., 1 gram of tissue in 10 mL of PBS) and homogenised for 10-15 minutes at 4°C. The homogenate was centrifuged at 15000 xg for 30 minutes at 4°C to remove debris. Clear supernatant was taken and stored at -80°C [29]. The supernatant obtained from the tissues was used to estimate biochemical parameters such as Malondialdehye, Nitric Oxide and Glutathione Peroxidase spectrophotometrically [Table/Fig-1] [30-32].

Parameters	Methods	Units
Malondialdehyde (MDA)	Thiobarbituric acid Method (TBARs) [30]	Serum: nmol/mL Tissue: nmol/mg of protein
Nitric Oxide (NO)	Modified Griess's method [31]	Serum: nmol/mL Tissue: nmol/mg protein
Glutathione Peroxidase (GPx)	Procedure of Rotruck et al. [32]	Serum: mmoles of GSH oxidised/min/mL Tissue: mmoles of GSH oxidised/min/mg protein

[Table/Fig-1]: Oxidative stress parameters [30-32].
GSH: Reduced glutathione

STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS) IBM, version 22.0 was used for analysis. Mean±SD was used for normally distributed data and the significance of difference was tested by independent t-test. Non normally distributed data were represented as Median (25^{th}-75^{th} Percentiles). Mann-Whitney U test were used for non parametric variables to check the difference in median between the groups. p-value <0.05 was considered statistically significant.

RESULTS

General toxicity: There was alteration in eating, drinking and defecation. Following three to five days of treatment with arsenic, we noticed a reduction in food and water intake for 15-20 days, thereafter consumption of food and water was almost similar compared to controls. After 45 days till decapitation, they reduced food and water intake. A few changes were also noticed in arsenic treated rats like alteration in texture of stool, bloated abdomen, restlessness.

Body weight, brain weight and arsenic concentration: Arsenic treated rats showed significant reduction in the body weight gain after 90 days when compared to body-weight gain of control rats after 90 days. There was also significant decrease in brain weight in arsenic-exposed rats compared to controls (p-value <0.001). Group II also increased arsenic concentration in serum and cerebellum of rats [Table/Fig-2].

Oxidative Stress (OS) markers: To check the impact of $NaAsO_2$ on OS- parameters, the activity of GPx and concentration of MDA and NO in serum and cerebellum of rats were assessed [Table/Fig-3].

Parameters	Group I (n=8)	Group II (n=8)	p-value
Initial body weight (grams)	225.38±6.78	229.63±5.98	0.303
Body weight after 90 days (grams)	317.25±6.99	286.87±6.6	**0.001**
Brain weight (grams)	2.17±0.19	1.36±0.12	**0.001**
Arsenic concentration in serum (ng/mL)	1.14±0.43	5.5±1.6	**0.001**
Arsenic concentration in cerebellum (µg/g)	0.65±0.29	2.76±0.56	**0.001**

[Table/Fig-2]: Comparison of differences in body weight, brain weight and arsenic concentration in Group I and Group II.
Values are Mean±SD. Independent t-test was done to calculate p-value. p<0.05 considered statistical significant

Oxidative stress parameters	Group I (n=8)	Group II (n=8)	p-value
Serum			
Malondialdehyde (nmol/mL)	8.81 (8.36-9.48)	16.84 (13.84-18.87)	**0.001**
Nitric-Oxide (nmol/mL)	17.66 (15.33-21.29)	33.79 (30.05-37.17)	**0.001**
Glutathione-Peroxidase (mmoles of GSH oxidised/min/mL)	15.16 (12.77-16.59)	6.89 (5.24-8.5)	**0.001**
Cerebellum			
Malondialdehyde (nmol/mg of protein)	3.02 (2.35-3.61)	7.98 (7.14-8.92)	**0.001**
Nitric-Oxide (nmol/mg of protein)	13.93 (11.0-16.16)	24.67 (21.4-28.22)	**0.001**
Glutathione-Peroxidase (mmoles of GSH oxidised/min/mg protein)	7.63 (7.08-9.19)	2.66 (1.19-3.86)	**0.001**

[Table/Fig-3]: Effect of Sodium-Arsenite on oxidative stress parameters.
Data is expressed as Median (25^{th}-75^{th} percentile). Mann-Whitney U test was used to calculate p-value. p<0.05 is considered significant difference between the groups

The current results documented that rats treated with arsenic (Group II) significantly increases the concentration of MDA and NO and decreased the activity of GPx in serum and cerebellum of rats in comparison with control rats. The neurobehavioural activities were measured in OFT and BWT in experimental rats.

