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PHARMACOLOGICAL EVALUATION OF STANDARDIZED EXTRACT OF *BACOPA MONNIERI* ON SODIUM ARSENITE-INDUCED COGNITIVE IMPAIRMENT IN EXPERIMENTAL RATS

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Abstract:

Background: *Chronic exposure to arsenic is associated with neurological dysfunction, cognitive impairment, oxidative stress, and alterations in neurotransmitter homeostasis. The present study was designed to investigate the neuroprotective potential of a standardized extract of* Bacopa monnieri *against sodium arsenite-induced cognitive and behavioral impairment in experimental rats.*

Methods: *Sprague–Dawley rats were randomly allocated into six groups (n = 6). Sodium arsenite (50 mg/kg, i.p.) was administered twice weekly for 7 weeks to induce neurotoxicity. Animals received standardized* B. monnieri *extract at doses of 20, 40, or 80 mg/kg orally for 7 weeks, while tocotrienols (150 mg/kg) served as the standard treatment. Cognitive and behavioral changes were assessed using the Morris water maze, rotarod, hot plate, elevated plus maze, and forced swimming tests. Following completion of the study, brain tissue was collected for estimation of oxidative stress and antioxidant parameters, including malondialdehyde, superoxide dismutase, catalase, reduced glutathione, and nitric oxide. Brain neurotransmitter levels and histopathological changes were also evaluated.*

Results: *Sodium arsenite exposure produced marked behavioral and neurochemical disturbances, including impaired spatial memory, reduced motor coordination, increased anxiety- and depressive-like responses, enhanced lipid peroxidation, and depletion of endogenous antioxidant defenses. Alterations in brain neurotransmitter levels were accompanied by pronounced histopathological changes. Treatment with standardized* B. monnieri *extract attenuated these arsenite-induced alterations in a dose-related manner, with the 80 mg/kg dose producing the most consistent improvement across behavioral, biochemical, neurotransmitter, and histological parameters. The high-dose extract improved spatial memory and motor performance, reduced behavioral abnormalities, enhanced antioxidant status, decreased oxidative damage, and improved the altered neurotransmitter profile.*

Conclusion: *The findings indicate that standardized* Bacopa monnieri *extract exerts a protective effect against sodium arsenite-induced neurobehavioral and biochemical alterations in rats. The observed activity appears to be associated with attenuation of oxidative and nitrosative stress, restoration of antioxidant defenses and neurotransmitter homeostasis, and preservation of brain tissue architecture. These findings support further investigation of standardized* B. monnieri *as a potential neuroprotective agent in arsenic-associated neurological dysfunction.*

Keywords: Bacopa monnieri*; sodium arsenite; cognitive impairment; neuroprotection; oxidative stress; antioxidant defense; neurotransmitters; Morris water maze; lipid peroxidation; neurotoxicity.*

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INTRODCUTION:

Arsenic is a naturally occurring metalloid and an important environmental toxicant to which humans may be exposed through contaminated drinking water, food, soil, and occupational sources. Chronic exposure to arsenic has been associated with adverse effects involving multiple organ systems, including the nervous, cardiovascular, gastrointestinal, hepatic, renal, and respiratory systems. The nervous system is particularly vulnerable to persistent arsenic exposure because arsenic can reach the brain and interfere with several cellular processes involved in neuronal survival and function. Increasing experimental evidence indicates that arsenic exposure is associated with cognitive and behavioral abnormalities, with oxidative stress, neuroinflammation, mitochondrial dysfunction, altered cellular signaling, and neurotransmitter disturbances contributing to the resulting neurological impairment.

Cognitive impairment represents an important consequence of chronic neurotoxic exposure and may manifest as deficits in learning, memory, attention, and behavioral adaptation. Experimental studies investigating arsenic-associated neurotoxicity have demonstrated that arsenic can adversely affect brain function through multiple interacting mechanisms rather than through a single toxicological pathway. Oxidative stress is considered an important component of this process, as excessive generation of reactive oxygen species can overwhelm endogenous antioxidant defenses and promote lipid, protein, and nucleic-acid damage. In parallel, arsenic-associated inflammatory signaling, mitochondrial dysfunction, and disruption of neuronal homeostasis may further compromise neuronal function and contribute to cognitive and behavioral abnormalities.

The brain is particularly susceptible to oxidative injury because of its high oxygen consumption, abundant lipid content, and relatively limited capacity to withstand sustained oxidative challenges. Excessive reactive oxygen species can alter neuronal membranes, impair mitochondrial function, and affect intracellular signaling pathways required for synaptic transmission and plasticity. Depletion of endogenous antioxidant defenses such as superoxide dismutase, catalase, and reduced glutathione can further intensify oxidative damage. Lipid peroxidation, commonly assessed through malondialdehyde formation, provides an important biochemical indicator of oxidative injury to neuronal membranes. Consequently, assessment of antioxidant enzymes, reduced glutathione, lipid peroxidation, and nitric oxide-related changes can provide useful information regarding the oxidative and nitrosative component of arsenic-induced neurotoxicity.

Arsenic exposure may also interfere with neurotransmitter systems involved in cognitive and emotional regulation. Alterations in dopaminergic, serotonergic, noradrenergic, and GABAergic signaling can influence learning, memory, motor activity, anxiety-related behavior, and affective responses. Such neurochemical changes may therefore contribute to the behavioral abnormalities observed following chronic arsenic exposure. The involvement of several interconnected pathways provides a rationale for investigating interventions capable of simultaneously influencing oxidative balance, neurotransmitter homeostasis, and neuronal integrity rather than targeting a single pathway.

There is increasing interest in naturally derived compounds with antioxidant and neuroprotective properties as potential approaches for mitigating chemically induced neuronal injury. *Bacopa monnieri* (L.) Wettst., commonly known as Brahmi, is a traditional medicinal plant that has long been associated with memory and cognitive health. Its pharmacological activity has been attributed primarily to a group of triterpenoid saponins collectively referred to as bacosides, together with other phytoconstituents including flavonoids and related bioactive compounds. Contemporary pharmacological research has investigated *B. monnieri* for its potential effects on

cognition, oxidative stress, neuronal signaling, inflammation, and neuroprotection.

Preclinical evidence suggests that *B. monnieri* may exert neuroprotective effects through multiple complementary mechanisms. These include attenuation of oxidative stress, modulation of antioxidant defenses, protection against cellular damage, and effects on neuronal signaling and synaptic function. A systematic review published in 2024 identified evidence supporting antioxidant, anti-inflammatory, anti-apoptotic, and neuroprotective actions of *B. monnieri* across experimental and clinical studies. The review also reported that research has investigated its potential effects on cognitive function and several neurological and behavioral outcomes. Other mechanistic reviews have similarly described the involvement of oxidative and cellular signaling pathways in the cognitive and neuroprotective effects associated with *B. monnieri* and its bioactive constituents.

