

Silver Nanoparticle-Based Delivery System Enhances the Neuroprotective Potential of Curcumin in a Mouse Model of Sleep Deprivation-Induced Memory Impairment

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Abstract

Sleep deprivation can induce oxidative stress and impair hippocampus-dependent memory, highlighting the need for effective therapeutic strategies. Curcumin has potent antioxidant properties but suffers from poor solubility and rapid metabolism, which limit its bioavailability. To address these limitations, curcumin was loaded onto silver nanoparticles (AgNPs) as a nanocarrier-based delivery system. In this study, the effects of curcumin at doses of 25 and 50 mg/kg, administered with or without AgNPs, were investigated in mice subjected to sleep deprivation. The synthesized AgNPs were spherical, with sizes ranging from 10 to 60 nm. The effects of treatment were evaluated using behavioral tests, including the novel object recognition test (NORT), object location test (OLT), and elevated-arm radial water maze (EARWM). Catalase activity was also measured in hippocampal tissue and blood plasma. The results demonstrated that 50 mg curcumin delivered via AgNPs produced the greatest improvement in behavioral performance and increased catalase activity. In contrast, curcumin administered without nanoparticles showed no effect, suggesting a potential contribution of the AgNP-based delivery system to the biological activity of curcumin under the conditions tested.

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1. Introduction

Sleep is a vital process for overall health and human well-being, influencing multiple systems, including the central nervous system (mood regulation, coordination, and cognitive function), immune system (regulated cytokine production and T-cell activity), and digestive system [1-3]. Inadequate sleep can negatively affect decision-

making, judgment, emotional regulation, and reaction time [4, 5]. A positive correlation exists between memory functions and sleep; insufficient sleep leads to memory impairments, while adequate sleep can enhance memory processes [6]. The hippocampus plays a critical role in memory function, and insufficient sleep can significantly affect this region [7]. One study demonstrated that sleep deprivation causes memory deficits by reducing dendritic spine density in the hippocampal CA1 area, which is essential for synaptic plasticity and memory consolidation [8]. The hippocampus is susceptible to redox conditions; insufficient sleep can increase reactive oxygen species (ROS) levels, leading to oxidative stress [9, 10].

Oxidative stress occurs when there is an imbalance between the production and scavenging of ROS within cells [11]. This imbalance can disrupt critical biochemical processes, such as the electron transport chain (ETC), ATP synthesis, and protein synthesis, or cause physical damage to cellular structures, including organelles, cell membranes, DNA, transcriptional machinery, and other macromolecules [11]. Oxidative stress has been implicated in the development of various neurological disorders, including Parkinson's disease, Alzheimer's disease, and depression [12-14]. Oxidative stress and neuroinflammation are closely interconnected processes in neurological pathophysiology. Excessive ROS production can promote inflammatory signaling, while inflammatory responses can further increase oxidative stress, resulting in a self-amplifying cycle that may contribute to neuronal dysfunction [15].

Conventional pharmacological agents used to improve cognitive function, such as donepezil, a cholinesterase inhibitor, may be associated with systemic adverse effects [16]. For a long time, plants have been excellent sources of various medicines which utilized for several diseases [17]. Therefore, it seems that natural compounds (e.g., phenols, terpenes, flavonoids) of medicinal plants could be desirable potent alternatives. So, in the present study, we employed curcumin (a major secondary metabolite of *Curcuma longa*) as a potent antioxidant that detoxifies toxins and ROS (e.g., H_2O_2) [18, 19]. Antioxidant compounds help prevent oxidative stress and may be beneficial for memory processing and hippocampal disorders [20-22]. Curcumin exhibits anti-inflammatory and antioxidant properties, as well as antidiabetic and antihypertensive effects [23]. However, curcumin has low bioavailability and undergoes rapid metabolism, resulting in poor absorption [24]. Nanoparticle-based delivery systems have therefore been investigated as a strategy to improve the delivery and biological performance of curcumin [25]. In particular, curcumin-conjugated or curcumin-loaded silver nanoparticles (AgNPs) have been developed and characterized, with previous studies reporting antibacterial, antibiofilm, and antioxidant activities of these hybrid systems [25, 26]. However, the potential effects of curcumin–AgNP formulations on memory-related outcomes under conditions of sleep deprivation remain insufficiently characterized. Therefore, in the present study, we investigated the effects of curcumin conjugated with AgNPs on memory-related behavioral and biochemical outcomes in sleep-deprived mice. Behavioral tests and biochemical assays were performed to evaluate these effects.

