Phosphodiesterase Inhibitors enhance the Arsenic-Induced memory impairment in the experimental rat model

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Abstract

Arsenic toxicity is a major health issue worldwide, known to cause neurotoxicity with complex etiology. PDE1 inhibitors and PDE4 inhibitors are known to enhance the brain condition manifesting similar neurological and behavioral phenotypes. The neurotoxicity was induced by administering sodium arsenite containing water. The pharmacological consequences of administering rolipram and vinpocetine in sodium arsenite induced behavioral phenotypes (spatial, short and long term memory, motor coordination) was assessed. The effect on brain and body weight was observed. Additionally, the effect of rolipram and vinpocetine on CREB and P-CREB expression was also analyzed by immunohistochemistry. The improvement in the behavioral phenotype was found and also a significant increase in the expression of CREB and P-CREB was observed. This improvement in the learning, memory power, reflexes and motor coordination, may be due to the enhanced CREB and P-CREB neurons expressions by rolipram and vinpocetine. Thus, PDE4 and PDE1 inhibition by rolipram and vinpocetine may be a possible target to study the arsenic induced neurotoxicity and behavioral deficit.

Keyword: Sodium arsenite, phosphodiesterase inhibitors, CREB/P-CREB pathway, cognitive impairment

1. Introduction

Arsenic is a well-known neurotoxin and has been convincingly linked to a variety of diseases, raising concerns about the issue on a global scale. The diseases can be cancerous and non-cancerous in nature such as hypertension, skin lesions, [1] peripheral vascular disease, and neurotoxicity.[2] Studies determining the harmful effects of arsenic exposure have suggested that arsenic plays a part in altering neurological processes and degrading cognition. Long-term ingestion of sodium arsenite via drinking water causes apoptosis in the hippocampus by initiating endoplasmic reticulum (ER), stress markers, and phosphorylating various proteins in the hippocampal area.[3] Perinatal exposure to arsenic cause neurobehavioral and neurochemical changes which affect cognition and motor impairment by arsenic, interrupting the cholinergic and dopaminergic functioning systems in the developing rat brain.[4]

Phosphodiesterase (PDE) is the enzyme responsible for the degradation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) or both. The PDE family comprises 11 subfamilies having specific substrate specificities such as PDE4, PDE7, and PDE8 are specific to cAMP, and PDE5, PDE6, and PDE9 are specific to cGMP while PDE1, PDE2, PDE3, PDE10, PDE11 act on both cyclic nucleotides.[5]

The cAMP and cGMP, both nucleotides are responsible for maintaining synaptic plasticity and are the essential component for neurodevelopment.[6] Cyclic AMP and cyclic GMP are primarily involved in the different brain processes and are responsible for preparing a milieu for the development of cognition power. Investigations were conducted into the significance of CREB and CREB-binding protein as a key component in long-term memory storage, [7] and confirms its association with several processes such as differentiation, proliferation, neurogenesis, neuronal plasticity, and long-term synaptic potentiation.[8, 9]

PDE4 is widely expressed in the different tissues of the brain.[10] PDE4 inhibitors are known to produce an anti-inflammatory effect and exert neuroprotective effects by enhancing the level of cAMP and by activating protein kinase A, a signaling pathway involved in the development of psychiatric disorders.[11] PDE4 can directly enhance neural plasticity, attenuating the degenerative and cognitive dysfunction and also reducing the BBB breakdown.[12]

Rolipram (PDE 4 Inhibitor) selectively acts on cAMP and has known to have an anti-inflammatory role, antidepressant, and cognitive enhancing characteristics.[13] Rolipram also had a protective effect on intracerebral hemorrhage associated with the cAMP/AMPK/SIRT1 signaling pathway.[14]According to a different report, rolipram reduced the level of TNF-a and IL-10 in type

2 diabetic rats, which improved their cognitive deficit by increasing the expression of CREB and pCREB in the hippocampal region.[15] As discussed earlier, CREB is one of the directed substrates for PKA/PKG which further increases the neurotropic factors such as BDNF enhancing neuronal growth and increasing long-term synaptic plasticity.[16]

Vinpocetine, a PDE1 inhibitor regulates both cAMP and cGMP levels. Vinpocetine is being used in the treatment of cerebral ischemia and is being used as a nootropic to enhance brain cognition power. Vinpocetine might act through the cholinergic pathway to enhance memory deficit. A previously published also demonstrated the effect of vinpocetine (2mg or 4mg/kg, p.o) for 28 days which improved the cognitive function in scopolamine-induced memory deficit in C57 BL/6J Mice.[17] Vinpocetine and rolipram are well-known PDE inhibitors, for improving several neurological and psychiatric conditions of CNS such as brain inflammation, increased oxidative stress, neural dysfunction, and altered synaptical structures.[18, 19]

Therefore, we hypothesized that long-term administration of vinpocetine and rolipram may improve the memory deficit in arsenic-induced memory impairment in SD rats. We used behavioral assessment methods including the Morris water maze, Y maze task, Surface Righting reflex, Negative Geotaxis reflex, and Rota Rod to test the effects of vinpocetine and rolipram. Furthermore, the immunohistochemistry of CREB/p-CREB expressions was studied in the cortex region of the brain.

