

Research Article

The Effect of Pretreatment with Hydroalcoholic Extract of *Cynodon dactylon* on Spatial Learning, Memory, and Motor Balance in Rats

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Abstract

Background: The incidence of Alzheimer's disease is rising at an alarming rate, becoming a major social and healthcare concern in many countries. Pharmacological strategies for Alzheimer's disease are categorized into three groups: (a) disease-modifying therapies, such as vitamin E and selegiline; (b) neurotransmitter-based therapies, including cholinesterase inhibitors; and (c) psychotropic agents aimed at managing behavioral symptoms. A key factor in the pathogenesis of Alzheimer's disease is oxidative stress, defined by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system. *Cynodon dactylon* (commonly known as Bermuda grass), a member of the Poaceae family, has been investigated for its potential antioxidant properties. Emerging evidence suggests that its bioactive compounds may counteract oxidative stress, making it a promising candidate for further research into neuroprotective therapies. **Methods:** In this experimental study, the hydroalcoholic (ethanolic) extract of *Cynodon dactylon* was prepared using the maceration method and concentrated with a rotary evaporator. Extract doses of 50, 100, and 200 mg/kg were dissolved in saline for administration. The effects of the extract on spatial learning and memory were evaluated using the Morris water maze test, while passive avoidance learning and memory were assessed through the shuttle box test. Psychomotor coordination was examined using the rotarod test. Serum antioxidant capacity was measured by the ferric reducing antioxidant power (FRAP) method via spectrophotometry, and serum malondialdehyde (MDA) levels were quantified using high-performance liquid chromatography (HPLC). Following the treatment period, the behavioral and biochemical assessments were conducted. Data were analyzed using SPSS and GraphPad Prism 6 software, with statistical significance set at $p < 0.05$. **Results:** *Cynodon dactylon* extract significantly improved memory retention and motor coordination. In the shuttle box test, doses of 100 and 200 mg/kg increased secondary delay time compared to control ($P < 0.05$, $P < 0.01$). In the rotarod test, motor balance and coordination were enhanced, especially at higher doses. Antioxidant assessments showed a dose-dependent increase in serum and brain FRAP values ($P < 0.05$ for 50 mg/kg; $P < 0.01$ for 100 and 200 mg/kg) and a significant reduction in MDA levels, indicating decreased oxidative stress. **Conclusion:** The extract's antioxidant compounds may reduce oxidative stress in the central nervous system, leading to better retention memory and motor coordination. Nonetheless, its negative effect on spatial memory suggests distinct underlying neural mechanisms. Further studies are needed to clarify these contrasting effects.

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Received: 25 May 2025; Accepted: 11 June 2025; Published: 21 July 2025



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Keywords

Cynodon Dactylon, Antioxidant, Spatial Memory, Motor Coordination, Passive Avoidance learning, Malondialdehyde (MDA), Oxidative Stress, Behavioral Tests

1. Introduction

Individuals with memory and learning impairments often begin with short-term memory loss, such as forgetting addresses and names, and this can progressively worsen to the point where an individual may forget even the way back home. Alzheimer's disease, unfortunately, has no cure, but its progression can be slowed down. The onset of Alzheimer's disease is 71% attributed to genetic factors and 21% to environmental factors, with the disease occurring in 95% of cases after the age of 60 [1]. The ability to learn and retain memories is one of the most prominent features of humans and is essential for daily living and scientific advancement. The increasing number of individuals with learning and memory impairments in aging populations has led to greater motivation for research in geriatric medicine to discover new and effective drugs that can enhance learning and memory and prevent memory disorders. As a result, in recent years, the use of herbal medicine and natural remedies for treating forgetfulness and enhancing memory has become the subject of many medical studies [2]. Diseases like Alzheimer's, characterized by progressive cognitive decline and behavioral disturbances, are the most common forms of dementia [3]. The synthesis of synthetic drugs and their widespread use in treatment has led to a complex issue known as drug side effects. In some cases, the adverse effects and side effects of these drugs can be more harmful to the patient than the disease being treated. Additionally, the development of sensitivities to some of these substances in patients and the increasing resistance of many disease-causing agents to synthetic drugs have led scientists to once again focus their attention on natural and herbal medicines. Given the proven presence of antioxidant compounds, flavonoids, phosphorus, iron, and other substances in the roots of *Cynodon dactylon* [4], and the impact of these compounds on memory and learning, this study was conducted.