The potential cognitive effects of *B. monnieri* have also been investigated clinically. Earlier systematic reviews of randomized controlled trials reported evidence of improvement in selected memory outcomes following administration of *B. monnieri* extracts. However, the clinical evidence is not completely consistent. A 2022 systematic review evaluating *B. monnieri* in Alzheimer disease reported very low-certainty evidence because of methodological limitations, small sample sizes, and heterogeneity among the available studies. More recent controlled research has likewise reported improvements in selected cognitive domains rather than consistent effects across all cognitive outcomes. These findings highlight the importance of evaluating standardized preparations under defined experimental conditions and of investigating specific pathological models in which oxidative and neurochemical disturbances are prominent.

Although the neuroprotective properties of *B. monnieri* have been investigated in several experimental models, comparatively less attention has been directed toward its protective effects against sodium arsenite-induced cognitive and behavioral dysfunction. Arsenic-associated neurological injury involves a complex interaction among oxidative stress, inflammation, mitochondrial dysfunction, neurotransmitter disturbance, and neuronal structural damage. Therefore, an experimental model combining behavioral assessment with biochemical, neurochemical, and histopathological evaluation can provide a broader assessment of potential neuroprotection.
The present study was therefore undertaken to pharmacologically evaluate a standardized extract of *Bacopa monnieri* against sodium arsenite-induced cognitive impairment in experimental rats. The study examined behavioral and functional changes using the Morris water maze, rotarod, hot plate, elevated plus maze, and forced swimming tests. In addition, brain oxidative and nitrosative status was evaluated through estimation of malondialdehyde, superoxide dismutase, catalase, reduced glutathione, and nitric oxide, while selected neurotransmitters and brain histopathology were investigated to further characterize the neuroprotective response. Different doses of standardized *B. monnieri* extract were compared with tocotrienols as a reference treatment to determine whether the extract could attenuate the multidimensional neurotoxic effects associated with repeated sodium arsenite exposure.

MATERIALS AND METHODS:

MATERIALS:

Standardized extract of *Bacopa monnieri* was procured from Natural Remedies Pvt. Ltd., Bengaluru, India. The chemicals and biochemical reagents used for the preparation of experimental solutions and biochemical estimations, including sodium citrate dihydrate, adenosine triphosphate, potassium hydroxide, sodium hydroxide, sulphuric acid, ammonium molybdate, Folin–Ciocalteu reagent, trichloroacetic acid, chloroform, boric acid, epinephrine, Tris-HCl, polysorbate 80, hydrogen peroxide, bovine serum albumin, Bradford reagent, ethanol, NADPH, sodium arsenite, sulphanilamide, phosphoric acid, magnesium sulphate, potassium chloride, EDTA, γ-aminobutyric acid, sodium chloride, Griess reagent, malondialdehyde bis-dimethyl acetal, Triton X-100, potassium and sodium phosphate salts, potassium dichromate, 2-thiobarbituric acid, aminoguanidine bicarbonate, dopamine hydrochloride, copper sulphate, 3,4-dihydroxyphenylacetic acid, acetylcholine iodide and 1,1-diphenyl-2-picrylhydrazyl (DPPH), were obtained from reputed commercial suppliers. Tocotrienol capsules (Evion) were procured from Procter & Gamble Health Ltd., Goa, India. All chemicals and reagents were of analytical or appropriate experimental grade and were used as received.

Animals:

Sprague Dawley rats weighing 180-200 gm were purchased from Global Bioresearch Solutions Private Limited, H No 251 Nhavi, Tal-Bhor, Dist-Pune. The animals were housed in polypropylene cages and maintained under the environmental condition of temperature 25±1 ºC and relative humidity of 45-55 % under a 12h light: 12 dark cycles. The animals had free access to food pellets (Nav Maharashtra Chakan oil mills Ltd., Pune) and

water ad libitum. The Institutional Animal Ethics Committee (IAEC) of 2168/PO/Re/S/22/CPCSEA approved all the experimental protocols under the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The protocol approval number is 2168/PO/Re/S/22/CPCSEA.

METHODS:

Preparation and Administration of Test and Standard Drugs

The standardized extract of *Bacopa monnieri* was freshly dispersed in distilled water before administration. The required quantity of extract was calculated according to the individual body weight of the experimental animals. Fresh preparations were made daily, maintained in airtight amber-colored containers, and kept at room temperature until administration. The extract was administered orally (p.o.) throughout the experimental period.

Tocotrienols were used as the standard treatment and were prepared using 1% sodium carboxymethylcellulose as the vehicle. The tocotrienol soft gelatin capsules were stored below 25 °C, and a fresh suspension was prepared on each day of treatment. The required dose was adjusted according to the body weight of each animal and administered orally.

Experimental Design and Induction of Cognitive Impairment

The experimental animals were randomly allocated into six groups, with six rats in each group. Group I served as the normal group and received distilled water (10 mg/kg, p.o.) throughout the 7-week experimental period. Group II served as the vehicle control and received sodium arsenite (50 mg/kg, i.p.) twice weekly for 7 weeks along with distilled water (10 mg/kg). Group III received sodium arsenite (50 mg/kg, i.p.) twice weekly together with tocotrienols at a dose of 150 mg/kg for 7 weeks and served as the standard-treated group. Groups IV, V, and VI received sodium arsenite (50 mg/kg, i.p.) twice weekly along with standardized *Bacopa monnieri* extract at doses of 20, 40, and 80 mg/kg, respectively, administered orally for 7 weeks. All treatment doses were adjusted according to the body weight of individual animals.

Assessment of Pharmacological Effects

The effects of standardized *Bacopa monnieri* extract on sodium arsenite-induced cognitive impairment were assessed using a series of behavioral and functional parameters, including changes in body weight, spatial learning and memory, motor coordination, nociceptive response, anxiety-like behavior, and depression-like behavior.

Body Weight

The body weight of each experimental animal was recorded daily using a calibrated animal weighing balance. Body-weight measurements were used for dose adjustment and to monitor changes in general physiological status throughout the experimental period.

Morris Water Maze Test

Spatial learning and memory were evaluated using the Morris water maze (MWM). A circular pool measuring 120 cm in diameter and 45 cm in height was filled with water to a depth of approximately 37.5 cm and maintained at 21 °C. The water was rendered opaque using black ink, and the pool was divided into four equal quadrants designated north, south, east, and west. A transparent platform measuring 10 cm in diameter was positioned in the east quadrant, approximately 40 cm from the pool wall, with its upper surface maintained 2 cm below the water level.

Each rat was introduced into the pool from one of four predetermined starting locations distributed around the circumference. The starting position was varied among trials. A computerized tracking system was used to monitor the swimming behavior and quantify the distance traveled within the target quadrant. Animals that did not locate the submerged platform within 90 s were manually guided to it. The time spent in the target quadrant and swimming distance during the 90-s observation period were recorded as measures of spatial learning and memory performance.

Motor Coordination Test

Motor coordination and balance were evaluated using a rotarod apparatus. Before the experimental assessment, animals were acclimatized to the rotating rod by training at a constant low speed for three consecutive days. During the test, each animal was placed individually on the rotating rod, which accelerated from 4 to 40 rpm over a 5-min period. The latency to fall from the rotating rod was recorded as an indicator of motor coordination and balance. Each animal underwent three trials, and the mean latency was considered for analysis.