2. Material and methods

2.1. Experimental Animals

Male Sprague Dawley (SD) rats (150-200g) were selected and acclimatized for 1week. The room temperature was maintained at (22 ± 2°C) and relative humidity at (50-70%) under a natural chronological day-night cycle and free access to food and water was provided to all animals. All experimental procedures were approved under protocol no. IIRT/IAEC/2121/01/47, conducted in conformity with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India).

2.2. Drugs and Chemicals

Rolipram, vinpocetine, and donepezil hydrochloride were bought from Sigma Aldrich (USA). Erba Mannheim (Germany) provided the assay kits for all antioxidants. All other chemicals and reagents were sourced from CDH. Rolipram was prepared by suspending it in 0.5% (w/v) sodium carboxymethylcellulose[20], vinpocetine was prepared by dissolving in 0.5% (w/v) sodium carboxymethylcellulose[21] and donepezil was prepared by dissolving in 0.9%saline, [22] administered once daily for 28days in the respected groups between 10am -12pm.

2.3. Experimental Protocol

36 SD rats were used in the study, choice of animals and the dose of sodium arsenite were based on previously published studies.[4] After 1 week of acclimatization, the animals were randomly divided into six groups with 6 rats in each group (n=6): Group I received distilled water without sodium arsenite. Group II – Group VI was exposed to sodium arsenite for 90 days. After 90 days, Group III received rolipram (0.5mg/kg, p.o), [23, 24] and Group IV received vinpocetine (4mg/kg, p.o) [25, 21] for 28 days daily. Group V received rolipram (0.5mg/kg/day vinpocetine (4mg/kg, p.o) for 28 days daily, Group VI received standard treatment as Donepezil (0.5mg/kg, p.o)[22,26] for consecutive 28days. Arsenic water was freshly prepared every day and the consumption of water by each group was measured on a daily basis. During the study, the physical condition of the animals was observed daily and the body weight of each animal was recorded after regular intervals. Rats from each study group were subjected to the Morris water maze (MWM), Y-electric maze, Surface righting reflex, Negative geotaxis reflex, and Rota rod test. After the behavioral assessment, animals were sacrificed under an overdose of anesthesia. Brain samples collected (hippocampus and cortex) were separated onto a cold plate, and washed with ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The samples were kept at -80°C for further use in biochemical parameters.

2.4. Behavioral assessment

Animals were subjected to behavioral assessment after treatment with the drugs, tests were conducted during the light phase that is between 09:00 and 18:00 h. Initially, MWM was conducted to evaluate the escape latency and probe tests were done to evaluate spatial memory, followed by Y-Maze, Surface righting reflex, Negative geotaxis, and Rota rod test performed to test the short-term memory, Vestibular functions, motor control, and coordination in the rats.

2.4.1. Morris water maze (MWM)

The spatial memory was evaluated using the Morris water Maze, described by Morris et al. (1984).[27] It consists of a spherical tank (1.2m diameter and 49cm height) and was filled with enough water, to prevent the reach of the rat to the bottom and was made opaque by adding milk powder to it. The temperature was controlled at 22–25 °C. The platform was placed into the fourth quadrant or target quadrant. The rats were given four trials per day for four consecutive days during the acquisition phase. The test was performed by placing a rat on the water pool slowly (virtually sectioned into four equal quadrants) and guided to swim freely. Each quadrant was used as a starting point in the trial session, while the position of the platform was fixed during the trial. The time set to navigate the platform was 120sec. If a rat was unable to reach the platform within the

given time period, the rat was manually directed to the platform and was allowed to stay there for 10-15sec, then dried off and kept in the holding cage for the next trial. There should be an interval of 2 min between each trial. On the fifth day, the hidden platform was removed and the probe trial was carried out to assess the retention ability of the rats. The rats were allowed to swim for 60sec and find the platform. The path opted by the rats was analyzed by an automated video tracking system (VJ Instruments, Maharashtra), mounted on the center of the platform. The time taken to find the platform (escape latency) and the time spent in the target quadrant (TSTQ) was noted as a measure of learning and retrieval ability.

