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To Evaluate the Protective Effect of *Murraya Koenigii* extract against Sodium Arsenite-induced Cognitive Dysfunction in Swiss Albino Mice

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ABSTRACT
Background: Arsenic is a ubiquitous environmental toxicant that can cause neurotoxicity and impairment in cognitive function by generating oxidative stress, neuroinflammation, and damage to neurons. The inorganic form, the highly toxic Sodium arsenite, has been known to exert detrimental effects on learning and memory functions of experimental animals. The natural products with anti-oxidative and neuroprotective properties have been found as promising agents against arsenic-induced neurotoxicity. Murraya koenigii (curry leaf) is one of the medicinal plants, characterized by the presence of carbazole alkaloids, flavonoids, and phenolic compounds, which are reported to possess potent antioxidant and neuroprotective activities.
Methods: Cognitive dysfunction was induced by giving sodium arsenite treatment to the Swiss albino mice. The neuroprotective activity of the extract of *Murraya koenigii* was tested by behavioural parameters for testing learning and memory. The biochemical estimation of oxidative stress parameters and endogenous antioxidant enzyme activities was carried out.
Results: The treatment of Sodium arsenite showed impaired learning and memory functions and increased the levels of oxidative stress markers and neuronal damage in mice. Administration of *Murraya koenigii* Extract to sodium arsenite-treated mice reversed the deficits in cognitive behaviour, and also significantly attenuated the markers of oxidative stress and improved the activities of endogenous antioxidant enzymes. The extract showed significant neuroprotective activity against arsenic-induced cognitive dysfunction.
Conclusion: *Murraya koenigii* extract exhibited significant protection against sodium arsenite-induced cognitive dysfunction in mice, mediated possibly through its antioxidant and neuroprotective activities. This study suggests that *Murraya koenigii* could serve as a potential natural agent for the treatment of arsenic-induced neurotoxicity and cognitive impairments.

KEYWORDS: *Murraya koenigii,* Sodium arsenite, Cognitive dysfunction, Neuroprotection, Oxidative stress.

1. INTRODUCTION

Arsenic, a metalloid, is a natural contaminant and one of the most toxic environmental pollutants known globally. It is exposed to humans through various media such as drinking water, food, industries, and agriculture. Human beings with chronic exposure to arsenic can suffer from many health problems such as cardiovascular system damage, cancer, liver, kidney, neural system diseases, and diabetes. Different forms of arsenic are present, but among them all inorganic arsenic forms are the most dangerous ones. [1,2]

Sodium arsenite (NaAsO), a pentavalent inorganic arsenic compound, is considered to be highly neurotoxic since it shows a strong affinity for the sulfhydryl groups of proteins and enzymes. The fact that it can easily pass across the blood-brain barrier and accumulate in brain tissues causes changes in the cellular structure and function of neurons. According to previous experimental and epidemiological studies, administration of sodium arsenite induces a spectrum of neurobehavioral effects in animals, impairs memory acquisition and retention processes, and affects overall cognitive performance, in particular in areas of the brain related to memory processing such as the hippocampus and cerebral cortex. [3,4]

The precise mechanisms through which arsenic induces cognitive impairment are diverse, encompassing oxidative stress, neuroinflammation, mitochondrial dysfunction, apoptosis, and alteration of neurotransmitter pathways. Oxidative stress, which arises when there is a significant imbalance between reactive oxygen species (ROS) generation and the ability of the antioxidant system to scavenge them, leads to damage to cellular molecules such as lipids, proteins, and DNA, resulting in altered communication and memory deficits. [5,6]

More recently, attention has also been paid to medicinal plants and natural antioxidants in the prevention and therapy of neurodegenerative diseases. Phytochemicals derived from plants could potentially be very helpful in managing these conditions, owing to their significant effect on inflammation and redox pathways, while exhibiting considerably lesser toxicity and side effects. Indeed, many phytochemicals (e.g., flavonoids, polyphenols, alkaloids, and terpenoids) could

exhibit protective effects in cognitive deficiency models. [7,8] *Murraya koenigii (*L.) Spreng., commonly called curry leaf, is an herb in the Rutaceae family that is native to and abundant on the Indian subcontinent. For a long time, curry leaf was a standard component of traditional Ayurvedic medicine used to manage a range of health problems. From phytochemical studies, carbazole alkaloids, flavonoids, glycosides, phenolic compounds, vitamins, and several mineral compounds were identified, contributing to various pharmacological activities. For example, it is known to possess antioxidant, anti-inflammatory, anti-diabetic, anti-microbial, hepatic, and anti-neuro effects. [9,10] The major contributor for the neuroprotective effects of Murraya koenigii lies in its strong antioxidants which can trap reactive oxygen species and strengthen the existing antioxidant defense system of the body. Various carbazole alkaloids present in this plant were demonstrated to confer neuronal protection against oxidative injury, promote cholinergic transmission and retain cognitive ability. Due to these medicinal properties, *Murraya koenigii* may have promising therapeutic potential for reducing arsenic-induced neuronal damage and memory impairment. [11,12] Although the neuroprotective effects and antioxidative capacity of the extract of Murraya koenigii have received much attention from researchers and were confirmed by numerous reports, only a few research works have been done so far to study its effect on arsenic toxicity. In the present study, we have aimed to study the protective effect of *Murraya koenigii* against sodium arsenite-induced cognitive deficits in Swiss albino mice. The present study could provide useful insight to establish a plant-based remedial method for the prevention of neurodegenerative disorders due to exposure to environmental toxins. [13,14]

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Sodium arsenite was bought from CDH. Ethanolic extract of *Murraya koenigii* leaves was bought from Vital Herbs Pvt. Ltd. Add- Z-26/27 Commercial Enclave, Mohan Garden, Uttam Nagar, Delhi, 110059 (GSTIN:07CIRPD7950H1ZV). All other chemicals and solvents used were of analytical grade and were purchased commercially.

2.2. Experimental Animals

Adult Swiss albino mice (Male; 25- 30 g), 6-8 weeks old, obtained from a CPCSEA-registered animal breeding unit, were kept in standard laboratory conditions (22±2°C temperature, 55±5% relative humidity, 12 h light/dark cycle). Animals were provided with a standard pelleted diet and drinking water ad libitum throughout the experiment. Institutional animal ethical committee clearance was obtained according to CPCSEA guidelines (1204/Po/ReBiBt/S/2008/ CCSEA/25-08

2.3. Induction of Cognitive Dysfunction

The cognitive impairment was generated by oral administration of sodium arsenite at a dose of 5mg/kg/day for seven weeks. Chronic toxicity of sodium arsenite could bring about oxidative stress, neuroinflammation, degenerating nerves, and loss of memory and cognition, such as aged cognitive impairment and Alzheimer's disease.

2.4. Experimental Design

Six animals were used per group (n = 6).
Group I- Normal Control
Group II- *M koenigii* extract (500 mg/kg p.o) Per se
Group III- Sodium arsenite (5 mg/kg p.o)
Group IV- *M koenigii* extract (250mg/kg p.o) + Sodium arsenite (5 mg/kg p.o)
Group V- *M koenigii* extract (500mg/kg p.o) + Sodium arsenite (5 mg/kg p.o)

2.5. Behavioural Assessment

Behavioural tests were conducted during the last week of treatment.