Effect of arsenic on OFT parameters: OFT was performed to assess the effect of oral administration of $NaAsO_2$ on locomotion and exploratory behaviour. Group II rats significantly reduced the frequency of square line crossed, rearing, wall rearing and no of grooming in comparison with group I. On the other hand, freezing time increased significantly in group II, but there was no difference in grooming duration between the groups [Table/Fig-4].

Parameters	Group I (n=8) Median (25^{th}-75^{th} percentile)	Group II (n=8) Median (25^{th}-75^{th} percentile)	p-value
Number of square line crossed	128.0 (121.67-141.75)	27.34 (24.5-28.25)	**0.001**
Number of rearing	13.67 (11.84-16.17)	3.0 (2.67-3.33)	**0.001**
Number of wall-rearing	25.17 (22.33-27.33)	10.67 (8.84-12.5)	**0.001**
Time spent in center (Seconds)	5.34 (3.5-13.75)	0.0 (0.0-0.75)	**0.001**
Freezing time (Seconds)	47.84 (39.5-62.0)	224.33 (210.67-247.92)	**0.001**
Number of grooming	10.5 (9.75-11.92)	4.0 (3.42-5.08)	**0.001**
Duration of grooming (Seconds)	26.34 (21.42-28.83)	24.33 (19.25-26.83)	0.312

[Table/Fig-4]: Effect of arsenic on Open Field Test (OFT) parameters.
Values are Median (25^{th}-75^{th} Percentile). Mann-Whitney U test was used to check significance difference. The p-value <0.05 considered statistically significant

Effect of arsenic on Beam Walking Test (BWT) parameters: BWT was done to assess the effect of oral administration of $NaAsO_2$ on motor co-ordination and balance of rats [Table/Fig-5a,b].

Parameters	Group I (n=8) Median (25^{th}-75^{th} percentile)	Group II (n=8) Median (25^{th}-75^{th} percentile)	p-value
Time spent to cross the beam (seconds)	19.7 (14.4-25.1)	48.8 (36.7-60.6)	**0.001**
Number of foot slip	1.17 (0.25-2.25)	6.17 (5.42-7.67)	**0.001**
Number of near-fall	0.0 (0.00-0.25)	2.5 (1.75-3.25)	**0.001**
Actual fall	0.0 (0.0-0.0)	0.17 (0.0-0.33)	**0.025**

[Table/Fig-5a]: Effect of Beam Walking Test (BWT) parameters in 5 cm thick beam.
Values are Median (25^{th}-75^{th} Percentile). Mann-Whitney U test was used to check significance difference. The p-value <0.05 considered statistically significant

The current study indicated that group II took longer time to traverse the beam and also experienced more number of foot slips and near fall in 2.5 cm and 5 cm thick beam compared to group I. This may suggest that $NaAsO_2$ alters the motor co-ordination and balance.

All rats (control and arsenic treated) took more time to traverse 2.5 cm square thick beam as compared to 5 cm thick beam. And

also number of foot slips and near falls was more in 2.5 cm thick beam as compared to 5 cm thick beam.

Parameters	Group I (n=8) Median (25^{th}-75^{th} percentile)	Group II (n=8) Median (25^{th}-75^{th} percentile)	p-value
Time spent to cross the beam (seconds)	9.0 (7.25-10.0)	39.67 (33.67-46.83)	**0.001**
Number of foot slip	0	2.5 (1.84-3.0)	**0.001**
Number of near-fall	0	1.33 (1.0-2.42)	**0.001**
Actual fall	0	0 (0.0-0.25)	0.144

[Table/Fig-5b]: Effect of Beam Walking Test (BWT) parameters in 2.5 cm thick beam. Data are depicted as Median (25^{th}-75^{th} Percentile). Mann-Whitney U test was done to calculate p-value. p<0.05 considered statistically significant.

DISCUSSION

There is an increasing concentration of arsenic in the environment due to rapid industrialisation and urbanisation, which contaminate the water, air and soil. The uses of arsenic contaminants are toxic to humans and animals even at low (<10 µg/L) concentration [33]. The current finding reported that the administration of $NaAsO_2$ causes an impact on neurobehavioural and neurochemical alterations in rat's cerebellum.

The present results revealed that arsenic treated rats cause a reduction in body weight gain which is similar to the study conducted by Sárközi K et al., and Rodríguez VM et al., [34,35]. The deficit in body weight gain could be due to reduced food consumption or damage to the gastric mucosa that causes gastrointestinal alterations and may alter the texture of stool [36]. The current study also distinguished that there was a significant reduction in brain weight which may indicate arsenic has crossed the BBB and altered the brain weight and changes in brain weight could indicate oxidative damage.