2.4.2. Y-maze test

Y maze test was done to evaluate the short-term memory in the animals. The apparatus consists of three arms inclined at an angle of 120° (shape of Y) connected to a central zone. Each arm is provided with a current supplied with the stainless steel grid fitted underneath. Any arm could be chosen as starting point, which was called to be a non-safe zone after training began. The outer end of the arms had a light source, which was the only indicator of the safe zone. Rats were acclimatized in the maze for 3min prior to the training session to explore the maze. After 3 min of acclimatization, the training started by placing rats into the Y maze. Rats have an innate tendency towards dark environments. On current stimulation, the conditioned reflex of the rat was to escape towards the dark arm with no light source, but after several continuous sessions, some rats actively respond to the conditioned reflex due to current stimulation and move towards a safe zone with a light source. The training sessions were considered to be completed when 9/10 correct entries were made by the rat with his half-upper body inside the safe arm, the light was turned off and the particular arm becomes the starting arm for the next session. The arms of the maze were cleaned after every session. After 24 hours, the rats were again put into the Y-maze and tested for10 times to assess the active avoidance learning ability and memory expressed as a correct rate.[28, 29]

2.5.3. Surface righting reflex

Righting reflex was performed to assess the motor control and coordination in the rats. It relies on alertness and vestibular response toward surroundings. A rat is placed on its back (supine position), and released. The time to restore a normal prone position with all four paws was noted. If a rat return to the normal position within 2sec, the response is noted as a positive else response is noted as negative. The training continued for three days (3 trials/day) and fourth day, the final experiment took place with the same procedure. The percent positive response was calculated.[30, 31]

2.5.4. Negative Geotaxis

A negative geotaxis test was conducted to assess the motor response, cerebral integrity, and vestibular functions of animals. It is based on unlearned response and directional movement against the gravitation cues by rats. All the rats in different interventional groups along with the control group undergo the test for five consecutive days (3 trials/day). Rat with its head down was placed on a platform (made up of wood) with a rough surface inclined at an angle of 25° and allowed to turn freely. The point at which the rat rotate its body at an angle of 180°, time was recorded. If the rat completes this within 60sec, the response was noted as a positive response.[30]

2.5.5. Rota rod performance test

The Rota rod test was conducted to assess the brain's motor coordination in rats. The Rota rod consists of four adjacent rods (10cm width, 40cm height), an automated apparatus revolved at 10rpm/min. The rats of all the groups were placed on the rotating rods and allowed to balance on the rotating rod. The fall-off time was noted for all groups, and the greater the time of falling, indicative of good motor coordination in the rats.[32]

3. Immunohistochemistry

After completion of the behavioral assessment, the cortex region was analyzed for the protein expressions of CREB/p-CREB in all study groups. All animals were decaptivated under sodium pentobarbital (60mg/kg, i.p) anesthesia. The cutout cortex samples were removed and post-fixed in 4% paraformaldehyde for 24 h and then in 10%, 20%, and 30% sucrose solution (in 0.1 M phosphate buffer, pH 7.4) serially till they settled. 20µm coronal brain sections containing Cortex were collected serially using a cryostat (Microm HM 525, Germany), which were washed with 0.01 M PBS were blocked in PBST (0.01 M, Phosphate buffer saline and 0.25% tween 20) containing 1% normal horse serum (NHS Vecta-stain Elite ABC kit, Vector Laboratories, Burlingame, CA, USA), 0.25% tween 20. These sections were incubated overnight with primary antibodies (rabbit monoclonal, 1:1000 dilutions). Finally, the sections were incubated with avidin-biotinylated-peroxidase complex (PK-6200, Vectastain Elite ABC Kit; Vector Laboratories) and followed by DAB staining (ab64238, DAB peroxidase substrate, Abcam). Stained sections were fixed on glass slides and images from the sections were developed by a Nikon Eclipse Ni microscope (Nikon, Tokyo, Japan). Expression was analyzed as the number of positive nuclei in the Cortex of the rat brain using the NIS-Basic Research image analysis system (Nikon, Tokyo).[33]

4. Statistical analysis

The data obtained from the neurobehavioral and biochemical parameters were statistically analyzed using Graph pad prism (Graph Pad Software, 9.3.1 version). All results are represented

as mean ±SD. The data from assessing change in body weight and escape latency was analyzed using two-way ANOVA followed by Bonferroni's multiple comparison post hoc test, while the remaining behavioral parameters and biochemical parameters were analyzed by one-way ANOVA followed by Tukey's post hoc test. The individual F value and degree of freedom (df) were examined. The accepted statistical significance value for all parameters was $p < 0.05$.

5. Results

5.5. Effect on Body Weight and brain weight of Rats

The rats of all groups were observed for any kind of abnormality. No obvious difference was found among all. The water intake for each group was noted, and the result was almost similar to the previous research which reports that a rat weighing 300g rat consumes 25-30ml of water a day. There was no change in water intake, was noted during the experiment. Though, a significant decrease in the body weight of the animal was observed in animals in the sodium arsenite group which was observed to increase after the drug therapy ($P < 0.05$, n=6) (df = 5, 24; F= 4.762). In comparing the brain weight, sodium arsenite significantly reduces the brain weight of the rats, when compared to the control group ($P < 0.0001$, n=6) (df = 5, 30; F= 26.25) (**Table 1**).