2. Materials and Methods

2.1 Nanoparticle preparation procedure

Curcumin powder (Merck, Germany) was dissolved in 5% DMSO, and the solution was analyzed by spectrophotometry. Additionally, 500 mg of polyvinyl alcohol (PVA) was dissolved in 100 mL of water to prepare a 5 mg/mL PVA solution. This solution was then processed using an ultrasonic homogenizer (30 min, 400 W, maximum 50% amplitude, six pulses per minute). Next, the PVA solution was added to the synthesized colloidal

AgNPs solution (pH 4.5–6.5). A fresh glutaraldehyde solution was prepared at a 1:10 ratio relative to the molecular weight of PVA, and PVA was then slowly added to the AgNP solution, which was then vigorously shaken. The resulting mixture was maintained at room temperature in the dark. After high-speed centrifugation (Sigma, Germany, $20,000 \times g$, 30 min), 5% of the initial AgNPs-PVA solution was concentrated. Curcumin was then added to this solution, and complete loading was performed under cold, dark conditions. Finally, the solution concentration in distilled water was adjusted to half the initial concentration.

2.2 Animals

Forty male BALB/c mice, 4 weeks of age (35–40 g), were obtained from the Animal House of Hakim Sabzevari University, Iran. The animals were housed under standard laboratory conditions for a 2-week acclimatization period before the initiation of the experimental procedures. Mice were housed in pairs in standard cages measuring approximately $30 \times 20 \times 18$ cm, with unrestricted access to standard laboratory chow and water. The animal room was maintained under an automatically controlled 12:12-h light/dark cycle (from 07:00 to 19:00). Room temperature was maintained at $22\text{--}23$ °C, and relative humidity was maintained at 50–60%. This study was conducted in accordance with the committee's guidelines for the care and use of laboratory animals and was approved by the ethics committee (approval ID: IR.HSU.AEC.1401.007).

Table 1: Forty 28-day-old male mice were divided into four groups of ten. They were subjected to sleep deprivation to investigate the effects of free curcumin and curcumin loaded onto silver nanoparticles, aiming to improve memory impairments caused by sleep deprivation. The treatment conditions for each group are described in the table below.

Group	Description
TCtrl	Under sleep deprivation stress without treatment
TNC25	Under sleep deprivation stress and treated with 25 mg/kg AgNPs-Curcumin
TC50	Under sleep deprivation stress and treated with 50 mg/kg curcumin
TNC50	Under sleep deprivation stress and treated with 50 mg/kg AgNPs-Curcumin

2.3 Sleep deprivation procedure

Sleep deprivation was induced using a water box measuring $50 \text{ cm} \times 30 \text{ cm} \times 30 \text{ cm}$, containing six evenly spaced round platforms (3 cm in diameter and 5 cm high). To prevent movement restriction, only three mice were placed in the box at any given time. The water level was maintained 1 cm below the platforms, and the temperature was kept at 22 ± 2 °C. Adequate food and water were provided on top of the box. Before sleep deprivation, the mice were acclimated to the environment by being placed on the platforms for two hours daily over three consecutive days. Sleep deprivation began at 9 AM. Afterward, the mice were removed from the sleep deprivation boxes and housed in cages from 5 PM to 9 PM each day, during which they had unrestricted access to food and water. The sleep deprivation protocol lasted for seven days, and the water in the boxes was changed daily [27, 28].

2.4 Behavioral testing

2.4.1 Object Location Test (OLT) and Novel Object Recognition Test (NORT)

Object Location Test (OLT) and Novel Object Recognition Test (NORT) were performed to evaluate spatial and recognition memory, respectively. Behavioral testing was conducted in a square open-field arena measuring $40 \times$

40 × 40 cm. The arena was constructed with a non-porous, polyester-insulated surface and was completely enclosed and white in color. Behavioral sessions were video-recorded for subsequent analysis.

Before behavioral testing, mice were acclimated to the testing room for 30 min. During habituation, each mouse was individually placed in the empty arena and allowed to freely explore for 6 min. The arena was thoroughly cleaned with 70% ethanol between animals to minimize the influence of olfactory cues. For the training session, two identical objects (approximately 6 × 6 cm) were placed at designated locations in two corners of the arena. Each mouse was allowed to freely explore the arena and the two objects for 6 min. At the end of the training session, the mouse was returned to its home cage.

For the OLT, conducted 24 h after the training session, one of the two familiar objects was relocated to a previously unoccupied corner of the arena, while the other object remained in its original location. The mouse was then allowed to explore the arena for 6 min. The time spent investigating the displaced and stationary objects was recorded. For the NORT, the stationary familiar object was replaced with a novel object at the same spatial location, whereas the other familiar object remained in its original position. The mouse was allowed to explore the arena for 6 min, and the time spent investigating the novel and familiar objects was recorded.