2.5.1. Morris Water Maze Test (MWM)

The Morris water maze test was conducted to assess spatial learning and memory. Escape latency time, duration spent in the target quadrant, and the frequency of platform crossings were analysed. The mice were positioned on the platform; the training duration was established at 60 seconds; if the mice failed to reach the terminal point within 60 seconds, it was recorded as 60 seconds. Furthermore, it must be manually directed to remain on the platform for 10 seconds if the mice fail to locate it within the first 4 days. Mice underwent training every 24 hours, and experimental results were gathered on the fifth day. On the sixth day, the platform was removed to assess the mice's spatial search capability, while recording the number of mice that traversed the original location and the time spent in the target quadrant. (15)

2.5.2. Passive Avoidance Test

Memory retention and learning ability were assessed using the passive avoidance apparatus. Step-through latency was recorded during acquisition and retention trials.The passive avoidance apparatus comprises a lit white compartment and a dark compartment linked by a moveable door, with unavoidable electrical shocks administered to the animals upon their entry into the electrified dark room. Mice were introduced to the apparatus for a duration of 5 minutes to acclimate to the environment. Twenty-four hours following the initial trial, the passive avoidance test was conducted. The mice were positioned in the illuminated compartment, the movable door was opened, and the latency period for entering the dark compartment was documented as "escape latency," while the frequency of entries into the dark box was recorded as "the number of errors. If the mouse did not enter the dark chamber within 300 seconds, a delay of 300 seconds was documented. (16)

2.5.3. Open Field Test (OFT)

Locomotor activity was assessed in a 50 cm × 50 cm open field arena (50 cm × 50 cm × 38 cm, length × breadth × height). The mice were positioned at the center of the apparatus, and their locomotor behaviours were documented for 5 minutes

utilizing a digital camera. Horizontal locomotor activity was quantified as the total distance travelled ambulatorily. The open field chamber was sanitized between trials using a 75% alcohol solution. (17)

2.6. Biochemical Assay

Animals were sacrificed sequentially following behavioural observation in the following order. Brains were washed with isotonic saline, weighed, then cut in half on a mirror. The remaining half was then used for biochemical calculation. In the course of the biochemical investigation, the homogenised tissues (10% (w/v) in 0.1 M phosphate buffer, pH 7.4) were centrifuged for 15 min at 4C (10,000g), supernatants were aliquoted and used for the measurement of biochemical components. Biochemical tests were conducted using a UV-vis Spectrophotometer 1800 [Shimadzu].

2.6.1. Reduced Glutathione (GSH)

The level of GSH was determined using the technique described by Jollow et al. (1974). Briefly, 0.25 mL of brain homogenate was incubated for 10 min in the presence of 0.25mL ice-cold 5% trichloroacetic acid (TCA) and subsequently centrifuged at 4000 rpm for 10 min. One milliliter of the supernatant was mixed with 0.25mL of 0.2 M phosphate buffer (pH 8.0) and 0.5mL of DTNB (0.6 mM), and absorbance was measured using a Shimadzu UV-1800 spectrophotometer at 412nm. It was represented as micromoles of GSH per mg of protein. (18)

2.6.2. Malondialdehyde (MDA)

Quantitative assessment of lipid peroxidation in brains of experimental mice was measured according to the Wills procedure and determined with the aid of the chromophore's molar extinction coefficient (1.58 X IO7 M -' cm ') as malondialdehyde nanomole/mg of protein. A double-beam UV-VIS spectrophotometer [UV spectrophotometer 1800 (Shimadzu)] was used to determine the concentration of Malondialdehyde (MDA) (the main component of lipid peroxidation) by a double reaction with thiobarbituric acid at 540 nm 25. (19)

2.6.3. Nitric Oxide (NO)

The amount of nitrite precipitated was estimated for determination of nitric oxide (NO) synthesis. A colorimetric assay of the nitrite (by using Griess reagent - 0.2% N-[1-naphthyl] ethylenediamine di-hydrochloride, 2.6% phosphoric acid, 1% sulphonamide) was prepared. The mixture of equal parts of the Greiss reagent and supernatant was allowed to stand for 10 min. The optical density was determined by a double-beam UV-VIS spectrophotometer [UV spectrophotometer 1800 (Shimadzu)] at 560 nm wavelength. The unknown amount of nitrite in the supernatant was extrapolated from the calibration curve using sodium nitrite standard and expressed as µg/mg of Protein. (20)

2.6.4. Catalase (CAT)

The activity of catalase was determined according to the procedure described by Luck, in which H2O2 is degraded. Briefly, the assay mixture containing 3 mL of H2O2 phosphate buffer and 0.05 mL of the supernatant of the tissue homogenate was monitored at 240 nm for 2 min at 30-second intervals using a UV Spectrophotometer 1800 (Shimadzu). It was represented as Units per mg of protein. (21)

2.6.5. Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) was measured according to Kono. SOD Assay system contains EDTA 0.1 mM, Na2CO3 50 mM, and 96 mM of nitro blue tetrazolium (NBT). Into cuvettes was added 2 mL of the mixture mentioned above, 0.05 mL of hydroxylamine, and 0.05 mL of the supernatant, and the auto-oxidation of hydroxylamine was measured for 2 minutes at intervals of 30 seconds using a UV spectrophotometer 1800 (Shimadzu) with measuring absorbance at 560 nm. The findings were represented as units per mg of protein. (22)

2.6.6. Acetylcholinesterase (AChE)

Loss of forebrain cholinergic neurons leads to down-regulation of AChE, so we measured activity of AChE in a 96-well plate using the Ellman assay. Acetylcholinesterase assay consists of the addition of 0.05 ml of supernatant in a buffer consisting of 3 ml of sodium phosphate buffer pH 8, 0.1 ml of acetylthiocholine iodide, and 0.1 ml of DTNB (Ellman's reagent); the change of absorbance in 2 minutes was monitored at 30-second intervals on a UV spectrophotometer 1800 (Shimadzu) at a wavelength of 412 nm. The enzyme activity was expressed as micromoles of acetylthiocholine hydrolysed per minute per milligram of protein. (23)

2.6.7. Estimation of Interleukin-1 Alpha (IL-1α)

A commercially available Rat IL-1 ELISA Kit from Elab Science Biotechnology Co., Ltd. Was used to determine the concentration of the pro-inflammatory cytokine Interleukin-1 alpha (IL-1) in the homogenized samples. Brain tissue IL-1 concentrations were evaluated by the sandwich ELISA (Enzyme-Linked Immunosorbent Assay (ELISA). The ELISA Kit was used as per the manufacturer's guidelines (Shimadzu). (24)

2.6.8. Estimation of Interleukin-10 (IL-10)

Detection of the anti-inflammatory cytokine Interleukin-10(IL-10). Brain IL-10 levels were detected by a commercial kit - Rat IL-10 ELISA Kit provided by Elab Science Biotechnology Co., Ltd. By the Sandwich ELISA method, IL-10 concentrations in brain tissue homogenates were detected according to the instructions of the kit. (24)

2.6.9. Estimation of Tumor Necrosis Factor-Alpha (TNF-α)

Tumor Necrosis Factor alpha (TNF) levels in rat brain tissues were determined using the commercially available Rat TNF ELISA Kit purchased from Elab Science Biotechnology Co., Ltd. The concentrations of TNF in rat brain homogenate were determined with the Sandwich ELISA method according to the manufacturer. (24)

2.7. Histopathological Examination

Brain tissues, particularly the hippocampus and cerebral cortex, were preserved in 10% neutral buffered formalin, sectioned, stained with H&E, and microscopically analyzed for neuronal degeneration, pyknosis, vacuolization, or other neuropathological alterations.