	Control	Sod. Ars	Sod. Ars + R	Sod. Ars + V	Sod. Ars + R+ V	Sod. Ars +D
Body weight	205.24±23.59	167.02±19.3*	166.38±9.99*	176.3±10.85*	169.46±10.00*	173.12±10.27*
Brain weight	1.35±0.02	1.23±0.02**	1.26±0.01**	1.26±0.01**	1.30±0.02**	1.30±0.01**

2.5. Table 1. Effect of rolipram and vinpocetine on Body weight and Brain weight of animals: Values are expressed in Mean ± SD (n=6),*p<0.05, **p<0.0001 when compared to the control group. Data for body weight was determined by using two-way ANOVA followed by Bonferroni's multiple comparison post hoc test while brain weight values were determined by using one-way ANOVA followed by Tukey's multiple comparison post hoc test. **Effect on spatial memory**

A two-way ANOVA followed by Bonferroni's multiple comparison post hoc test determined the significant delay in the escape latency in the rats exposed to sodium arsenite evaluated by the Morris water maze test, which was significantly enhanced by rolipram and vinpocetine ($p<0.0001$, n=6) (df = 5, 15; F= 11.81) and a significant difference was also observed with the interaction between drug treatment and days ($p<0.001$, n=6) (df = 3, 15; F= 104.4). Sodium arsenite significantly drops swimming speed in the rats when compared to the control group, found to be

increased in the rats administered rolipram and vinpocetine ($p<0.001$, n=6) (df = 5, 30; F= 129.5). This illustrates that the rats treated with 28 days therapy of rolipram and vinpocetine showed a potential to improve spatial learning in rats. After 24 hours of the acquisition test, a probe test was conducted which showed that rats treated with rolipram and vinpocetine spend more time in the target quadrant in comparison to the rats in the sodium arsenite group ($p<0.001$, n=6) (df = 5, 30; F= 44.03), analyzed by one-way ANOVA followed by Tukey's post hoc test ($p<0.001$). The result indicates that rolipram and vinpocetine increased the learning memory retention and consolidation in the probe trial test (**Figure 1A-C**).