In the past two decades, extensive research has been conducted to identify suitable therapeutic methods on one hand and to investigate the causes and risk factors associated with Alzheimer's disease on the other. This research is ongoing. It seems that alternative therapies, especially medicinal plants, have opened a new avenue for the treatment of Alzheimer's disease. Cognitive impairment in Alzheimer's disease may not only result from chemical damage to the nucleus basalis of Meynert (NBM) due to the degeneration of cholinergic branches from NBM to the septum and cortex, but also from the extensive damage to non-cholinergic cells within the

NBM. Additionally, other cholinergic pathways in the anterior basal forebrain may also be affected. It is also possible that the activity of the enzyme acetylcholinesterase in the cortex is reduced [5]. Neuronal degeneration in the NBM, which sends cholinergic projections to various brain regions, including the neocortex, leads to cognitive decline. Using animal models of this disease, significant progress has been made in cholinergic-based strategies for treating cognitive deficits resulting from NBM lesions, including the use of acetylcholinesterase inhibitors [6]. Several acetylcholine-enhancing drugs were later developed for the treatment of Alzheimer's, including tacrine, donepezil, rivastigmine, and galantamine. All of these cholinergic enhancers have been approved for the treatment of mild to moderate Alzheimer's disease but are associated with many side effects [7]. Nowadays, significant efforts are being made to develop new drugs and suitable alternatives, with a particular focus on herbal medicines. *Cynodon dactylon*, also known as Bermuda grass, belongs to the family Poaceae. This plant has a stem length ranging from 10 to 30 centimeters. The leaves are grayish to bluish-green in color. The inflorescence is a spike-like cluster, 14 to 30 centimeters long, containing 4 to 6 spikes connected at the tip of the stem. Bermuda grass grows rapidly in fertile soils with sufficient depth. It is known as "doob" in India. This plant has multiple applications in medicine and is recognized for its antimicrobial properties. It is commonly found in temperate regions, especially in Iran. The root of this plant is mainly used, while the leaves and aerial stem are tasteless, and the root has a slightly sweet taste [8].

2. Implications

In this study, the assessment of motor balance (motor learning) was carried out using the Rotarod device [9], the evaluation of passive avoidance learning was performed using the Shuttle box device [10], and spatial memory was measured using the Morris Water Maze device [11].

In a study investigating the effects of *Cynodon dactylon* extract at doses of 250, 500, and 750 mg/kg orally, its effect on modulating blood sugar levels was proven [12].

In a study examining the anti-inflammatory and analgesic effects of *Cynodon dactylon* with doses of 200, 400, and 600 mg via hot plate and acetic acid models, it was concluded that the plant showed significant analgesic and anti-inflammatory effects [13].

A study by Rai, P. K and colleagues on the antioxidant potential of *Cynodon dactylon* oral extract in diabetes induced by oxidative stress demonstrated that a dose of 500 mg/kg administered for 30 days via oral route in rats had therapeutic effects [14].

In a study by Alireza Garjan and colleagues, the hydroalcoholic extract of *Cynodon dactylon* rhizomes was tested for its effect on right heart infarction in rats. The study used doses of 100, 200, and 50 mg/kg orally for 50 days and found beneficial effects of the rhizome extract on improving right heart infarction [15].

A study conducted by SK Singh and colleagues found that *Cynodon dactylon* extract at doses of 250, 500, and 750 mg/kg orally effectively reduced blood sugar levels [16].

In research by Ahmed, S. and colleagues, the anti-inflammatory, anti-nausea, and antimicrobial effects of *Cynodon dactylon* extract were evaluated and confirmed in rats [17].