Object investigation was defined as active exploration of an object, with the animal's head oriented toward the object and its nose positioned within approximately 2 cm of the object. Behaviors such as sitting on, climbing over, or passing by an object without directed investigation were not considered exploratory behavior [29]. The steps and procedures for these tests are detailed in Table 2.

Table 2: Forty 28-day-old male mice subjected to sleep deprivation stress were divided into four groups of ten. To investigate the effects of curcumin alone and curcumin loaded onto silver nanoparticles on memory impairments caused by sleep deprivation, behavioral tests—Object Location Test (OLT) and Novel Object Recognition Test (NORT)—were conducted to assess memory performance. The following table outlines the steps and details of these behavioral tests.

Phase	Timing	Procedure	Duration
Habituation	Before training	Mice were acclimated to the behavioral testing room for 30 min and subsequently placed individually in the empty arena for free exploration.	6 min
Training	After habituation	Two identical objects (approximately 6 × 6 cm) were placed in two designated corners of the arena. Mice were allowed to explore the arena and both objects freely.	6 min
OLT	24 h after training	One familiar object was moved to a previously unoccupied corner, while the second object remained in its original location. Mice were allowed to explore the arena and both objects.	6 min
NORT	After the OLT phase	The stationary familiar object was replaced by a novel object at the same spatial location. Mice were allowed to explore the arena and both objects.	6 min

Behavior was analyzed by measuring the time spent investigating each object within a 2 cm radius. Results were expressed as the Percent of Total Investigation Time (PTIT) and the Discrimination Index (DI).

$$PTIT = \frac{\text{Time with novel location or object}}{(\text{Time with novel location or object} + \text{Time with familiar location or object})} \times 100$$

$$DI = \frac{(\text{Time with novel location or object} - \text{Time with familiar location or object})}{(\text{Time with novel location or object} + \text{Time with familiar location or object})}$$

2.4.2 Elevated Radial Arm Water Maze (ERAWM) Test

The maze consisted of eight arms (10 cm wide, 20 cm high, 29 cm long) radiating from a central platform (85 cm in diameter), placed in a water-filled pan with platforms (15 cm high) submerged 1 cm below the water surface at the ends of four arms. The water temperature was maintained at room temperature. Each mouse was placed in the starting arm and given up to 120 seconds to locate a hidden platform. The location of the platforms remained constant throughout testing. On the first day, mice were allowed to remain on the platform for 10 seconds after every four trials to facilitate spatial orientation. The number of first and repeated entries into each arm was recorded to assess working- and reference-memory errors (RME). These entries were classified as correct working memory errors. Additionally, the number of first entries into each arm without a platform was counted for each animal and categorized as RME. The number of repeated entries into each arm that never included a platform was also recorded, resulting in working memory incorrect errors (WMICE). Latency to reach the platform was also measured. Mice that failed to swim or exhibited abnormal behavior were excluded from analysis [30].

2.5 Biochemical analyses: catalase activity assay

After behavioral testing, the animals were euthanized, and blood and brain tissues were collected. Brains were preserved in cold phosphate buffer (50 mM, pH 7.4), and hippocampal tissues were homogenized in 50 mM phosphate buffer (pH 7.4) on ice. Total protein concentrations in plasma and hippocampal tissue homogenates were determined using the Bradford protein assay, based on the binding of Coomassie Brilliant Blue G-250 to proteins [31]. Briefly, bovine serum albumin (BSA) was used as the protein standard, and a series of BSA standard solutions covering the concentration range of 0.1–1.0 mg/mL was prepared. The absorbance of the standards and samples was measured at 595 nm after addition of the Bradford reagent. A calibration curve was generated by plotting absorbance at 595 nm against the corresponding BSA concentration. The resulting standard curve was described by the linear regression equation, $y = 0.1195x + 0.0132$ ($R^2 = 0.9749$), and the protein concentrations of the unknown samples were calculated from this calibration curve. The Bradford assay was performed according to the principle originally described by Bradford (1976), in which protein binding to Coomassie Brilliant Blue G-250 produces an increase in absorbance at 595 nm.