Hot Plate Test

The hot plate test was performed to assess the nociceptive response of the experimental animals. The temperature of the heated plate was maintained at 55 ± 0.5 °C throughout the experiment. Each rat was individually placed on the heated surface, and the latency to the first characteristic nociceptive response, such as hind-paw licking or jumping, was recorded in seconds. A maximum response cut-off time of 30 s was used to minimize the possibility of tissue injury.

Elevated Plus Maze Test
An elevated plus maze consisting of two open arms and two enclosed arms elevated approximately 50 cm above the floor was used to assess anxiety-like behavior. Each rat was placed individually in the central area of the apparatus with its orientation toward an open arm and allowed to explore freely for 5 min. The number of entries into the open and closed arms and the time spent in each type of arm were recorded. The apparatus was thoroughly cleaned with 70% ethanol between animals to minimize the influence of residual olfactory cues.

Forced Swimming Test
Depressive-like behavioral changes were evaluated using the forced swimming test. Individual animals were placed in a transparent cylindrical container containing water maintained at 25 ± 1 °C and at a depth sufficient to prevent contact of the tail with the bottom. The total test duration was 6 min, and the behavioral observations obtained during the final 4 min were used for analysis. Immobility was defined as the absence of active escape-directed movements, apart from the movements required to maintain the head above the water surface. Following the test, animals were removed from the container, dried, and returned to a warm cage.

***Ex-vivo* Biochemical and Tissue Parameters**
At the end of the experimental period, the animals were sacrificed and the brains were rapidly isolated. The tissue was homogenized in 0.1 M Tris-HCl buffer (pH 7.4), and the homogenate was centrifuged to obtain the supernatant. The resulting fraction was used for estimation of lipid peroxidation, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), nitric oxide (NO), and total protein. The biochemical parameters were normalized to the corresponding protein content where applicable.

Lipid Peroxidation
Brain lipid peroxidation was assessed by estimating malondialdehyde (MDA) using the thiobarbituric acid reactive substances approach described by Slater and Sawyer (1971). Briefly, tissue supernatant was treated with 10% trichloroacetic acid and centrifuged to remove the precipitated material. The resulting supernatant was reacted with thiobarbituric acid and heated in a boiling water bath for 10 min. After cooling, the absorbance of the developed chromogen was measured at 532 nm against an appropriate reagent blank. MDA concentrations were determined using a standard curve prepared with known concentrations of malondialdehyde and expressed as nmol MDA/mg protein.

Superoxide Dismutase Activity
SOD activity was determined according to the method of Misra and Fridovich (1972), based on inhibition of epinephrine oxidation to adrenochrome. Brain homogenate was treated with chilled ethanol and chloroform and centrifuged to obtain the enzyme-containing supernatant. An aliquot of the supernatant was mixed with carbonate buffer and EDTA, and the reaction was initiated by addition of epinephrine. The change in absorbance was monitored at 480 nm. SOD activity was calculated from the degree of inhibition of epinephrine autoxidation and expressed as units/mg protein.

Reduced Glutathione
Reduced glutathione (GSH) content was estimated using the method reported by Moron et al. (1979). An aliquot of brain tissue supernatant was mixed with 20% trichloroacetic acid and centrifuged to remove precipitated proteins. The clear supernatant was reacted with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) in phosphate buffer. The intensity of the resulting yellow-colored product was measured spectrophotometrically at 412 nm. GSH content was calculated using a standard glutathione calibration curve and expressed as µg GSH/mg protein.

Catalase Activity
Catalase activity was determined in brain tissue homogenate prepared in cold phosphate buffer. The tissue extract was centrifuged to obtain the enzyme-containing supernatant, which was incubated with a reaction mixture containing hydrogen peroxide and phosphate buffer. The decomposition of hydrogen peroxide was monitored spectrophotometrically by measuring the reduction in absorbance over a defined time interval. Catalase activity was calculated using the appropriate molar extinction coefficient and expressed as units/mg protein.

Nitric Oxide Estimation
Nitric oxide production was estimated indirectly by determining nitrite concentration using the Griess reaction, following the method of Miranda et al. (2001). Tissue samples were processed for conversion of nitrate to nitrite, followed by reaction with sulfanilamide and N-(1-naphthyl)ethylenediamine to generate a colored azo compound. Absorbance was measured at 543 nm, and nitrite concentration was calculated from a sodium nitrate standard curve. The results were expressed as µg/mg protein.

Total Protein Estimation
Total protein concentration in the brain tissue samples was determined by the Lowry method using bovine serum albumin as the reference standard (Lowry et al., 1951). Appropriate aliquots of the tissue preparation were reacted with alkaline

copper reagent followed by Folin–Ciocalteu phenol reagent. After development of the characteristic color, absorbance was measured at 660 nm. Protein concentration was calculated from the BSA calibration curve and expressed as mg/g wet tissue.

Estimation of Brain Neurotransmitters
Brain neurotransmitter levels were determined using established biochemical procedures. Brain tissue was rapidly isolated, homogenized, and processed under chilled conditions to minimize degradation of the analytes.

Brain GABA Estimation
Gamma-aminobutyric acid (GABA) was estimated following the reported paper chromatographic and colorimetric procedure. Brain tissue was homogenized in 0.01 N hydrochloric acid and extracted with ice-cold absolute alcohol. Following centrifugation, the alcoholic supernatant was collected, concentrated, and subjected to solvent partitioning. The aqueous fraction was applied to Whatman No. 41 paper and chromatographically separated using n-butanol, acetic acid, and water as the mobile phase. Following development and drying, the GABA-containing region was treated with ninhydrin, and the developed color was measured at 570 nm. Quantification was performed using a standard GABA calibration curve prepared over the appropriate concentration range.

Brain Dopamine Estimation
Dopamine concentration in brain tissue was estimated using the fluorometric oxidation procedure described by Fleming et al. (1965). Brain homogenate was reacted with iodine under controlled conditions, followed by treatment with alkaline sulfite and acetic acid. The reaction mixture was heated in a boiling water bath and subsequently cooled. Dopamine-derived fluorescence was measured at an excitation wavelength of 378 nm and an emission wavelength of 335 nm. Dopamine concentration was calculated using an appropriate standard calibration procedure.

Brain Serotonin (5-HT) Estimation
Brain serotonin (5-hydroxytryptamine; 5-HT) was estimated using a fluorometric procedure based on o-phthalaldehyde derivatization. Brain homogenate was extracted using n-heptane and cysteine solution, followed by centrifugation and separation of the aqueous phase. The aqueous fraction was reacted with hydrochloric acid containing o-phthalaldehyde and incubated at 70 °C for 15 min. After cooling, fluorescence was measured at an excitation wavelength of 470 nm and an emission wavelength of 360 nm. Serotonin concentration was determined using an appropriate standard calibration curve.