2.8. Statistical Analysis

Data were presented as Mean ± SEM. Statistical analysis was conducted with one-way ANOVA, followed by Tukey's multiple comparison test, performed with GraphPad Prism software. Values of $p<0.05$ were deemed statistically significant.

3. RESULT AND DISCUSSION

3.1. Morris Water Maze Test

The effect of treatment on Learning and memory was tested over six consecutive days through the Morris Water Maze test. On days 1–5, learning ability was measured in terms of elapsed time taken to find the hidden platform (escape latency time) by each animal. The decrease in elapsed time from day 1 to day 5 indicates proper learning ability of the animal, whereas the decrease in elapsed time on day 5 when compared with day 1 indicates no significant learning impairment. On day 6, the platform was removed, and retention of memory was analysed by calculating time spent in the target quadrant (TSTQ). More time in the target quadrant shows retention of memory, whereas time spent in the target quadrant decreases, as it shows cognitive impairment. Control shows a significant ($p < 0.05$) elapsed time on Day 5 when compared with Day 1. Control shows significantly more time spent in the target quadrant (Q4) on Day 6 when compared with the treatment group, thereby indicating proper learning and memory retention capacity. Animals in Sodium arsenite-treated groups had a significant elapsed time on day 5F (4,25) =9.84, * $p < 0.05$), and time spent in the target quadrant on day 6 F (4,25) =11.26, * $p< 0.05$), which was decreased significantly, showing impairment of the learning and memory process. Treatment of MKE reversed these effects significantly and improved cognitive functions in a dose-dependent manner.

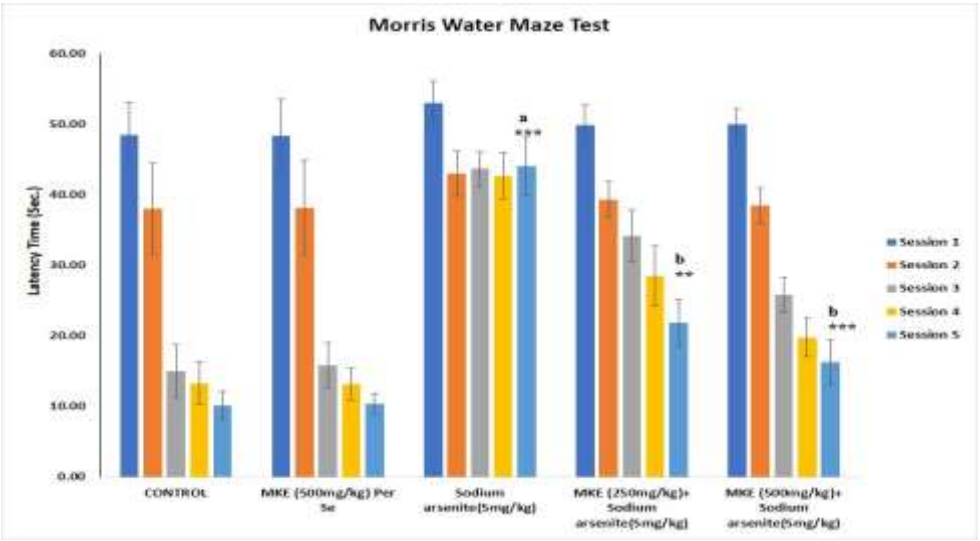

Figure 1: Effect of MKE on spatial learning and memory in Sodium arsenite-induced experimental animals using the Morris Water Maze test.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. MKE (Murraya koenigii Extract).

3.2. Time Spent in Target Quadrant (TSTQ)

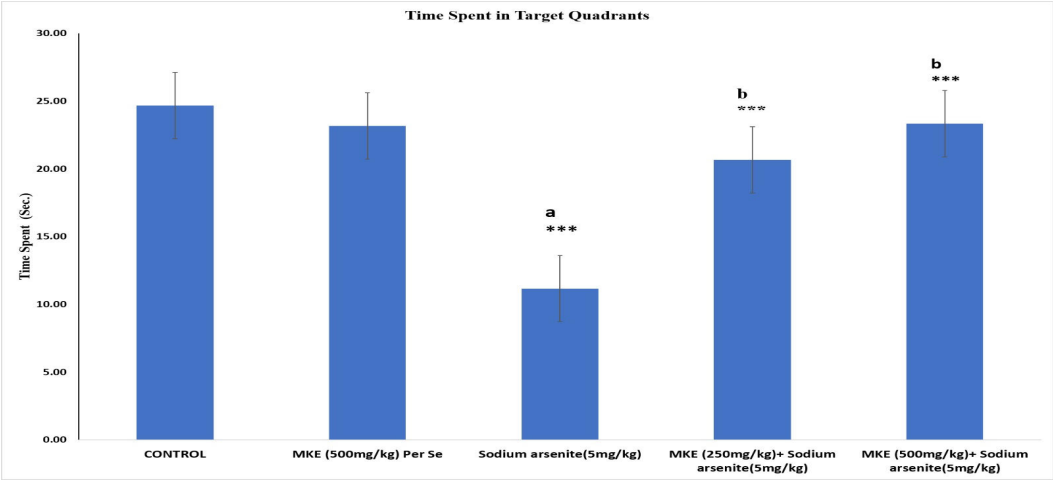

Figure 2: Effect of MK on time spent in the target quadrant (TSTQ) in Sodium arsenite-induced experimental animals using the Morris Water Maze test.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. TSTQ: Time spent in target quadrant; MKE (Murraya koenigii Extract).

3.3. Passive Avoidance Test

When compared to the control group, the Sodium arsenite-treated group shows a significant reduction in learning and memory, as evidenced by a reduction in step-through latency $F(4,25) = 9.67$, $^*p < 0.05$. When compared to the Sodium arsenite group, both treatment groups show a statistically significant enhancement in step-through latency ($p < 0.05$), indicating improved memory retention. A dose-dependent recovery of cognitive performance was observed after the treatment of MKE. This result indicated that MKE showed a significant protective effect against Sodium arsenite-induced cognitive dysfunction and memory enhancement.