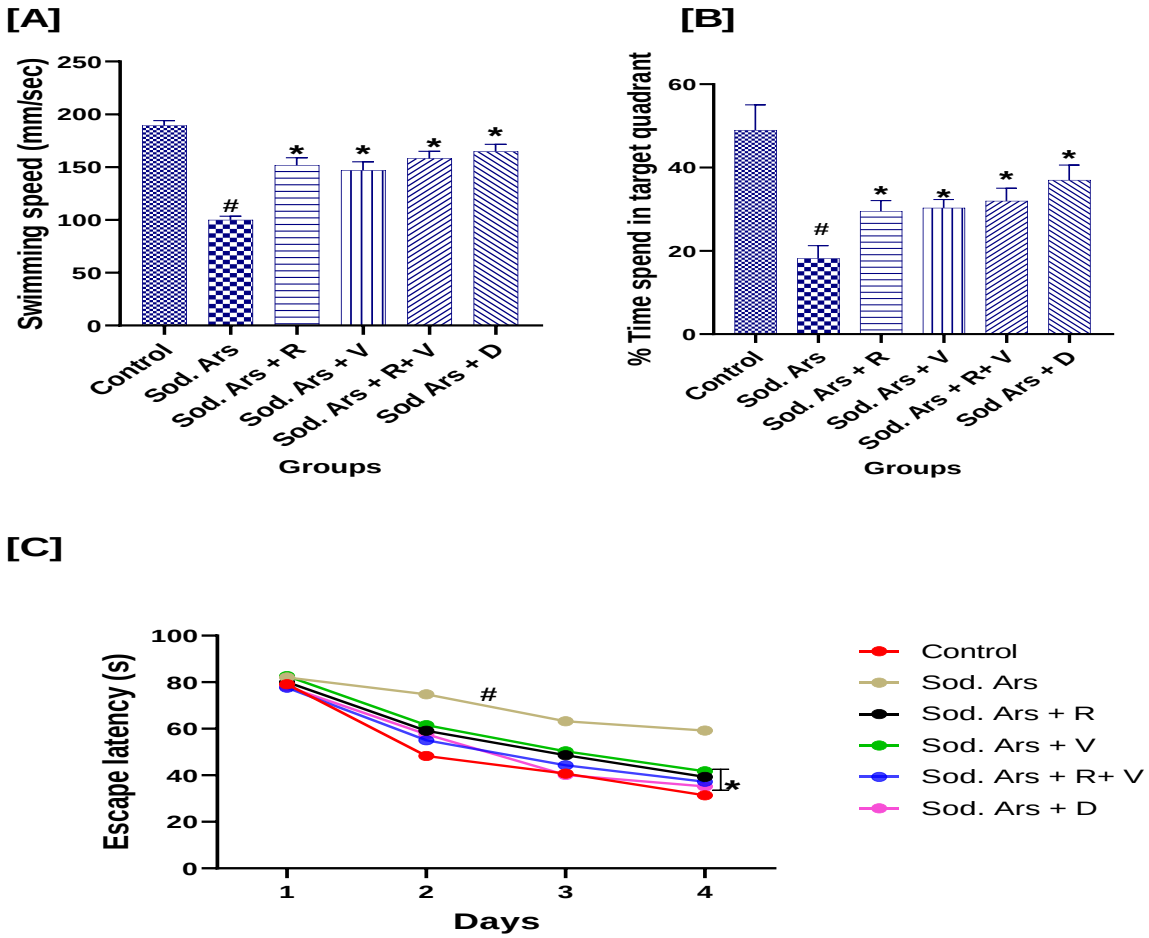


Figure 1. Effect of rolipram and vinpocetine on spatial memory in sodium arsenite exposed rats: Rolipram and vinpocetine had an effect on the swimming speed of the rats **(A),** time spent by the rats in the target quadrant **(B),** and Escape latency to find the hidden platform **(C).** Values are expressed in Mean ± SD (n=6), # $p<0.0001$ compared to Control and *$p<0.0001$ compared to the sodium arsenite group. The results of the escape latency were determined by two-way ANOVA followed by Bonferroni's multiple comparison post hoc test and the results for the estimation of swimming speed and probe trial were determined using one-way ANOVA followed by Tukey'spost hoc test.

2.6. Effect on short-term and spatial memory and reflex action

As analyzed by one-way ANOVA followed by Tukey's post hoc test, a lesser number of rats were found to reach the safe zone upon current stimulation from the group exposed to sodium arsenite in comparison to the control group ($p<0.0001$, n=6). After 28days administration with rolipram and vinpocetine, a significant improvement in the number of rats that responded to the current stimulation was found and rats were able to direct themselves toward the safe zone ($p<0.001$, n=6) (df = 5, 30; F= 17.66). This indicates that rolipram and vinpocetine administration alone or in combination improves short and spatial memory (**Figure 2A**). The percent positive response in the Surface righting reflex was also found to be increased in the groups administered with rolipram and vinpocetine when compared to the sodium arsenite group zone ($p<0.001$, n=6) (df = 5, 30; F= 16.01) (**Figure 2B**).

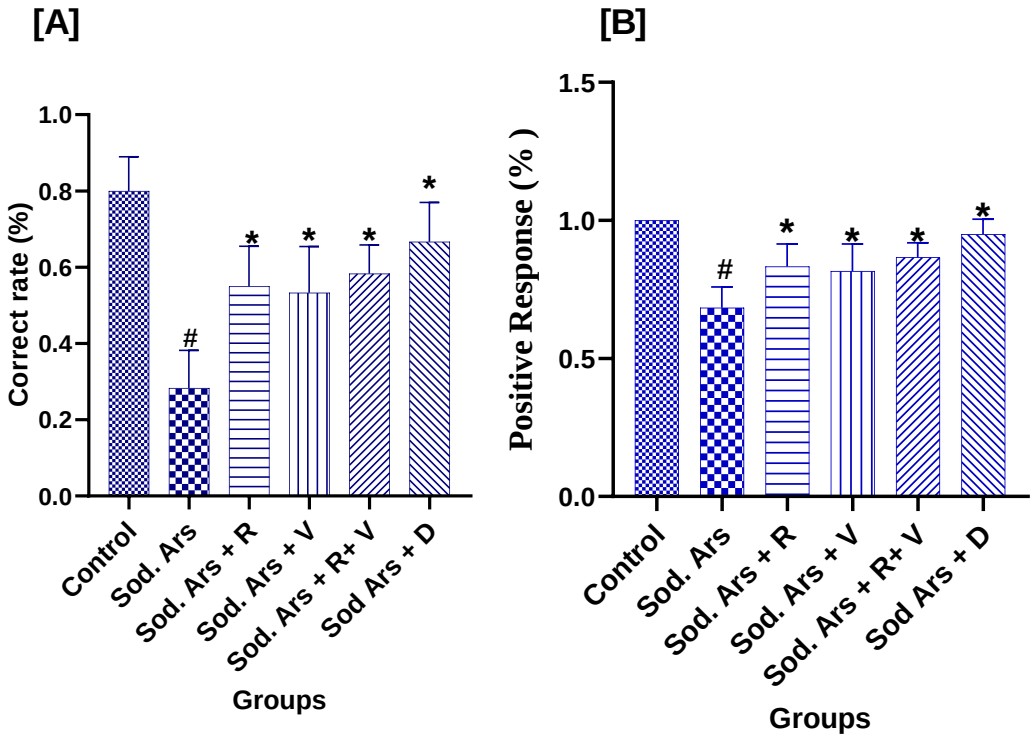
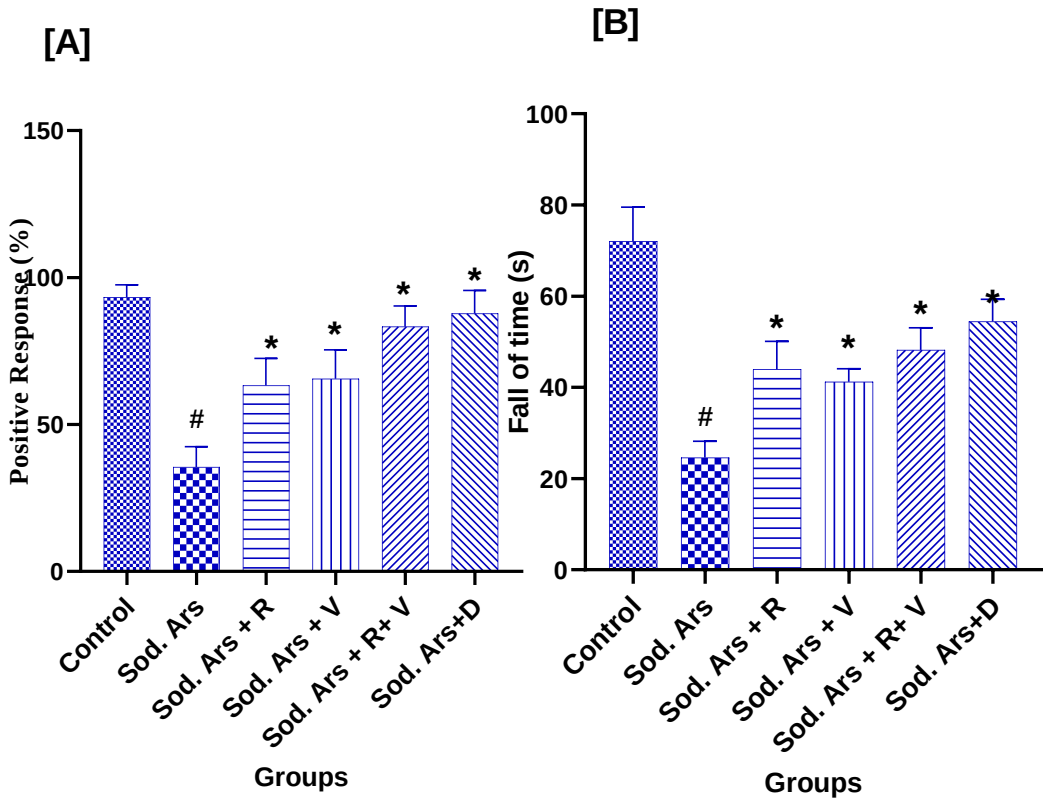