Histopathological Examination
For histopathological assessment, representative brain tissue samples were immediately fixed in 10% formalin. The fixed specimens were processed through graded alcohol, cleared with xylene, and embedded in paraffin wax. Sections approximately 5 µm thick were prepared and stained with hematoxylin and eosin. The stained sections were examined under a light microscope (Nikon E200) at 100× magnification. Histological alterations, including inflammatory cell infiltration, vascular congestion, edema, and neuronal or tissue necrosis, were assessed qualitatively and compared among the experimental groups.

Statistical Analysis
All experimental data were analyzed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Results were compared among the experimental groups using the statistical test appropriate to the experimental design. Differences were considered statistically significant when $P < 0.05$.

RESULTS:

Effect of *Bacopa monnieri* on Body Weight
Sodium arsenite administration produced a significant reduction in body weight compared with the normal group (165.30 ± 19.63 vs. 224.20 ± 22.57 g; $P < 0.001$). Treatment with tocotrienols (150 mg/kg) significantly improved body weight (215.20 ± 9.58 g; $P < 0.001$ vs. vehicle control). *Bacopa monnieri* produced a dose-related improvement, with significant increases observed at 40 mg/kg (195.50 ± 10.77 g; $P < 0.01$) and 80 mg/kg (211.70 ± 10.78 g; $P < 0.001$). The 20 mg/kg dose (175.50 ± 10.97 g) did not significantly differ from the vehicle control group.

Effect on Absolute and Relative Brain Weight
The sodium arsenite-treated group exhibited a significant reduction in absolute brain weight compared with the normal group (1.13 ± 0.01 vs. 1.34 ± 0.03 g; $P < 0.001$), accompanied by an increase in the brain-to-body weight ratio (7.26 ± 0.76 vs. 6.35 ± 0.79 × 10^{-3}; $P < 0.001$). Tocotrienols significantly restored both parameters. *B. monnieri* at 40 and 80 mg/kg significantly increased absolute brain weight to 1.29 ± 0.03 and 1.31 ± 0.02 g, respectively ($P < 0.001$), while also significantly modifying the relative brain weight. The 20 mg/kg dose did not significantly improve absolute brain weight.

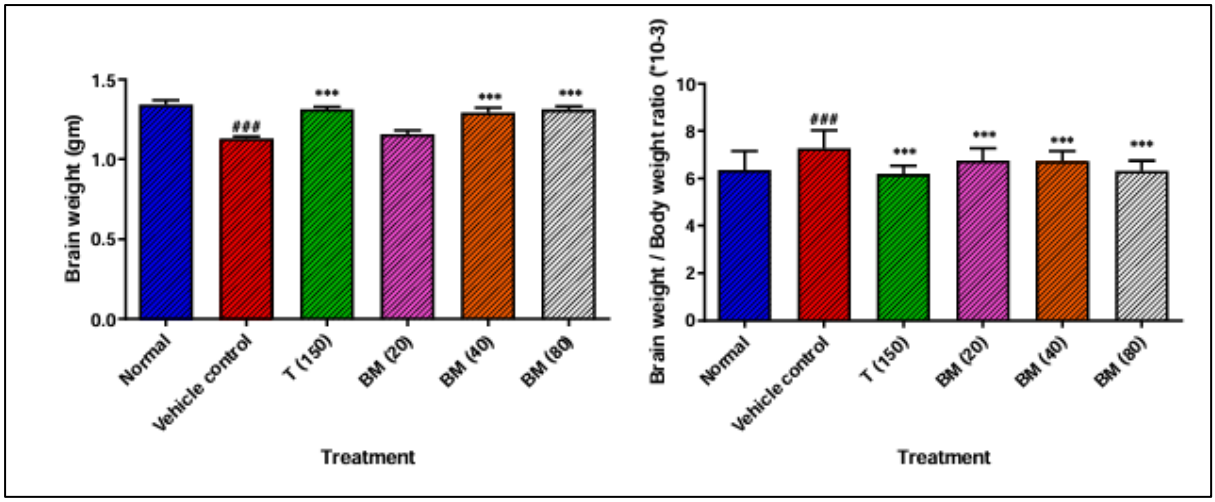

Figure 1: Effect of *Bacopa monnieri* on arsenic-induced alteration in absolute and relative brain weights

*Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group*

Effect on Morris Water Maze Performance

Sodium arsenite exposure significantly reduced the total swimming distance compared with the normal group (4721.00 ± 503.60 vs. 5179.00 ± 450.30 cm; $P < 0.001$). Tocotrienols significantly improved this parameter (5022.00 ± 319.50 cm; $P < 0.001$). Treatment with *B. monnieri* at 40 and 80 mg/kg increased the total distance travelled to 4969.00 ± 601.00 and 5125.00 ± 702.00 cm, respectively ($P < 0.001$), whereas the 20 mg/kg dose did not produce a significant change.

Effect on Target-Quadrant Performance

The vehicle control group showed marked impairment in spatial memory, with significant reductions in both time spent and distance travelled in the target quadrant compared with normal animals ($P < 0.001$). Target-quadrant time decreased from 22.00 ± 3.07 sec in the normal group to 11.17 ± 2.02 sec in the vehicle control group, while target-quadrant distance decreased from 1804.00 ± 303.80 to 891.30 ± 145.80 cm. Tocotrienols significantly improved both parameters ($P < 0.001$). *B. monnieri* at 40 mg/kg increased target-quadrant time and distance to 14.67 ± 2.50 sec and 1355.00 ± 235.80 cm, respectively ($P < 0.01$), whereas the 80 mg/kg dose produced further improvement to 17.83 ± 2.71 sec and 1614.00 ± 239.50 cm ($P < 0.001$). The 20 mg/kg dose did not significantly alter either parameter.

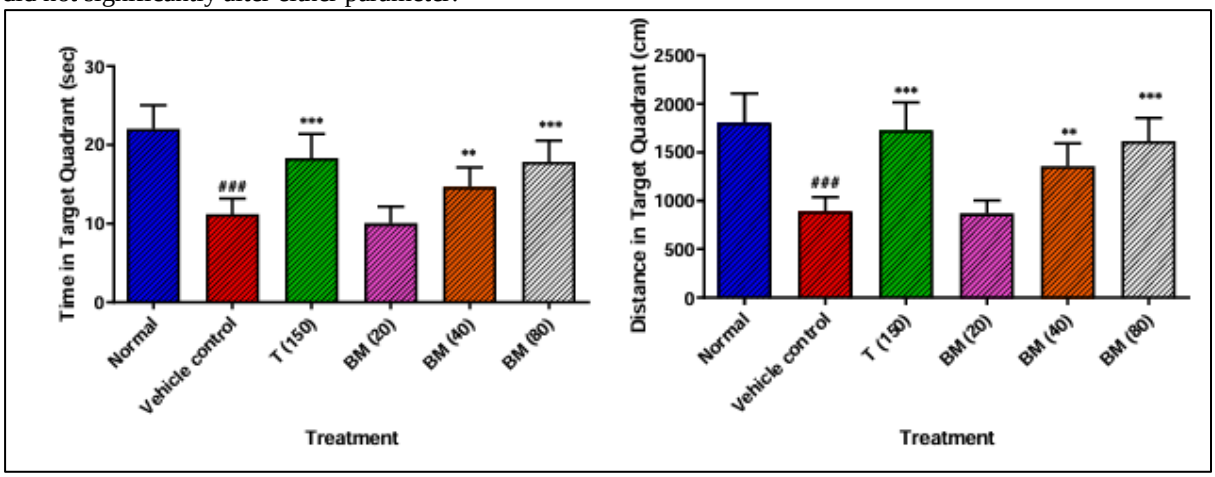

Figure 2: Effect of *Bacopa monnieri* on arsenic-induced alteration in time in target quadrant and distance in target quadrant in Morris water maze test

*Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.*

Effect on Motor Coordination

Sodium arsenite significantly reduced the time spent on the rotarod compared with the normal group (13.00 ± 1.39 vs. 28.83 ± 1.08 sec; $P < 0.001$). Tocotrienol treatment significantly improved rotarod performance to 27.00 ± 0.93 sec ($P < 0.001$). *B. monnieri* treatment resulted in dose-related improvement, with significant increases observed at 40 mg/kg (19.50 ± 0.76 sec; $P < 0.01$) and 80 mg/kg (24.50 ± 1.23 sec; $P < 0.001$). No significant improvement was observed with 20 mg/kg.

Effect on Hot-Plate Response

Sodium arsenite significantly increased hot-plate response latency compared with the normal group (52.83 ± 3.54 vs. 20.00 ± 0.86 sec; $P < 0.001$). Tocotrienols markedly reduced the latency to 20.67 ± 1.20 sec ($P < 0.001$). *B. monnieri* at 40 and 80 mg/kg significantly reduced the response time to 32.67 ± 2.11 and 22.83 ± 2.70 sec, respectively ($P < 0.01$ and $P < 0.001$). The 20 mg/kg dose did not produce a significant change.

Effect on Elevated Plus Maze Performance

Sodium arsenite exposure significantly decreased open-arm exploration. Open-arm entries and time spent in the open arms were reduced to 2.00 ± 0.37 entries and 43.17 ± 12.24 sec, respectively, compared with 9.50 ± 0.76 entries and 140.20 ± 11.26 sec in the normal group ($P < 0.001$). Tocotrienols significantly restored both parameters. *B. monnieri* at 40 mg/kg increased open-arm entries to 6.00 ± 0.26 and open-arm time to 82.67 ± 2.40 sec ($P < 0.01$), while 80 mg/kg produced greater improvement, with 8.17 ± 1.20 entries and 127.70 ± 14.34 sec ($P < 0.001$). The 20 mg/kg dose showed no significant effect.

Conversely, sodium arsenite increased closed-arm exploration, with closed-arm entries and time increasing to 10.00 ± 0.52 and 172.70 ± 5.18 sec, respectively ($P < 0.001$ vs. normal). Tocotrienols significantly reduced both measures. *B. monnieri* at 40 and 80 mg/kg also significantly reduced closed-arm entries and duration, whereas the 20 mg/kg dose was ineffective.

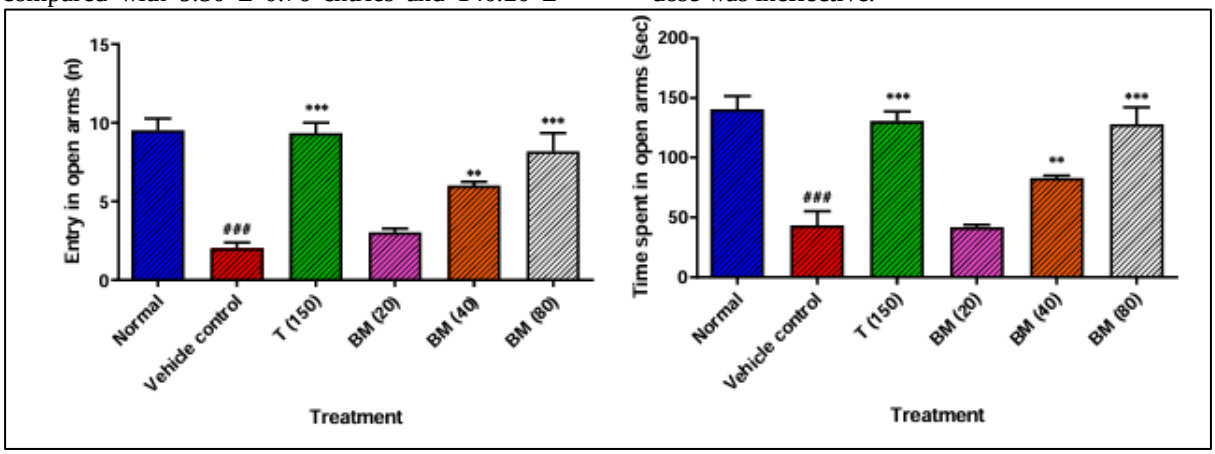

Figure 3: Effect of Bacopa monnieri on arsenic-induced alteration entry in open arms and time spent in open arms in elevated plus maze test

*Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.*

Effect on Forced Swimming Test

The sodium arsenite-treated group demonstrated a significant increase in immobility time compared with normal animals (205.00 ± 17.95 vs. 56.83 ± 20.51 sec; $P < 0.001$). Tocotrienols reduced immobility to 68.67 ± 21.52 sec ($P < 0.001$). *B. monnieri* at 40 and 80 mg/kg significantly decreased immobility to 117.50 ± 23.65 and 81.67 ± 16.41 sec, respectively ($P < 0.01$ and $P < 0.001$). The 20 mg/kg dose did not significantly modify immobility time.

Effect on Brain Norepinephrine Levels

Sodium arsenite significantly reduced brain norepinephrine concentration compared with the normal group (313.10 ± 66.93 vs. 474.80 ± 77.44 ng/g; $P < 0.001$). Tocotrienols significantly increased norepinephrine levels to 459.60 ± 81.58 ng/g ($P < 0.001$). Treatment with *B. monnieri* at 40 and 80 mg/kg increased norepinephrine concentrations to 398.30 ± 88.11 and 400.30 ± 85.04 ng/g, respectively, with significant differences compared with the vehicle control. The 20 mg/kg dose did not significantly alter norepinephrine levels.

Effect on Brain Serotonin and Dopamine Levels

Sodium arsenite exposure significantly decreased serotonin and dopamine concentrations. Serotonin decreased from 334.40 ± 16.26 ng/g in normal animals to 259.40 ± 31.07 ng/g in the vehicle control group, while dopamine decreased from 188.70 ± 29.09 to 73.43 ± 12.35 ng/g ($P < 0.001$). Tocotrienols significantly restored both neurotransmitters. *B. monnieri* at 40 mg/kg significantly increased serotonin and dopamine to 286.10 ± 25.12 and 118.50 ± 17.19 ng/g, respectively ($P < 0.01$). The 80 mg/kg dose produced a greater increase to 310.00 ± 22.20 ng/g serotonin and 160.90 ± 23.41 ng/g dopamine ($P < 0.001$). No significant effect was observed at 20 mg/kg.