It is documented in animal experiments that arsenic can cross BBB and invade the brain parenchyma [20]. In present study, arsenic concentration in serum and cerebellum significantly increased in arsenic-treated rats which might be a contributing factor to cerebellar neurotoxicity. The present study is parallel with the results of Guan H et al., and Markowski VP et al., [37,38]. However, the change in serum and cerebellum arsenic concentration in the current and previous study could be due to different durations and routes of exposure and also to different groups of rodents.

OS is considered a deciding factor that contributes and triggers the induction of arsenic toxicity and has solid relationship with accumulation of arsenic in many organs such as heart, kidneys, lungs, liver, muscles, spleen and brain. Its accumulation has a persistent potential to damage organs and tissues [39]. To study the toxic signs caused by arsenic and its effect on oxidative stress, we assessed MDA, NO and GPx in serum and cerebellum.

The results of the present study indicated that $NaAsO_2$ causes significant neurotoxicity as confirmed by increase in OS markers such as MDA and NO as well as decrease in GPx activity in the serum and cerebellum of rats. In the current study, the concentration of MDA and NO level increased significantly in cerebellum which indirectly reflects the body cells by the severity of free radicals attacks. The present results also points out a positive relationship between MDA and NO levels and arsenic concentration in serum and cerebellum of rats. Simultaneously, the oxidative damage parameters i.e., GPx found to be significantly reduced due to antioxidant enzyme consumption and the antioxidant enzyme; GPx had the ability to scavenge oxygen free radicals. These results may show that arsenic can induce oxidative stress in rats' cerebellum. These findings are consistent with the earlier reports indicating an increase in MDA and NO level and decrease in GPx activity in the cerebellum [10,40,41].

Ingested arsenic accumulates in different targeted tissues and can interfere with many biochemical reactions and alter numerous physiological activities in various organs of body [42]. To substantiate the motor coordination, balance, locomotion and exploratory behaviour in arsenic induced rats, we performed BWT and OFT.

Rats treated with $NaAsO_2$ showed marked impairment in locomotor activities as evidenced by the decreased frequency of square line crossed and increased freezing time as addressed in OFT which was similar to the study conducted by Kaushal P et al., and Saritha S et al., [43,44]. The reduction in locomotion might be due to a cerebellar disorder because the cerebellum is especially linked to voluntary nervous system and can result improper coordination between the nervous system and the muscular junction and might also be due to the oxidative stress detected in the of arsenic treated cerebellum which decreased motility in open field [20,34,45]. However, the present study data is contradictory to the study done by Bikashvili T et al., and stated that there was no difference in locomotion between the arsenic exposed and control rodents [46].

In addition, we also evaluated the exploratory activities and found that there was significant reduction in exploratory activities such as rearing and wall-rearing in arsenic-treated rats, which is parallel to the study conducted by Saritha S et al., [44]. The reduction in rearing in arsenic treated rats may indicate weakening of the limb muscles and leads to motor impairment and balance of the body.

Damage to the cerebellar structures promotes impairment in motor tasks such as coordination of movements, balance, and timing to traverse the beam. The present study showed that $NaAsO_2$ treated rats showed a significant impairment in fine motor co-ordination and balance by increasing time to cross the beam, number of foot-slips and near fall which may indicate arsenic induced cerebellar neurotoxicity. This is in consonance with the study done by Kim H et al., and stated that arsenic treated mice reduced the motor coordination compared to control [47]. This impairment in motor dysfunction may be linked with cerebellar markers of lipid and DNA oxidation [45].

Limitation(s)

Less number of animals was used in each group, microscopic study and other biochemical parameters has not been evaluated, rats were given sodium Arsenite orally through oral gavage, hence further research can be carried out in different experimental animals with other routes of administration.

CONCLUSION(S)

The oral administration of sodium arsenite ($NaAsO_2$) at the doses of 50 PPM once in a day for 90 days showed reduction in body weight gain. The arsenic treated rats had high concentration of arsenic in serum and cerebellar tissue which indicates accumulation of arsenic in cerebellum of rats and may reduce the brain weight and probably could have increased the production of free radicals and might cause oxidative stress. The present study also confirms that reduction in locomotion and exploratory behaviours and impairment in motor coordination and balance which indicate arsenic induced cerebellar neurotoxicity. Further, molecular studies may confirm the cerebellar neurotoxicity which may strengthen the present study.

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