A study by Miraldi E and colleagues on the root and rhizome of *Cynodon dactylon* showed its beneficial effects on depression and epilepsy [18].

Research conducted by Atmani, F. and colleagues demonstrated the preventive and therapeutic effects of aqueous rhizome extract of *Cynodon dactylon* at doses of 125, 250, and 500 mg/kg over three weeks for nephrolithiasis treatment [19].

In a study by Patil, MB. and colleagues, the anti-ulcer effect of alcoholic *Cynodon dactylon* extract in rats was observed at doses of 200, 400, and 600 mg/kg orally [20].

One of the observed symptoms in Alzheimer's disease is the reduction of neurons in key areas for learning and memory, especially in the hippocampus. One of the factors contributing to the pathogenesis of Alzheimer's disease is oxidative stress, which is an imbalance between free radicals and the antioxidant system. Oxygen-free radicals can attack proteins, nucleic acids, and lipid membranes, disrupting cellular integrity and function. Brain tissue contains a large amount of unsaturated fatty acids, which are particularly vulnerable to attacks from free radicals [21].

In the past two decades, extensive research has been conducted to find appropriate treatment methods and explore the causes and factors affecting memory decline. Research results have shown that antioxidants may play a significant role in improving memory. Antioxidants prevent the action of free radicals and could be useful in preventing and treating memory-degenerative diseases [22].

Improvement in passive avoidance learning and motor balance retention as indicated by the time spent on the Rotarod for motor coordination in groups receiving the extract may be attributed to the antioxidant properties of the plant, as well as the inhibitory effect on acetylcholinesterase activity, which requires further studies for clarification.

3. Sample Size and Sampling Method

This study was an experimental study conducted on a population of healthy male Wistar rats with a sample size of 100 rats. The sampling method used was convenient and accessible. The study procedures are outlined as follows:

3.1. Animals

A total of 100 male Wistar rats were used in this study. The rats were housed in standard conditions with a 12-hour light/dark cycle and free access to food and water. The animals were acclimatized to the laboratory environment for one week prior to the experiment.

3.2. Grouping

The rats were randomly assigned to different experimental groups based on the doses of *Cynodon dactylon* extract administered. Control groups and treatment groups were established as follows:

- 1) Control group: No treatment.
- 2) Treatment groups: Received *Cynodon dactylon* extract at varying doses.

3.3. Extraction Method

The rhizomes of *Cynodon dactylon* were obtained from the Herbal Medicine and By-products Research Unit, Agricultural and Natural Resources Research Center of Isfahan Province. The plant was then dried in the shade and ground into a fine powder. The powder was soaked in 90% ethanol. The flask containing the plant material was placed on a magnetic stirrer and kept at room temperature for one week. After this period, the mixture was filtered, and the filtered solution was stored at 37 °C. The extract was concentrated using a rotary evaporator [23].

3.4. Experimental Animals

Adult male Wistar rats, weighing between 200 and 250 grams, were selected for this study. The rats were housed under controlled environmental conditions (temperature: 21 ± 2 °C, 12-hour light/dark cycle) with free access to food and water. The rats were divided into the following groups:

Control group: This group received only distilled water and was not subjected to any drug injections or surgeries. The rats underwent behavioral testing (n=7).

Healthy group with 50 mg/kg dose of extract: This group received the extract via intraperitoneal injection for 7 days and was subjected to behavioral testing (n=7).

Healthy group with 100 mg/kg dose of extract: This group received the extract via intraperitoneal injection for 7 days and underwent behavioral testing (n=7).

Healthy group with 200 mg/kg dose of extract: This group received the extract via intraperitoneal injection for 7 days

and underwent behavioral testing (n=7).