Effect on Brain Total Protein

Brain total protein was significantly reduced following sodium arsenite exposure, declining from 2.38 ± 0.47 mg/g in the normal group to 0.46 ± 0.20 mg/g in the vehicle control group ($P < 0.001$). Tocotrienols significantly restored protein concentration to 2.63 ± 0.51 mg/g ($P < 0.001$). *B. monnieri* at 40 and 80 mg/kg increased protein

levels to 1.33 ± 0.49 and 1.88 ± 0.51 mg/g, respectively, with significant differences compared with the vehicle control. The 20 mg/kg dose did not produce a significant change.

Effect on Brain SOD and GSH

Sodium arsenite significantly depleted both SOD and GSH compared with the normal group. SOD activity decreased from 15.44 ± 1.12 to 4.02 ± 0.46 U/mg protein, while GSH declined from 35.17 ± 2.78 to 12.29 ± 1.79 µg/mg protein ($P < 0.001$). Tocotrienols significantly restored both antioxidant parameters. *B. monnieri* at 40 mg/kg increased SOD to 7.22 ± 0.62 U/mg protein and GSH to 19.89 ± 1.71 µg/mg protein ($P < 0.01$), whereas 80 mg/kg produced greater restoration to 9.54 ± 1.31 U/mg protein and 28.65 ± 2.57 µg/mg protein, respectively ($P < 0.001$). The 20 mg/kg dose did not significantly alter either parameter.

Effect on Brain Catalase Activity

Catalase activity was significantly reduced by sodium arsenite exposure, from 10.58 ± 1.45 U/mg protein in the normal group to 4.25 ± 0.74 U/mg protein in the vehicle control group ($P < 0.001$). Tocotrienols significantly restored catalase activity to 9.53 ± 1.16 U/mg protein ($P < 0.001$). *B. monnieri* at 40 and 80 mg/kg significantly increased catalase activity to 7.02 ± 0.66 and 8.01 ± 0.76 U/mg protein, respectively ($P < 0.01$ and $P < 0.001$). The 20 mg/kg dose did not significantly affect catalase activity.

Effect on Brain MDA and Nitric Oxide Levels

Sodium arsenite significantly increased MDA and nitric oxide levels compared with the normal group. MDA increased from 95.31 ± 12.77 to 197.20 ± 11.08 nM/mg protein, while nitric oxide increased from 0.96 ± 0.03 to 1.74 ± 0.09 µg/mL ($P < 0.001$). Tocotrienols significantly reduced both parameters to 106.20 ± 5.98 nM/mg protein and 1.03 ± 0.11 µg/mL, respectively ($P < 0.001$). Treatment with *B. monnieri* at 40 mg/kg reduced MDA to 159.70 ± 8.01 nM/mg protein and nitric oxide to 1.45 ± 0.12 µg/mL ($P < 0.01$). The 80 mg/kg dose produced a greater reduction, with MDA and nitric oxide levels of 126.60 ± 11.01 nM/mg protein and 1.24 ± 0.07 µg/mL, respectively ($P < 0.001$). The 20 mg/kg dose did not significantly modify either parameter.

Table 1: Summary of the effects of standardized *Bacopa monnieri* extract on sodium arsenite-induced behavioral, neurochemical and biochemical alterations in rats

Parameter	Normal	Sodium arsenite control	Tocotrienols 150 mg/kg	*B. monnieri* 20 mg/kg	*B. monnieri* 40 mg/kg	*B. monnieri* 80 mg/kg
Body weight (g)	224.20 ± 22.57	165.30 ± 19.63###	215.20 ± 9.58***	175.50 ± 10.97	195.50 ± 10.77**	211.70 ± 10.78***
Absolute brain weight (g)	1.34 ± 0.03	1.13 ± 0.01###	1.31 ± 0.02***	1.16 ± 0.02	1.29 ± 0.03***	1.31 ± 0.02***
Relative brain weight (×10⁻³)	6.35 ± 0.79	7.26 ± 0.76###	6.18 ± 0.35***	6.75 ± 0.52***	6.73 ± 0.43***	6.30 ± 0.44***
MWM total distance travelled (cm)	5179.00 ± 450.30	4721.00 ± 503.60###	5022.00 ± 319.50***	4463.00 ± 420.30	4969.00 ± 601.00***	5125.00 ± 702.00***
MWM time in target quadrant (sec)	22.00 ± 3.07	11.17 ± 2.02###	18.33 ± 3.07***	10.00 ± 2.19	14.67 ± 2.50**	17.83 ± 2.71***
MWM distance in target quadrant (cm)	1804.00 ± 303.80	891.30 ± 145.80###	1726.00 ± 288.90***	866.80 ± 138.60	1355.00 ± 235.80**	1614.00 ± 239.50***
Rotarod latency (sec)	28.83 ± 1.08	13.00 ± 1.39###	27.00 ± 0.93***	14.83 ± 2.41	19.50 ± 0.76**	24.50 ± 1.23***
Hot-plate latency (sec)	20.00 ± 0.86	52.83 ± 3.54###	20.67 ± 1.20***	49.83 ± 3.01	32.67 ± 2.11**	22.83 ± 2.70***
EPM open-arm entries (n)	9.50 ± 0.76	2.00 ± 0.37	9.33 ± 0.67***	3.00 ± 0.26	6.00 ± 0.26**	8.17 ± 1.20***
EPM open-arm time (sec)	140.20 ± 11.26	43.17 ± 12.24###	130.30 ± 8.25***	41.83 ± 2.24	82.67 ± 2.40**	127.70 ± 14.34***
EPM closed-arm entries (n)	5.67 ± 0.61	10.00 ± 0.52###	4.83 ± 0.31***	9.83 ± 0.60	7.50 ± 0.34**	5.00 ± 0.68***
EPM closed-arm time (sec)	86.00 ± 6.42	172.70 ± 5.18###	93.50 ± 4.35***	171.30 ± 5.35	147.80 ± 15.61**	116.50 ± 14.29***
Forced swimming immobility (sec)	56.83 ± 20.51	205.00 ± 17.95###	68.67 ± 21.52***	196.00 ± 15.02	117.50 ± 23.65**	81.67 ± 16.41***