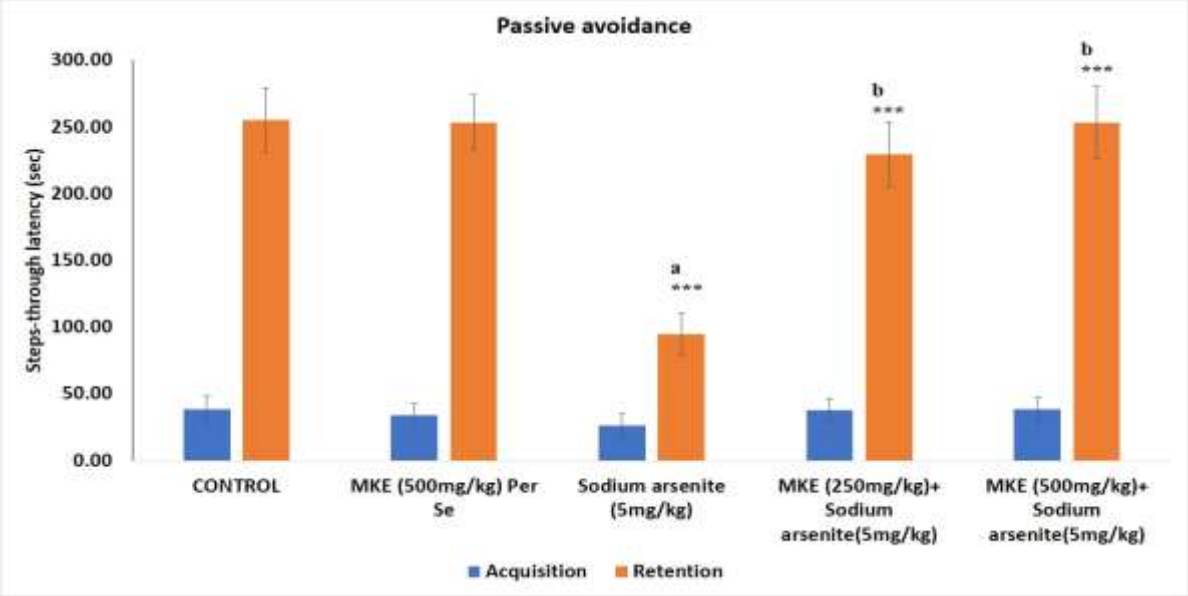

Figure 3: Effect of MKE on learning and memory in Sodium arsenite-induced experimental animals using the Passive Avoidance test.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. MKE (Murraya koenigii Extract).

3.4. Open Field Test (OFT)

When compared to the control group, the Sodium arsenite-treated group showed no significant differences in locomotor and exploratory activity, as evidenced by line crossings, rearing, and grooming behaviour ($F(4,25) = 0.9707$, $^*p > 0.05$). Similarly, treatment with MKE (Murraya koenigii Extract) did not produce any significant alteration in behavioural parameters when compared with the Sodium arsenite group. These findings suggest that neither Sodium arsenite nor MKE treatment significantly affected general locomotor activity.

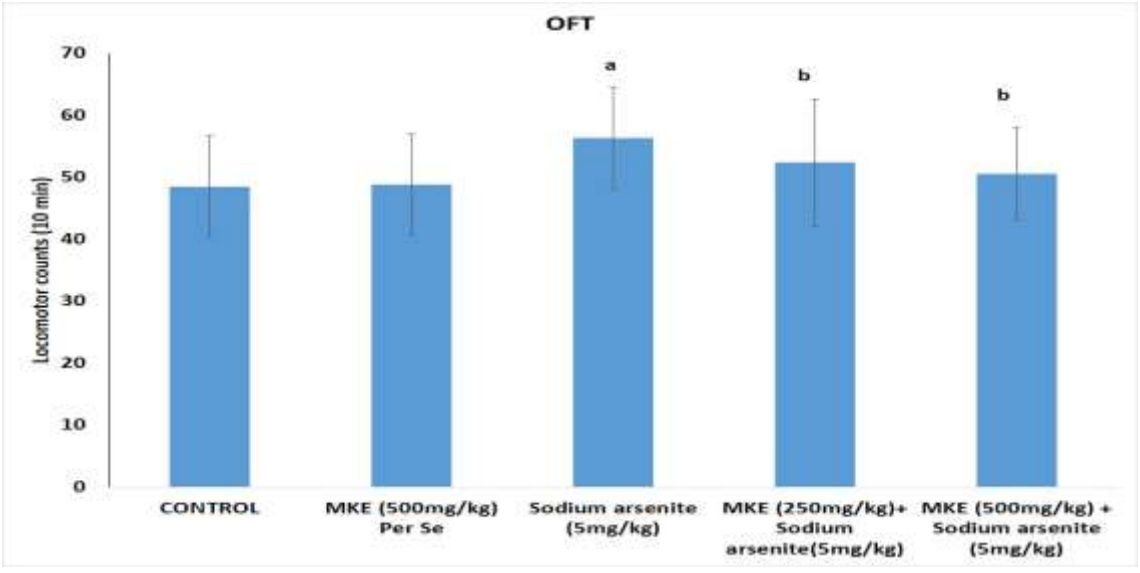

Figure 4: Effect of MKE on locomotor and exploratory behaviour in Sodium arsenite-induced experimental animals using the Open Field Test.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p > 0.05$ was considered statistically non-significant. MKE (Murraya koenigii Extract).

3.5. Estimation of Reduced Glutathione (GSH)

When compared to the control group, the level of GSH was significantly decreased in the Sodium arsenite-treated group $F(4,25) = 10.36^{*}p<0.05$. When compared with the Sodium arsenite group, both treatment groups significantly increased the level of GSH ($p<0.05$). And a dose- dependent improvement in GSH levels was observed in the following treatments with MKE (Murraya koenigii Extract)

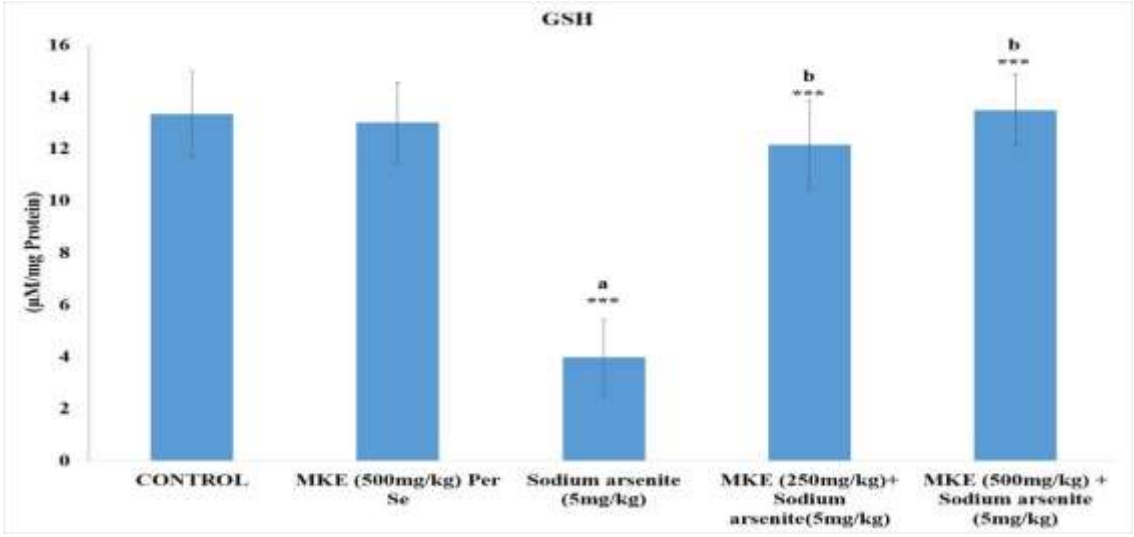

Figure 5: Effect of MKE on reduced glutathione (GSH) levels in Sodium arsenite-induced experimental animals.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. GSH: Reduced glutathione; MKE (*Murraya koenigii* Extract).

3.6. Estimation of Malondialdehyde (MDA)

When compared to the control group, the level of MDA was significantly increased in the Sodium arsenite-treated group $F(4,25) = 16.83^{*}p<0.05$. When compared with the Sodium arsenite group, both treatment groups significantly reduced the level of MDA ($p<0.05$). A dose-dependent reduction in MDA levels was observed in the following treatments with MKE (Murraya koenigii Extract).