3.5. Psychomotor Coordination Test with the Rotarod Device

The ability of the rats to maintain balance and their motor resistance was assessed using the Rotarod device. Initially, the rats were familiarized with the device, and they were trained to move on the rotating rod. The Rotarod device is used to measure the balance and motor resistance of animals. The device consists of a rotating drum, and the speed of rotation can be adjusted from 0 to 40 rpm. The device has a belt that, when moved, adjusts the speed of the drum. For this study, the speed was set to 10 rpm with an acceleration of 7 rpm², which corresponds to approximately 10-11 rotations per minute. Two hours after receiving the respective treatment, the rats from each experimental group (treatment or control) were placed on the rotating drum. The drum rotated for 60 seconds (maximum), and the time it took for the rats to maintain balance and resist the motion of the drum was recorded as the "resistance time." This procedure was repeated three times for each rat, and the average time was calculated [24].

3.6. Passive Avoidance Test Using the Shuttle Box

For this test, a shuttle box was utilized. The device consists of a chamber with a lighted compartment connected to a dark compartment via a guillotine door. Electric shocks were delivered to the floor grid rods of the shuttle box by a separate stimulator. This test was conducted over four days for each mouse.

On the first and second days of the experiment, each mouse was placed in the device and allowed to habituate to the environment for five minutes. On the third day, an acquisition trial was conducted. Each mouse was placed individually in the lighted compartment. After a two-minute adaptation period, the guillotine door was opened, and upon the mouse entering the dark compartment, the door was closed. A mild electric shock (1 mA, 1 second, single pulse) was then administered. During this session, the initial latency (time taken to enter the dark compartment) was recorded.

Twenty-four hours later, each mouse was returned to the lighted compartment to continue the test. The latency period between being placed in the lighted compartment and entering the dark compartment was measured and recorded as the step-through latency (with a maximum latency of 60 seconds) [25].

3.7. Spatial Memory Test Using the Morris Water Maze

The *Morris Water Maze* was utilized to assess learning, memory, and motor performance in rats. The device components included a water tank with a diameter of 136 cm and a height of 60 cm, filled with water at a height of 25 cm and

maintained at a temperature of 20 ± 1 °C. A circular plexiglass platform, 10 cm in diameter, was placed in the center of the southwest quadrant, submerged approximately 1 cm below the water surface. Additional equipment included an infrared light source, a specialized recording camera, and a computer connected to the camera.

The maze was situated in a room with external visual cues (such as a clock, posters, tables, etc.) to aid spatial navigation. Various training protocols were applied to evaluate indices related to learning, memory, and motor performance.

Each trial allowed the rat 60 seconds to locate the platform. If unsuccessful, the rat was guided to the platform by the researcher. A 30-second rest period was provided between trials, during which the rat could explore its surroundings. Between training blocks, there was a 10-minute break, during which the rat was removed from the water and allowed to rest in its cage.

Using this setup, factors such as movement speed, visual-motor coordination, and spatial learning were evaluated. Each rat underwent training four times per day for four consecutive days. On the fifth day, a *probe trial* was conducted without the platform to assess spatial memory [26].

3.8. Measurement of Antioxidant Capacity (Serum)

Blood was collected from the hearts of healthy rats at the end of the behavioral tests. The serum was separated using a centrifuge, and the antioxidant capacity of the serum was measured. The antioxidant power of the serum is determined by its ability to reduce Fe⁺² to Fe⁺³. Ferrous sulfate (FeSO₄) is used as the standard for the FRAP method [27].

3.9. Measurement of Serum MDA using HPLC

Fifty microliters (50 µl) of the standard sample were mixed with 50 µl of 0.05% Butylated HydroxyToluene (BHT), followed by the addition of 400 µl of 0.44M H₃PO₄ and 100 µl of 42 mM TBA. After vortexing, the mixture was heated at 100 °C for one hour. After cooling the solution at 0 °C for five minutes, 250 µl of normal butanol was added, and the solution was vortexed again.

The mixture was centrifuged (5 minutes at 14,000 rpm), and 20 µl of the upper layer was injected into the HPLC device for analysis [28].

4. Statistical Analysis

The behavioral activities of the animals in the Morris water maze, shuttle box, and rotarod devices, along with the results of serum antioxidant tests and serum malondialdehyde levels, were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. Data are presented as Mean \pm SEM, and statistical significance was considered at (P<0.05).