Brain norepinephrine (ng/g)	474.80 ± 77.44	313.10 ± 66.93###	459.60 ± 81.58***	303.60 ± 72.19	398.30 ± 88.11***	400.30 ± 85.04***
Brain serotonin (ng/g)	334.40 ± 16.26	259.40 ± 31.07###	327.80 ± 19.24***	260.70 ± 28.07	286.10 ± 25.12**	310.00 ± 22.20***
Brain dopamine (ng/g)	188.70 ± 29.09	73.43 ± 12.35###	174.50 ± 23.90***	78.09 ± 17.89	118.50 ± 17.19**	160.90 ± 23.41***
Brain total protein (mg/g)	2.38 ± 0.47	0.46 ± 0.20###	2.63 ± 0.51***	0.50 ± 0.22	1.33 ± 0.49**	1.88 ± 0.51***
SOD (U/mg protein)	15.44 ± 1.12	4.02 ± 0.46###	14.51 ± 0.82***	5.04 ± 0.71	7.22 ± 0.62*	9.54 ± 1.31***
GSH (µg/mg protein)	35.17 ± 2.78	12.29 ± 1.79###	32.87 ± 2.62***	14.26 ± 2.55	19.89 ± 1.71**	28.65 ± 2.57***
CAT (U/mg protein)	10.58 ± 1.45	4.25 ± 0.74###	9.53 ± 1.16***	4.80 ± 0.45	7.02 ± 0.66**	8.01 ± 0.76***
MDA (nM/mg protein)	95.31 ± 12.77	197.20 ± 11.08###	106.20 ± 5.98***	190.30 ± 10.21	159.70 ± 8.01**	126.60 ± 11.01***
Nitric oxide (µg/mL)	0.96 ± 0.03	1.74 ± 0.09###	1.03 ± 0.11***	1.61 ± 0.13	1.45 ± 0.12**	1.24 ± 0.07***

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test.
P < 0.001 versus normal group; P < 0.05, P < 0.01, P < 0.001 versus sodium arsenite-treated control group.
Abbreviations: *MWM: Morris water maze; EPM: elevated plus maze; SOD: superoxide dismutase; GSH: reduced glutathione; CAT: catalase; MDA: malondialdehyde; SEM: standard error of mean.*

Histopathological Alterations in Brain Tissue
Histopathological examination revealed marked differences among the experimental groups (Figure 7.16). Brain sections from the normal group showed largely preserved tissue architecture with no evident congestion or necrotic changes, although mild inflammatory infiltration and edema were observed. In contrast, sodium arsenite-exposed rats demonstrated pronounced pathological alterations, characterized by extensive neutrophilic infiltration and edema (grade 4), together with severe congestion (grade 3) and necrotic changes (grade 4).

Tocotrienol treatment at 150 mg/kg substantially attenuated the arsenite-associated histological alterations. Brain sections showed only mild inflammatory infiltration and congestion (grade 1), with no detectable necrotic changes. Treatment with *Bacopa monnieri* produced an apparent dose-related improvement in tissue morphology. The 40 mg/kg group exhibited moderate reduction in inflammatory infiltration and edema, with grade 2 changes, whereas the 80 mg/kg group showed comparatively preserved tissue architecture with only mild inflammatory infiltration, edema, and necrotic changes. Histopathological findings therefore supported the protective effects observed with the higher dose of *B. monnieri* in the behavioral and biochemical assessments.
The 20 mg/kg *B. monnieri* group was not subjected to histopathological evaluation because the dose did not demonstrate significant protective effects across the major biochemical and behavioral parameters.

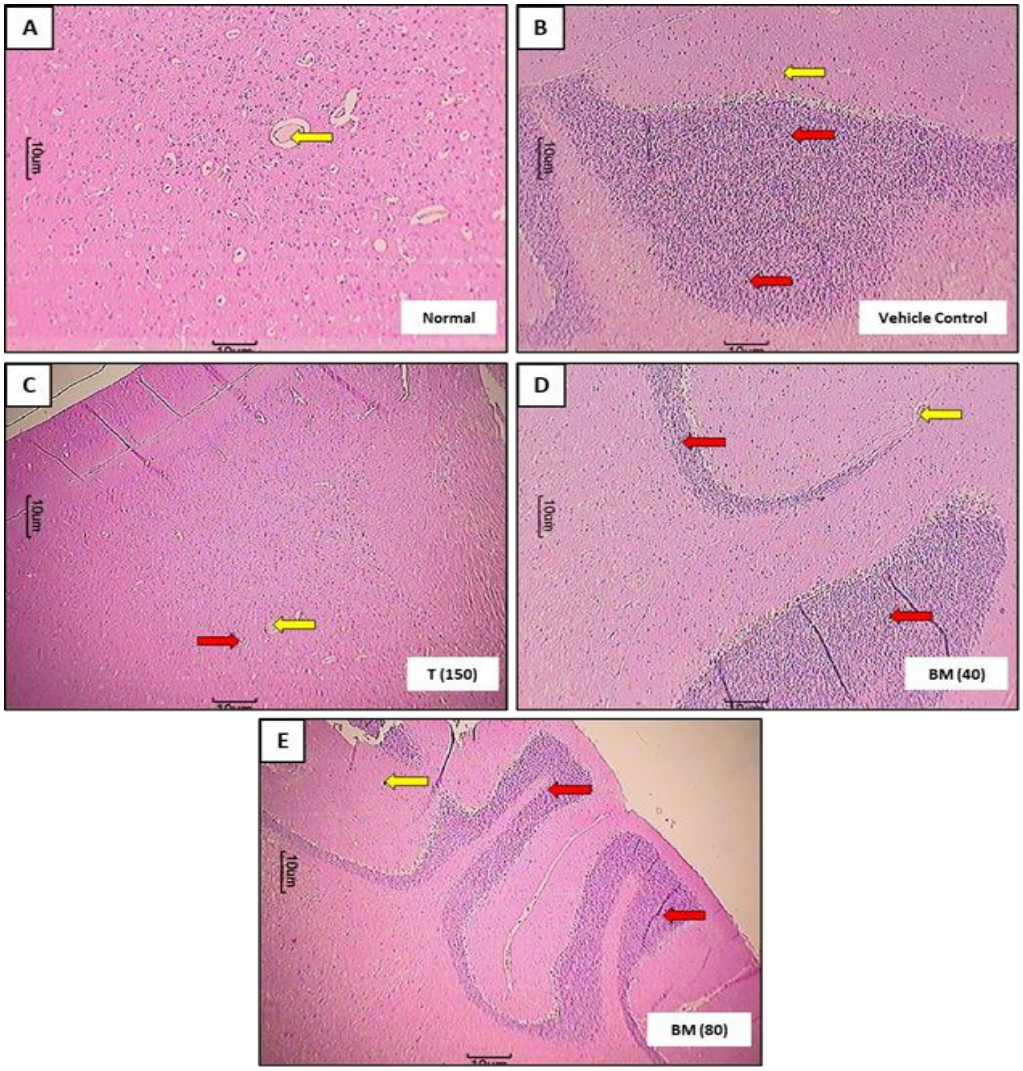

Figure 4: Histopathological representation of brain tissue from normal rats (A), Vehicle Control rats (B), Tocotrienols (150 mg/kg) treated rats (C), BM (40 mg/kg) treated rats (D) and BM (80 mg/kg) treated rats (E). Stained with H&E (at 100 X).

0 = no abnormality; + (grade 1) = <25% tissue involvement; ++ (grade 2) = <50%; +++ (grade 3) = <75%; ++++ (grade 4) = >75%.