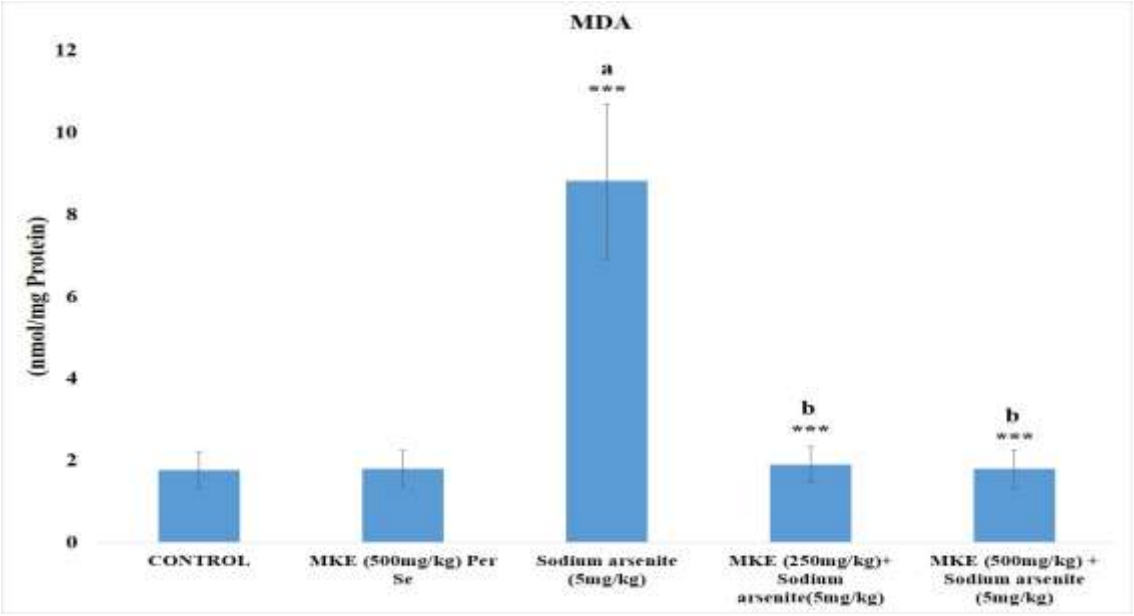

Figure 6: Effect of MKE on malondialdehyde (MDA) levels in Sodium arsenite-induced experimental animals.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. MDA: Malondialdehyde; MKE (Murraya koenigii Extract).

3.7. Estimation of Nitric Oxide (NO)

When compared to the control group, the level of nitric oxide (NO) was significantly increased in the Sodium arsenite-treated group F (4,25) =23.62*p<0.05. When compared with the Sodium arsenite group, both treatment groups significantly reduced the level of NO (p<0.05). And a dose- dependent reduction in NO levels was observed in the following treatments with MKE (*Murraya koenigii* Extract).

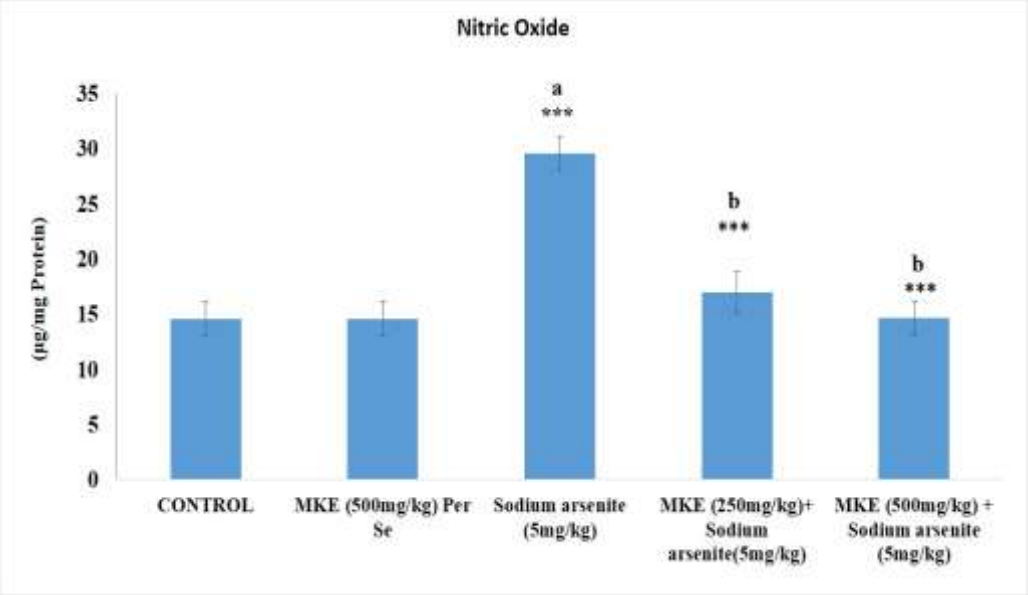

Figure 7: Effect of MKE on nitric oxide (NO) levels in Sodium arsenite-induced experimental animals.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. p < 0.05 was considered statistically significant. NO: Nitric oxide; MKE (*Murraya koenigii* Extract).

3.8. Estimation of Catalase (CAT)

When compared to the control group, the level of catalase (CAT) was significantly decreased in the Sodium arsenite-treated group F (4,25) = 14.18, *p<0.05. When compared with the Sodium arsenite group, both treatment groups significantly increased the level of catalase (CAT) (p<0.05). A dose-dependent improvement in CAT levels was observed in the following treatments with MKE (*Murraya koenigii* Extract)

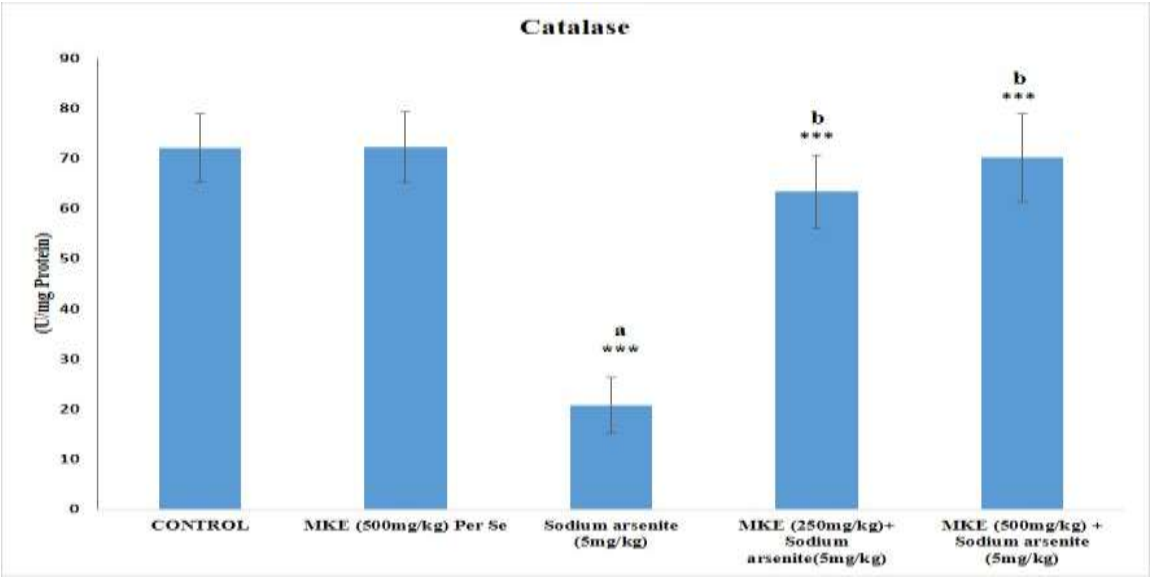

Figure 8: Effect of MKE on catalase (CAT) levels in Sodium arsenite-induced experimental animals.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. p < 0.05 was considered statistically significant. CAT: Catalase; MKE (Murraya koenigii Extract).