5. Results

Chemical Analysis of Ethanol Extract of *Cynodon dactylon* Rhizome.

5.1. Effect of Hydroalcoholic Extract of *Cynodon dactylon* Rhizome on Retention Memory via the Shuttle Box Test in Rats

The results of the shuttle box test for evaluating learning and retention memory are shown in Figure 1. As illustrated in the graph, the secondary latency time in the groups receiving 100 mg/kg and 200 mg/kg doses of the extract significantly increased compared to the control group ($P<0.05$ and $P<0.01$, respectively). However, the 50 mg/kg dose did not show a significant difference compared to the control group ($P>0.05$). No significant difference was observed between the extract-treated groups and the control group in the initial latency time ($P>0.05$).

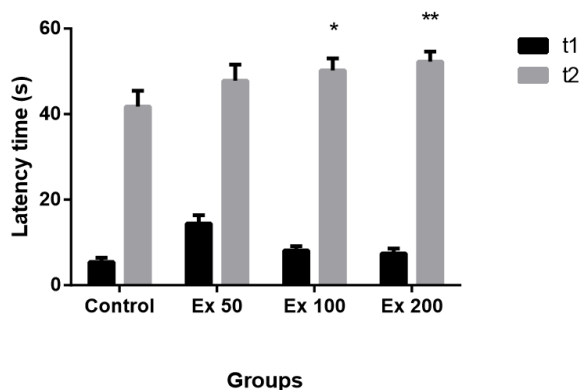


Figure 1. Comparison of the effects of different doses of the *Cynodon dactylon* rhizome extract on the initial and secondary latency times compared to the control group. * indicates $P<0.05$ and ** indicates $P<0.01$ in comparison to the control group.

5.2. The Effect of Hydroalcoholic Extract of *Cynodon dactylon* Rhizomes on Spatial Learning and Memory in the Morris Water Maze Test

The results from the Morris water maze test for evaluating learning and spatial memory, shown in Figures 2 and 3, indicate that rats receiving the *Cynodon dactylon* extract were able to reach the hidden platform as effectively as the control group during the 4-day learning period. By the fourth day, these rats reached the platform in a shorter time compared to the first day. However, a significant difference was observed only on the second day, where the group receiving a dose of 50 mg/kg showed less learning ability compared to the control group. No significant differences were observed on other days.

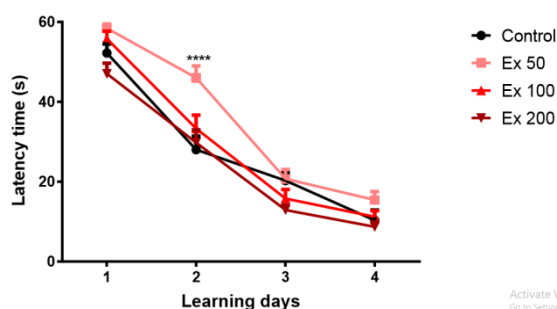


Figure 2. Comparison of the effects of different doses of *Cynodon dactylon* rhizome extract on the latency time to reach the hidden platform in the Morris water maze test compared to the control group. $P<0.001$ indicates a significant difference when compared to the control group.

The results of spatial memory assessment in the probe test (on the fifth day) for the groups receiving the plant extract showed that they spent less time in the target quadrant of the Morris water maze to find the hidden platform compared to the control group. These results indicate a decrease in spatial memory compared to the control group. As shown in Figure 3, the groups receiving doses of 50, 100, and 200 mg/kg of the plant extract had a significant difference from the control group.