DISCUSSION:

Chronic exposure to sodium arsenite produced marked behavioral, biochemical, neurochemical, and histological alterations in the experimental rats, indicating substantial arsenic-associated neurotoxicity. The Morris water maze findings demonstrated impaired spatial learning and memory in the sodium arsenite-treated control group, as reflected by the reduced time spent in the target quadrant compared with the normal group. The decline in target-quadrant exploration suggests impairment of hippocampus-dependent spatial memory, which is consistent with the broader pattern of behavioral dysfunction observed following arsenite exposure. Treatment with the standardized extract of *Bacopa monnieri* produced an improvement in spatial performance, with the 80 mg/kg dose showing the most evident recovery among the tested extract doses. The increased target-quadrant time observed at this dose suggests that the extract may attenuate the cognitive consequences associated with sodium arsenite exposure.

Sodium arsenite exposure was also associated with alterations in behavioral responses related to anxiety and depressive-like behavior. Increased immobility in the forced swimming test and reduced exploration of the open arms of the elevated plus maze indicated substantial behavioral disturbance in the arsenite-treated group. Administration of *B. monnieri*, particularly at 80 mg/kg, reduced these behavioral abnormalities. Improvement in forced swimming and elevated plus maze performance, together with the Morris water maze findings, indicates that the protective effect of the extract was not restricted to a single

behavioral domain. These observations may be related to the pharmacological properties of bioactive constituents present in standardized *B. monnieri* extract, although the present study does not establish a specific molecular mechanism.

The rotarod findings further demonstrated functional impairment following sodium arsenite administration. Reduced latency to fall in the arsenite-treated group indicated compromised motor coordination and balance. Treatment with the higher dose of *B. monnieri* improved rotarod performance, suggesting preservation of neuromotor function. The improvement in motor performance is important when interpreting the cognitive findings because severe motor impairment can independently affect performance in behavioral paradigms requiring swimming or coordinated movement. The improved motor coordination following treatment therefore supports the overall behavioral recovery observed with the standardized extract.

The behavioral alterations were accompanied by pronounced oxidative and nitrosative disturbances in brain tissue. Arsenite exposure increased lipid peroxidation, as demonstrated by the elevated MDA concentration, while endogenous antioxidant defenses were adversely affected. Reduction in SOD, CAT, and GSH indicates disruption of both enzymatic and non-enzymatic antioxidant mechanisms. Because neuronal membranes contain high concentrations of polyunsaturated fatty acids and the brain has considerable metabolic activity, increased oxidative burden may contribute substantially to neuronal dysfunction. The elevated MDA level observed in the arsenite-treated group further suggests enhanced membrane lipid oxidation.

Treatment with standardized *B. monnieri* extract, particularly at 80 mg/kg, improved the altered antioxidant profile. The restoration of SOD, CAT, and GSH together with attenuation of lipid peroxidation suggests that the extract may reduce oxidative damage by strengthening endogenous antioxidant defenses. The reduction in NO-related changes further indicates a possible attenuation of nitrosative stress. These biochemical findings correspond with the behavioral improvements, providing evidence that modulation of oxidative and nitrosative imbalance may contribute to the neuroprotective effect observed with the standardized extract. Nevertheless, the present findings demonstrate an association between antioxidant restoration and behavioral improvement rather than establishing a direct causal molecular pathway.

Alterations in brain neurotransmitter levels provided additional evidence of arsenite-induced neurochemical dysfunction. Sodium arsenite exposure resulted in depletion of important neurotransmitters associated with cognitive, emotional, and behavioral regulation. Changes in dopamine, serotonin, and norepinephrine may contribute to the impaired spatial performance, increased immobility, and anxiety-related behavioral alterations observed in the experimental animals. Treatment with *B. monnieri* improved the altered neurotransmitter profile, with the 80 mg/kg dose producing more pronounced restoration than the lower dose groups. In particular, the increased norepinephrine and dopamine levels following high-dose treatment suggest that preservation or normalization of monoaminergic neurotransmission may contribute to the observed behavioral recovery. However, the exact mechanism responsible for these neurochemical changes requires further investigation.

Histopathological findings provided structural support for the behavioral and biochemical observations. Sodium arsenite exposure was associated with pronounced pathological changes in brain tissue, including inflammatory infiltration, edema, and necrotic alterations. Such structural abnormalities provide a plausible anatomical basis for the observed functional deficits. Treatment with the higher dose of *B. monnieri* resulted in comparatively improved tissue architecture and reduced pathological alterations. The parallel improvement in antioxidant status, neurotransmitter levels, behavioral performance, and histological appearance suggests that the neuroprotective activity of the extract may involve multiple interconnected mechanisms rather than a single pharmacological target.

A dose-related pattern was evident among the *B. monnieri* treatment groups. The 20 mg/kg dose produced limited improvement across several assessed parameters, whereas the 80 mg/kg dose produced more consistent protective effects. This difference suggests that the pharmacological response to the standardized extract may be dose dependent within the range investigated. The findings therefore indicate that the higher dose was associated with greater protection against sodium arsenite-induced alterations under the experimental conditions used. The tocotrienol-treated group also demonstrated marked improvement across several parameters and served as a useful reference for comparison with the plant extract.

Overall, the findings indicate that sodium arsenite-induced neurotoxicity involves interconnected behavioral, oxidative, neurochemical, and structural disturbances. The ability of standardized *B. monnieri* extract to improve several of these

alterations, particularly at 80 mg/kg, supports its potential neuroprotective activity in this experimental model. The observed effects are consistent with a multifactorial protective response involving attenuation of oxidative stress, improvement of endogenous antioxidant defenses, modulation of neurotransmitter alterations, and preservation of brain tissue architecture. Further mechanistic studies are required to identify the specific constituents and molecular pathways responsible for these effects.

CONCLUSION:

The present study demonstrated that repeated sodium arsenite exposure produced substantial neurobehavioral, biochemical, neurochemical, and histopathological alterations in rats. Arsenite-associated toxicity was characterized by impaired spatial memory, altered anxiety- and depressive-like behaviors, reduced motor coordination, increased lipid peroxidation, disruption of endogenous antioxidant defenses, altered nitric oxide status, disturbances in brain neurotransmitters, and pathological changes in brain tissue.

Administration of the standardized extract of *Bacopa monnieri* attenuated several of these arsenite-induced alterations, with the 80 mg/kg dose producing the most consistent protective response among the investigated extract doses. The improvement in Morris water maze performance and motor coordination, together with reduced behavioral abnormalities, was accompanied by restoration of SOD, CAT, and GSH, reduction of oxidative damage, improvement in altered neurotransmitter levels, and preservation of brain tissue architecture. The comparatively limited response observed with the 20 mg/kg dose suggests a dose-related pharmacological effect within the tested range.

Taken together, the findings support the potential of standardized *B. monnieri* extract as a neuroprotective intervention against sodium arsenite-associated cognitive and behavioral disturbances. Its protective effects appear to involve coordinated modulation of oxidative stress, endogenous antioxidant defenses, neurotransmitter homeostasis, and tissue integrity. However, further studies involving molecular biomarkers, identification of the active constituents, mechanistic investigations, pharmacokinetic evaluation, and confirmation in additional experimental models are warranted before the findings can be extrapolated to clinical applications.

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