3.9. Estimation of Superoxide Dismutase (SOD)

When compared to the control group, the level of superoxide dismutase (SOD) was significantly decreased in the Sodium arsenite-treated group F (4,25) =10.77, * p<0.05. When compared with the Sodium arsenite group, both treatment groups

significantly increased the level of SOD ($p<0.05$). A dose-dependent improvement in SOD levels was observed in the following treatments with MKE (*Murraya koenigii* Extract)

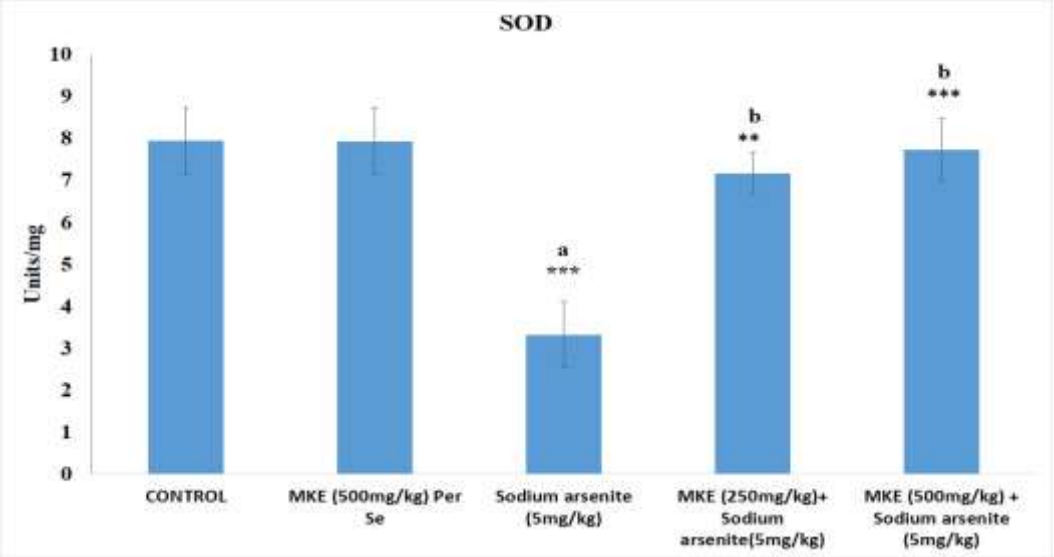

Figure 9: Effect of MK on superoxide dismutase (SOD) levels in Sodium arsenite-induced experimental animals.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. SOD: Superoxide dismutase; MKE (*Murraya koenigii* Extract).

3.10. Estimation of Acetylcholinesterase (AChE)

When compared to the control group, the level of acetylcholinesterase (AChE) was significantly increased in the Sodium arsenite-treated group $F(4,25) = 5.224$, $^*p<0.05$. When compared with the Sodium arsenite group, both treatment groups significantly reduced the level of AChE ($p<0.05$). A dose-dependent reduction in AChE levels was observed with the following treatments using MKE (*Murraya koenigii* Extract).

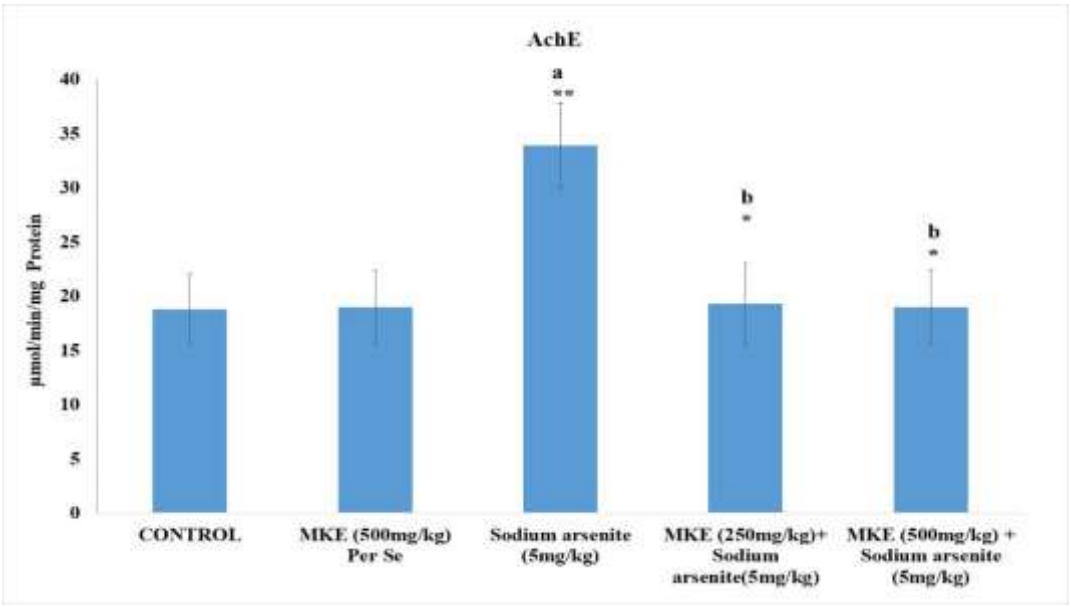

Figure 10: Effect of MK on acetylcholinesterase (AChE) levels in Sodium arsenite-induced experimental animals.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. AChE: Acetylcholinesterase; MKE (*Murraya koenigii* Extract).

3.11. Estimation of Tumor Necrosis Factor-Alpha (TNF-α)

When compared to the control group, the level of TNF-α was significantly increased in the Sodium arsenite-treated group $F(4,25) = 15.32$, $^*p<0.05$. When compared with the Sodium arsenite group, both treatment groups significantly reduced the level of TNF-α ($p<0.05$). A dose-dependent reduction in TNF-α levels was observed in the following treatments with MKE (*Murraya koenigii* Extract).

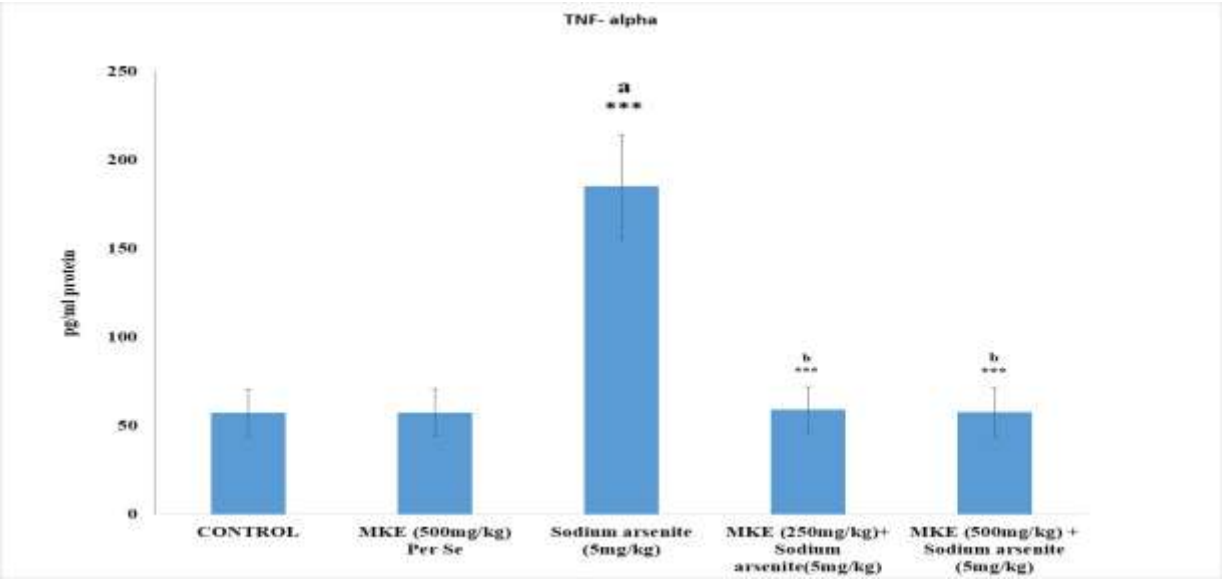

Figure 11: Effect of MKE on tumor necrosis factor-alpha (TNF-α) levels in Sodium arsenite-induced experimental animals.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. TNF-α: Tumor necrosis factor-alpha; MKE (*Murraya koenigii* Extract).