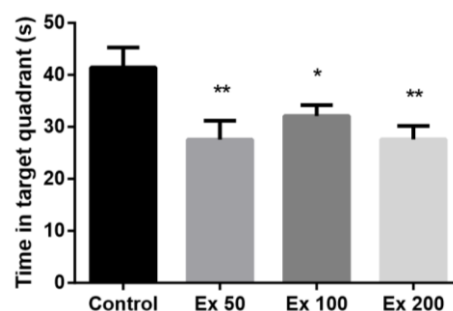


Figure 3. Comparison of the effects of different doses of plant rhizome extract on the time to reach the hidden platform in the Morris water maze probe test compared to the control group. * denotes $P<0.05$ and ** denotes $P<0.01$ compared to the control group.

5.3. The Effect of the Hydroalcoholic Extract of the Plant Rhizome on Motor Balance Using the Rotarod Device

As shown in Figure 4, the results of the one-way analysis of variance test indicated that the hydroalcoholic extract of the plant increased motor balance in the studied rats. The Tukey post-hoc test results showed that this increase was only significant at the dose of 200 mg/kg ($p<0.01$), while the doses of 50 and 100 mg/kg of the extract did not have a significant effect on motor balance ($p>0.05$).

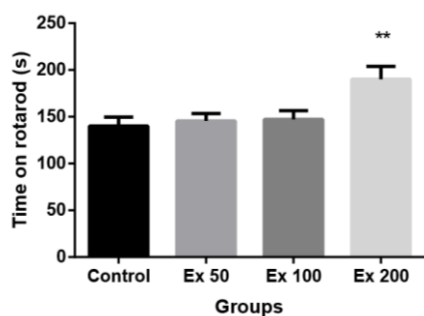


Figure 4. Comparison of the effects of different doses of the rhizome extract of the *Cynodon dactylon* plant on the duration of stay on the rotating rod device with the control group. ** indicates $P < 0.01$ compared to the control group.

Thus, animals that received the 200 mg/kg dose of the extract showed better balance compared to the group that received only saline, as they were able to maintain their position on the rotating rod for a longer period of time.

5.4. The Effect of the Hydroalcoholic Extract of *Cynodon dactylon* Rhizome on Serum and Brain Antioxidant Capacity in the Studied Groups

Pre-treatment of animals with the hydroalcoholic extract of *Cynodon dactylon* led to an increase in plasma antioxidant levels in all three groups receiving the extract (50, 100, and 200 mg/kg doses) compared to the control group. Tukey's post hoc test results showed that this increase was significant for the 50 mg/kg dose at $P < 0.01$, and for the 100 and 200 mg/kg doses, it was significant at $P < 0.001$ (Figure 5).

Figure 6 shows the results of measuring the antioxidant capacity in the brain for the studied groups. This study revealed that the brain antioxidant capacity in the groups receiving the 100 and 200 mg/kg doses was significantly higher compared to the control group ($P < 0.001$), while the 50 mg/kg dose did not show any significant effect ($P > 0.05$).

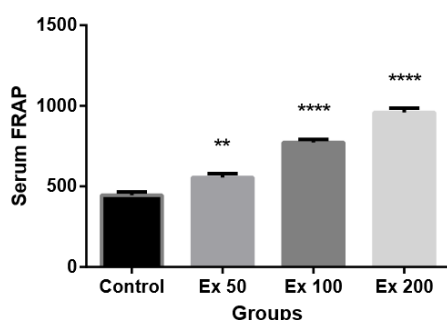


Figure 5. Effects of different doses of *Cynodon dactylon* rhizome extract on serum antioxidant capacity compared to the control group. ** indicates $P < 0.01$ and **** indicates $P < 0.0001$ compared to the control group.

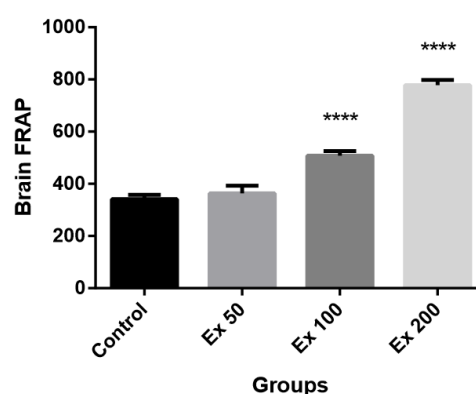


Figure 6. Effects of different doses of *Cynodon dactylon* rhizome extract on brain antioxidant capacity compared to the control group. **** indicates $P < 0.0001$ compared to the control group.