Figure 2. Effect of rolipram and vinpocetine on short-term memory and reflex response in sodium arsenite exposed rats: The figure depicts the correct rate (%) in Y-maze **(A),** Positive response (%) in Surface righting reflex **(B).** Values are expressed in Mean ± SD (n=6), # $p<0.0001$ compared to the control group, *$p<0.05$ compared to the sodium arsenite group. Results were determined by using one-way ANOVA followed by Tukey's post hoc test.

2.7. Effect on vestibular functions, motor coordination, and grip strength

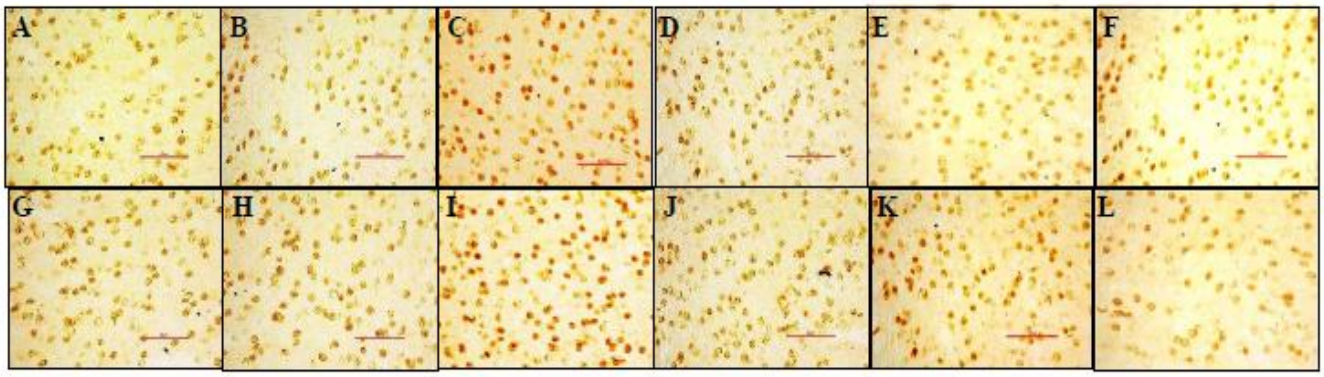
Animals showed a lower percent positive response representing a delayed reflex response in the negative geotaxis reflex test (**Figure 3A**) compared to the control group. Administration with rolipram and vinpocetine, significantly improved the percent positive response in the rats, when compared with the sodium arsenite group ($p<0.0001$, n=6) (df = 5, 30; F= 46.08). Low motor coordination and balance were observed in the sodium, arsenite group when compared with the control group ($p<0.0001$) (**Figure 3B**). The administration with rolipram and vinpocetine for 28days leads to a significant improvement in motor coordination in the rats when compared to the sodium arsenite group ($p<0.0001$, n=6) (df = 5, 30; F= 54.63). One-way ANOVA followed by Tukey's multiple comparison tests was used to determine the data obtained in negative geotaxis and rota rod test.