3.12. Estimation of Interleukin-1 Alpha (IL-1α)

When compared to the control group, the level of IL-1α was significantly increased in the Sodium arsenite-treated group $F(4,25) = 71.54$, $^*p<0.05$. When compared with the Sodium arsenite group, both treatment groups significantly reduced the level of IL-1α ($p<0.05$). A dose-dependent reduction in IL-1α levels was observed in the following treatments with MKE (*Murraya koenigii* Extract).

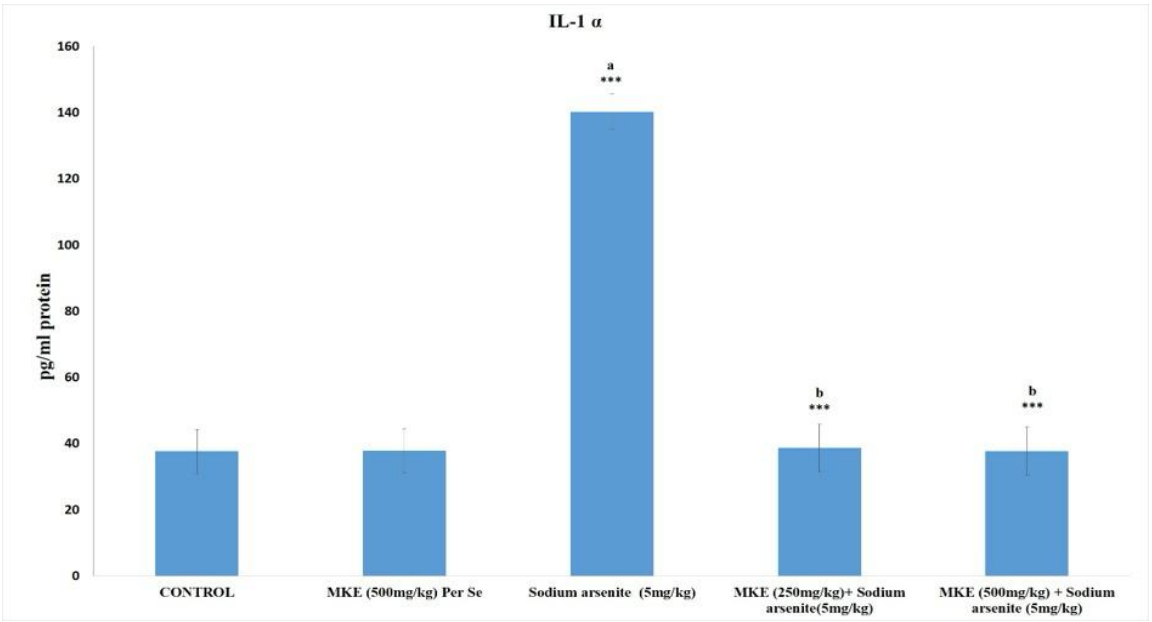

Figure 12: Effect of MKE on interleukin-1 alpha (IL-1α) levels in Sodium arsenite-induced experimental animals.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. IL-1α: Interleukin-1 alpha; MKE (*Murraya koenigii* Extract).

3.13. Estimation of Interleukin-10 (IL-10)

When compared to the control group, the level of IL-10 was significantly decreased in the Sodium arsenite-treated group $F(4,25) = 7.452$, $^*p<0.05$. When compared with the Sodium arsenite group, both treatment groups significantly increased the level of IL-10 ($p<0.05$). And a dose- dependent improvement in IL-10 levels was observed in the following treatments with MKE (*Murraya koenigii* Extract)

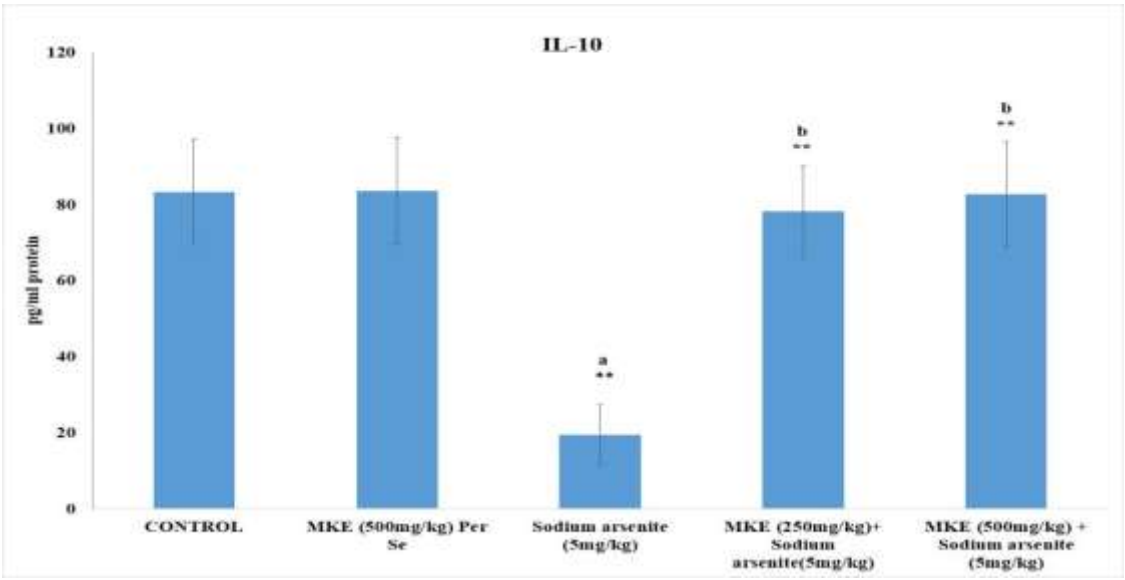

Figure 13: Effect of MKE on interleukin-10 (IL-10) levels in Sodium arsenite-induced experimental animals.

Results are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. IL-10: Interleukin-10; MKE (*Murraya koenigii* Extract).