5.5. Effect of Hydroalcoholic Extract of *Cynodon Dactylon* Rhizome on Serum and Brain Malondialdehyde Levels in the Studied Groups

The results of the one-way analysis of variance test showed that pre-treatment with the hydroalcoholic extract of *Cynodon dactylon* rhizome significantly reduced serum MDA levels in the groups receiving doses of 50, 100, and 200 mg/kg compared to the control group (Figure 7).

However, the Tukey post hoc test showed that the extract reduced serum malondialdehyde levels in all groups with a difference of $p < 0.001$.

Similar results were obtained for the malondialdehyde levels in the brain across the studied groups (Figure 8).

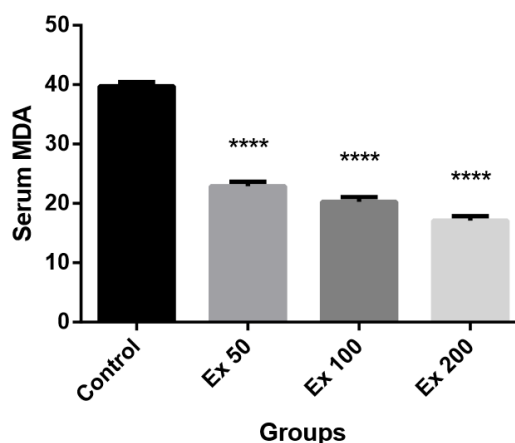


Figure 7. Effects of different doses of *Cynodon dactylon* rhizome extract on serum malondialdehyde levels compared to the control group. **** indicates $p < 0.0001$ compared to the control group.

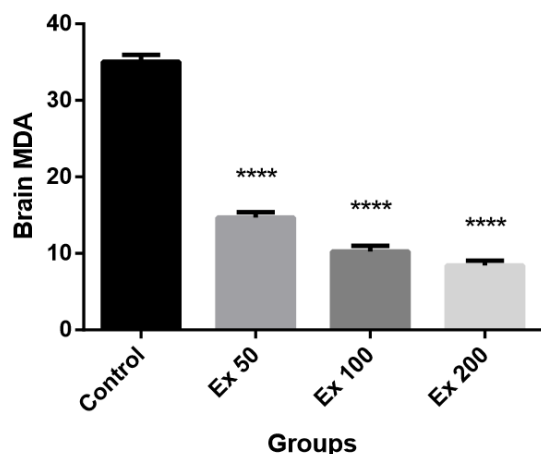


Figure 8. Effects of different doses of plant rhizome extract on brain malondialdehyde levels compared to the control group. **** denotes $p < 0.0001$ compared to the control group.

6. Discussion

In this study, the effects of *Cynodon dactylon* extract on memory, motor coordination, and antioxidant capacity in rats were investigated. The results demonstrated significant improvements in cognitive function, motor performance, and antioxidant levels in the serum and brain.

Effect on Memory

Memory and learning tests, including the Morris Water Maze and Passive Avoidance tests, revealed that rats receiving the *Cynodon dactylon* extract performed significantly better than the control group. Specifically, rats administered the extract at doses of 100 mg/kg and 200 mg/kg showed a notable decrease in the time required to locate the hidden platform in the Morris Water Maze, indicating improved spatial memory. Additionally, the increase in secondary delay time in the passive avoidance test suggests enhanced memory retention. These findings suggest that *Cynodon dactylon* may enhance memory through its antioxidant properties and potential modulation of the cholinergic system. The antioxidant compounds in *Cynodon dactylon* may activate the Nrf2/ARE (Nuclear factor erythroid 2-related factor 2/Antioxidant Response Element) pathway, known for regulating the expression of cytoprotective proteins that combat oxidative stress [29]. Furthermore, the inhibition of acetylcholinesterase could play a crucial role in improving memory retention by increasing acetylcholine levels, a neurotransmitter essential for learning and memory processes [30].