2.5. Figure 3. Effect of rolipram and vinpocetine reflex response and motor coordination in rats exposed to sodium arsenite: The figure depicts a positive response (%) in the negative geotaxis test **(A),** fall-off time in the Rota rod test **(B).** Values are expressed in Mean ± SD (n=6), $^{\#}p<0.0001$ compared to Control and $*p<0.0001$ compared to the sodium arsenite group. The results obtained from the negative geotaxis and rota rod test were

analyzed using the one-way ANOVA test followed by Tukey's multiple comparison test.**Effect of rolipram and vinpocetine on the level of CREB/ p-CREB**

Immunostaining of CREB and p-CREB was observed which showed a significantly low number of positive neurons against CREB exposed to sodium arsenite when compared with the control group ($p<0.0001$), which was relatively higher in the groups administered with rolipram and vinpocetine alone and in combination ($p<0.0001$, n=1) (df = 5, 30; F= 58.55). The number of positive neurons against p-CREB in the cortex region of all studied groups has been shown in Figures 4G and 4H indicating the less number of neurons in the sodium arsenite exposed group as compared with the control ($p<0.0001$). Further, the positive neurons against p-CREB showed a significant increase in the groups administered with rolipram and vinpocetine alone and combination of rolipram and vinpocetine, when compared with sodium arsenite group ($p<0.0001$, n=1) (df = 5, 30; F= 57.68). One-way ANOVA followed by Tukey's post hoc test has been used to determine the CREB and p-CREB level in the cortex region of the brain of rats exposed to sodium arsenite (**Figure 4A-H**).



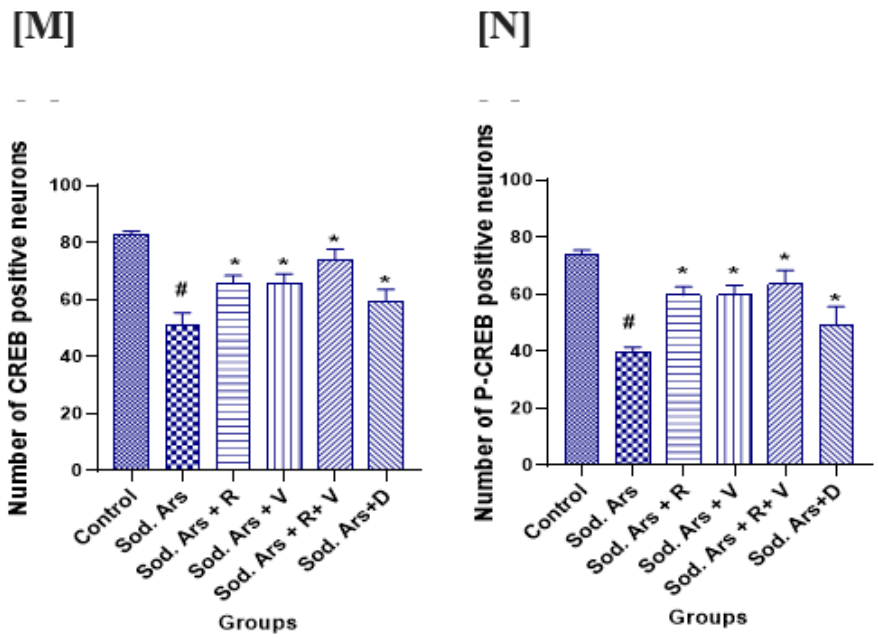


Figure 4. Effect of rolipram and vinpocetine on CREB and p-CREB expression in the cortex region of rat brain exposed with sodium arsenite. The picture shows the immunostaining of CREB in the control group **(A)**, immunostaining of CREB in sodium arsenite group **(B)**, immunostaining of CREB in sodium arsenite + rolipram group **(C)**, immunostaining of CREB in sodium arsenite + vinpocetine group **(D)**, immunostaining of CREB in sodium arsenite + rolipram + vinpocetine group **(E)**, immunostaining of CREB in sodium arsenite + donepezil group **(F)**, immunostaining of p-CREB in the control group **(G),** immunostaining of p-CREB in sodium arsenite group **(H)**, immunostaining of p-CREB in sodium arsenite + rolipram group **(I)**, immunostaining of p-CREB in sodium arsenite + vinpocetine group **(J)**, immunostaining of p-CREB in sodium arsenite + rolipram + vinpocetine group **(K)**, immunostaining of p-CREB in sodium arsenite + donepezil group **(L),** Number of positive neurons against CREB in cortex **(M)** and Number of positive neurons against p-CREB in Cortex region **(N).** Values are expressed in Mean ± SD (n=6), $^{\#}p<0.0001$ compared to control group, $*p<0.0001$ compared to sodium arsenite group. Results were determined by using one-way ANOVA followed by Tukey's post hoc test.