3.14. Histopathological Observations of Brain Tissue

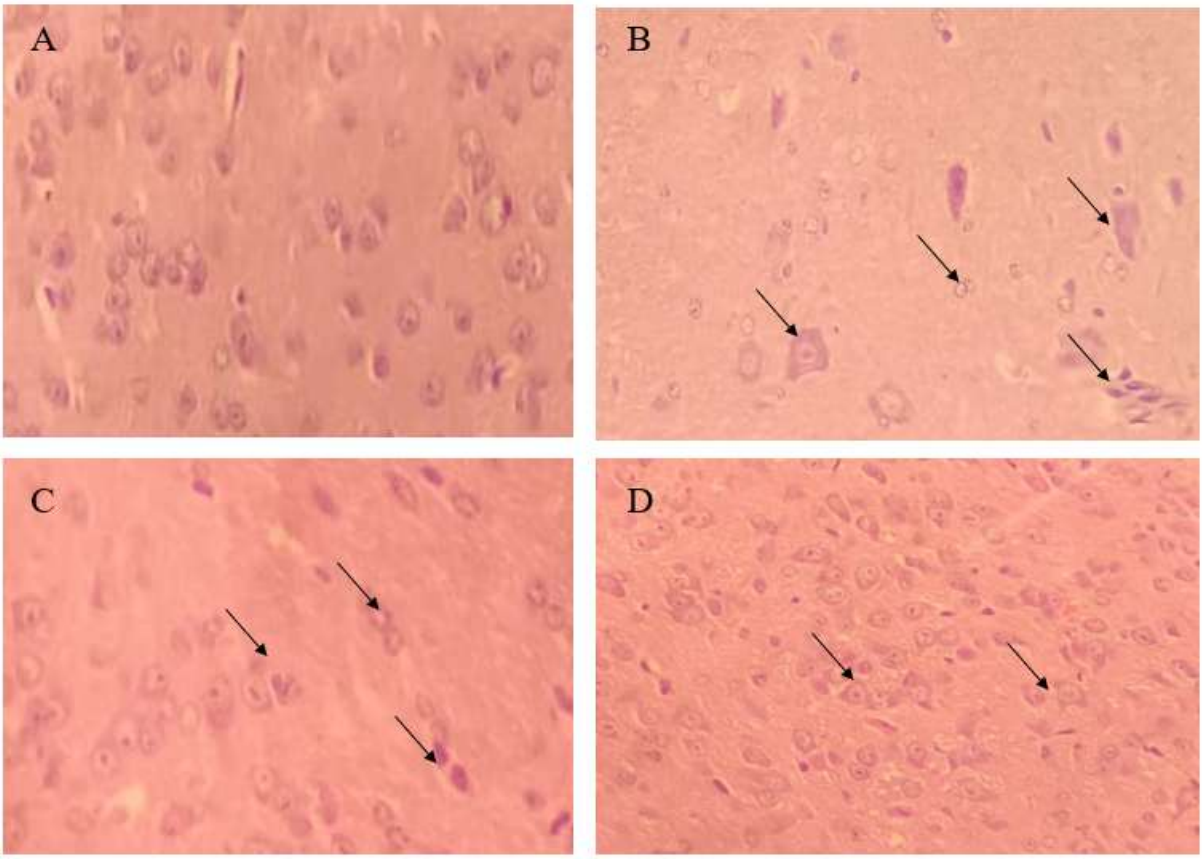

Figure 14: Illustrative pictures of the H&E-stained (Magnification x 400) in the Cortex area.

Group A: Normal Control

The hippocampus of normal control group showed normal cytoarchitecture, arranged in an orderly pattern for pyramidal cells. The neurons did not show damaged cell membrane; nuclei were well defined, and cytoplasmic architecture seemed normal. No sign of neuronal degenerations, vacuolizations, edema or any inflammatory cells was detected in any group.

Group B: Sodium Arsenite (5 mg/kg)
In the group administered sodium arsenite there were noticeable changes in the histological features of the hippocampus. Severe neuronal degeneration was found, presenting as shrunk pyrimidal neurons with picnotic nuclei and eosinophilic cytoplasm, marked vacuolization and disorganization of the neuronal layers. Substantial perivascular and pericellular edema with gliosis and inflammatory infiltrate cells were noticeable. Arsenic causes widespread neuron degeneration and loss. Was found.

Group C: *Murraya koenigii* (250 mg/kg) + Sodium Arsenite
Treatment with 250 mg/kg of *Murraya koenigii* partially alleviated the histopathological alterations caused by sodium arsenite, and, in the hippocampal tissue, the general structure was partially recovered with lesser neuronal degeneration and vacuolization; the edematous, the infiltrative inflammation remained, but the quantity of the healthy neurons increased in relation to that observed in the group exposed to sodium arsenite.

Group D: *Murraya koenigii* (500 mg/kg) + Sodium Arsenite
High-dose *Murraya koenigii*-treated groups showed preserved architectural integrity of hippocampus with normal appearing neuronal cells, nuclear and cytoplasm with significant decrease in neuronal degenerative changes, edema, vacuolation, gliosis, and inflammatory infiltration in compared with sodium arsenite treated groups. Neuronal organisation of brain tissues in high dose Murraya koenigii-treated animals were comparable with the control animals, suggesting its neuroprotective role against sodium arsenite mediated toxicity.

4. DISCUSSION
Our study found chronic exposure to sodium arsenite caused significant learning and memory deficits, oxidative stress, neuroinflammation, and neuronal loss in the mice. Behavioural tests including the Morris Water Maze test, Passive Avoidance test, and Open Field test indicated severe impairment of learning and memory capacity of the treated mice. These findings support previous studies that demonstrate arsenic accumulates in the brain tissue and affects the blood-brain barrier, overproduces reactive oxygen species (ROS), impairs cholinergic neurotransmission, and promotes neurodegeneration. **(25,26)**
Biochemical analysis results showed that sodium arsenite led to the obvious reduction in endogenous antioxidant enzymes such as GSH, SOD, and catalase, as well as an increase in AChE activity. Impaired antioxidant defense system implies serious oxidative stress leading to lipid peroxidation, impaired mitochondrial function, and neuronal apoptosis. Increased AChE activity further exacerbates the impairments in cholinergic neurotransmission, leading to memory deficit. **(27,28)**
Anti-inflammatory IL-10 decreased in sodium arsenite–exposed group while inflammatory cytokines TNF-alpha and IL-1 alpha remarkably increased, suggesting that neuroinflammation was induced in the sodium arsenite-exposed group. Chronic neuroinflammation further promoted neuronal damage and cognitive impairment via activation of microglial cells and induction of more pro-inflammatory mediators. **(29,30)**
Administration of Murraya koenigii attenuated behavioural dysfunction, normalized the antioxidant enzymes, abolished AChE and pro-inflammatory cytokines, and amplified IL-10. Carbazole alkaloids, flavonoids, and phenolic constituents of *Murraya koenigii* that possess remarkable antioxidant and anti-inflammatory characteristics could underlie these neuroprotective effects. **(31,32)**
Histopathological examination also showed that sodium arsenite administration caused damage in the hippocampus of animals, with cytoplasmic vacuolization, neuronal degeneration, pyknotic nuclei, and inflammatory cell infiltration, whereas treatment with Murraya koenigii at both doses preserved neuronal structure significantly and ameliorated degenerative effects compared to other groups. **(33,34)**

5. CONCLUSION
The findings of the present study indicate that *Murraya koenigii* holds the potential to exert a favourable neuroprotection on cognitive impairments caused by exposure to sodium arsenite, and the main mechanism seems to be the resultant antioxidant, anti-inflammatory, and cholinergic modulating effects. Thus, this herb may serve as a natural therapeutic to prevent and treat some neurodegenerative diseases like AD. However, future investigations are needed to elucidate the mechanism of the neuroprotective action and characterize the responsible phytoconstituents before any clinical application.

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