Effect on Motor Coordination

In the Rotarod test, rats administered 200 mg/kg of *Cynodon dactylon* extract exhibited significantly improved motor coordination, spending more time on the rotating rod than the control group. These findings suggest that *Cynodon dactylon* may help restore motor coordination, likely through its antioxidant and anti-inflammatory effects, as oxidative stress and neuroinflammation are often implicated in motor dysfunction [31]. Studies have shown that medicinal plants with antioxi-

dant properties can positively affect motor function, providing further support for the potential therapeutic use of *Cynodon dactylon* in neurodegenerative diseases associated with motor impairments [32].

Effect on Antioxidant Capacity and Malondialdehyde (MDA)

The study also demonstrated that the extract increased antioxidant capacity in both serum and brain tissues, while significantly reducing the levels of malondialdehyde (MDA), a marker of oxidative stress and lipid peroxidation. This suggests that the extract has a protective effect against oxidative damage in neuronal tissues. The reduction in MDA levels, in particular, indicates that *Cynodon dactylon* may help prevent cellular damage associated with neurodegenerative diseases such as Alzheimer's disease [33]. The antioxidant activity could be attributed to the presence of bioactive compounds such as flavonoids and polyphenols, which have been shown to scavenge free radicals and enhance cellular defense mechanisms [34].

Limitations and Future Research Directions

While the results of this study are promising, there are limitations that should be addressed in future research. First, the active compounds responsible for the observed effects remain unidentified, and future studies should aim to isolate and characterize these compounds using advanced techniques such as chromatography [35]. Furthermore, long-term studies investigating the chronic effects of *Cynodon dactylon* extract on cognitive function and motor coordination are needed to determine the sustainability of its beneficial effects.

It is also important to explore the molecular mechanisms behind the effects of *Cynodon dactylon* on the central nervous system, particularly its interaction with key signaling pathways involved in neuroprotection and neurogenesis [36]. Research into the synergistic effects of *Cynodon dactylon* extract combined with other pharmacological treatments, such as acetylcholinesterase inhibitors, could lead to the development of more effective therapies for Alzheimer's disease and other cognitive impairments [37].

Given the growing prevalence of neurodegenerative diseases and cognitive dysfunctions, *Cynodon dactylon* presents a promising natural therapeutic approach. Future clinical trials in humans are necessary to assess its efficacy and safety, particularly in aging populations at risk for cognitive decline. The combination of herbal medicine and traditional pharmacological treatments may offer a cost-effective and safer alternative, reducing the potential side effects associated with synthetic drugs [38].

7. Conclusion

Overall, the findings of this study indicate that the hydroalcoholic extract of *Cynodon dactylon* exhibits positive effects on passive avoidance learning and memory, motor coordination, and enhances antioxidant levels in both serum and brain. Additionally, it reduces malondialdehyde (MDA)

levels in serum and brain tissues of rats receiving the extract. However, the extract demonstrated negative effects on spatial memory. The antioxidant compounds present in the plant appear to reduce oxidative stress in the central nervous system by mitigating.

free radical production caused by the oxidation of proteins and lipids. This mechanism may underlie the beneficial effects of the extract on passive avoidance memory and motor coordination. Nevertheless, the differing effects of the extract on spatial memory compared to passive avoidance memory suggest that the underlying mechanisms may vary. Further studies are needed to investigate these mechanisms in more detail and explore the causes behind the negative impact on spatial memory.

Abbreviations

ROS	Reactive Oxygen Species
FRAP	Ferric Reducing Antioxidant Power
NBM	Nucleus Basalis of Meynert
BHT	Butylated HydroxyToluene
MDA	Malondialdehyde
HPLC	High-performance Liquid Chromatography

Acknowledgments

This study was financially supported by Shahrekord University of Medical Science.

Conflicts of Interest

The authors declare no conflicts of interest.

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