3. Discussion

The ameliorative effect of rolipram (PDE 4 inhibitor) and vinpocetine (PDE 1 inhibitor) on an animal model of sodium arsenite-induced behavioral phenotypes was investigated. The model was established by administering sodium arsenite through drinking water, the dose was employed according to the previously published study. Ingestion of sodium arsenite did not cause any significant alterations in the eating or drinking habits or appearances of the animals, but when body weight and brain weight were recorded there was a significant decrement in brain as well as body weight.

Different studies evaluated the phenotypical behavior of adult male rats, pregnant rats, and lactating rats using different maze models[34, 35,36] implicating the involvement of arsenic in the alteration of spatial reference memory. In the study we measured the escaped platform acquisition ability, swimming speed, and retrieval ability by using the Morris water maze, suggesting that rolipram and vinpocetine attenuate the prolongation of escape latency, and enhance the retrieving ability and swimming speed in the arsenite-exposed rats. Prolonged latency was the measure of low spatial memory. However, the difference in swimming speed is due to low spatial memory and disrupted locomotor activity due to arsenic.

Y–Maze was used to evaluate the short-term memory of rats of all groups, and showed a decrease in positive response in the sodium arsenite exposed groups, which was significantly enhanced in the groups administered with rolipram and vinpocetine individually or in combination. The

vestibular reflex response, motor coordination, and motor response were evaluated using surface righting reflex, negative geotaxis effect, and rota rod, which showed a significant improvement in the reflexes, motor response, and gripping strength in the rats administered with rolipram and vinpocetine.

These results obtained show that the short-term, spatial working, reference memory, and motor coordination were decreased by the administration of sodium arsenite which was significantly ameliorated by administering rolipram and vinpocetine.

In addition, arsenic exposure was also found to decrease the ERK/ CREB signaling in rat offspring. This decrease in the level of ERK, p-ERK, and CREB was associated with the cognitive decline in the offsprings.[30]It is well documented that CREB promotes neuronal growth and has an important role in the regulation of synaptic plasticity and long-term memory formation. P-CREB has been reported to be associated with hippocampal-based learning as well.[37] Altogether, these studies reveal the involvement of CREB in hippocampal-based memory formation [38], and also some research revealed that the inhibition of the PKA /CREB pathway plays a crucial role in contributing to cognition deficit in patients with the AD.[39] Aforementioned literature suggests that, both CREB and PKA signaling pathways are involved in altered neurobehavioral and cognitive dysfunctioning.[40,41]

In the published literature, we found that PDE4 inhibition by rolipram was reported to activate CREB and p-CREB levels in the hippocampus which improved the cognitive function in diabetic rats.[42] Rolipram at a dose of 0.1mg/kg acts as a neuro-protectant and prevents the loss of CA3 hippocampal neurons compared to a higher dose of 0.3 mg/kg.[43] Inhibition of PDE4 by rolipram ameliorates the memory impairment induced by Aβ1-42 by mediating the hypothalamic-pituitary-adrenal (HPA) axis by increasing the expression of p-CREB/CREB and BDNF.[44] Inhibition of PDE1 by Vinpocetine was observed to ameliorate locomotor activity, social deficits, and repetitive behavior in a ketamine-induced experimental model of ASD.[45]Vinpocetin improves the learning and memory deficits in the Morris water maze test induced by chronic cerebral hypoperfusion.[46] Vinpocetine enhances the cAMP and cGMP levels and upgrades the P-CREB levels via the cAMP/CREB and PKG pathway in the cerebral cortex and hippocampus ameliorating the deleterious effects of nicotine and ethanol-induced hyperactivity and cognition deficit.[47]

There are ample evidence that support the ameliorative effect of PDE 4 and PDE1 inhibition on the cognitive deficit model mediated through the cyclic AMP/CREB signaling pathway. In line with these observations, the present study also confirms the enhanced expression of CREB and P-CREB in the rat brain's cortex region, which has never been studied in the arsenic-induced memory impairment rat model. Therefore, the study reports that the administration of rolipram (0.5mg/kg) and vinpocetine (4mg/kg) for 28days and their combination attenuates the arsenite-induced low

CREB/P-CREB neuronal activity and ameliorates the arsenic-induced behavioral deficits which might be a consequence of increased CREB and P-CREB expression in the cortex region.

4. Conclusion

We can conclude that rolipram and vinpocetine could be potential targets for treating arsenic-induced cognitive deficit and could be a topic of investigation to explore more possible pathways for the inhibitory effect of PDE4 and PDE1 in arsenic-induced cognitive impairment.

5. Acknowledgment

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6. Conflict of interest

The authors declare that they have no conflicts of interest.

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