Catalase (CAT) activity was determined using the spectrophotometric ammonium molybdate method described by Góth (1991) [32]. Briefly, 0.2 mL of plasma or hippocampal tissue homogenate was mixed with 1.0 mL of hydrogen peroxide (H_2O_2 ; 65 μ M final concentration) prepared in 60 mM sodium–potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 60 s to allow enzymatic decomposition of H_2O_2 . The reaction was then terminated by adding 1.0 mL of ammonium molybdate (32.4 mM). The residual H_2O_2 reacted with ammonium molybdate to form a stable yellow complex, and its absorbance was measured spectrophotometrically at 405 nm. Appropriate reaction controls were included to account for background absorbance. CAT activity was calculated based on the amount of H_2O_2 remaining after the reaction and was normalized to the total protein content of each sample. The results were expressed as kU/mg protein.

2.6 Statistical analysis

Data were analyzed using SPSS version 26 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Shapiro–Wilk test, and Q–Q plots were additionally examined to evaluate normality. Data were considered normally distributed when the Shapiro–Wilk test indicated no significant

deviation from normality ($P > 0.05$). Normally distributed data were analyzed using analysis of covariance (ANCOVA), with body weight included as a covariate, followed by Tukey's post hoc test for multiple comparisons. For data that did not meet the assumption of normality, the Kruskal–Wallis test was used for comparisons among groups, followed by appropriate post hoc pairwise comparisons. For the OLT and NORT data, repeated-measures ANCOVA was performed to account for within-subject repeated measurements, with body weight included as a covariate where appropriate. Statistical significance was set at $P < 0.05$.

3. Results and Discussion

3.1 Physical properties of nanoparticles and curcumin

Maximum absorption for curcumin (Figure 1-A), AgNPs (Figure 1-B), and AgNPs-Curcumin (Figure 1-C) were observed at 400–450 nm, 420 nm, and 420–430 nm, respectively. Absorption is closely related to a substance's physical and chemical characteristics. Typically, curcumin's optimal absorption range as a therapeutic agent is between 400 and 600 nm [33, 34]. Furthermore, the physical and chemical properties of nanoparticles, which are associated with their absorption range [35], must align with an optimal wavelength to facilitate effective interactions with biological elements (e.g., cell membranes, surface receptors) [36, 37].

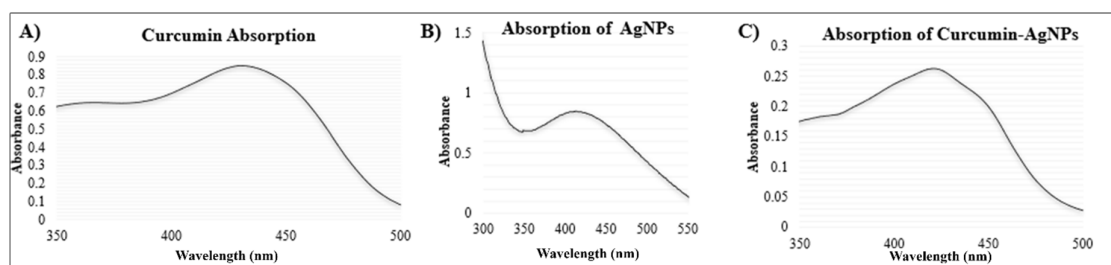


Figure 1. Figure A shows the absorption spectrum of curcumin spans 400–450 nm, with a characteristic peak at 425 nm. Figure B presents the absorption spectrum of synthesized silver nanoparticles (AgNPs); the absorption peak is at approximately 420 nm. Figure C displays the absorption spectrum of silver nanoparticles loaded with curcumin (AgNPs-Curcumin), while the absorption peak shifts slightly to around 420–430 nm

The Dynamic Light Scattering (DLS) method was used to determine the size and particle size distribution of the nanoparticles. The results showed that the nanoparticles ranged in size from 10 to 60 nm (Figure 2-A). After loading with curcumin, the DLS analysis showed a predominant particle size distribution in the range of roughly 300–400 nm, with a peak at around 320 nm (Figure 2-B). Scanning Electron Microscopy (SEM) confirmed that the nanoparticle sizes were consistent with the results obtained from DLS. SEM images revealed that the nanoparticles were spherical (Figure 2-C). Additionally, Energy Dispersive X-ray Spectroscopy (EDX) was employed to identify the nanoparticles as AgNPs (Figure 2-D).

AgNPs can effectively load and release both hydrophilic and hydrophobic drugs. They also possess distinctive optical, electrical, and thermal properties, along with a high surface-to-volume ratio, which enables functionalization for targeting specific cells or tissues. Various synthesis methods—biological, physical, and chemical—allow precise control over size, surface chemistry, and shape, thereby minimizing toxicity and optimizing bioavailability [38, 39].

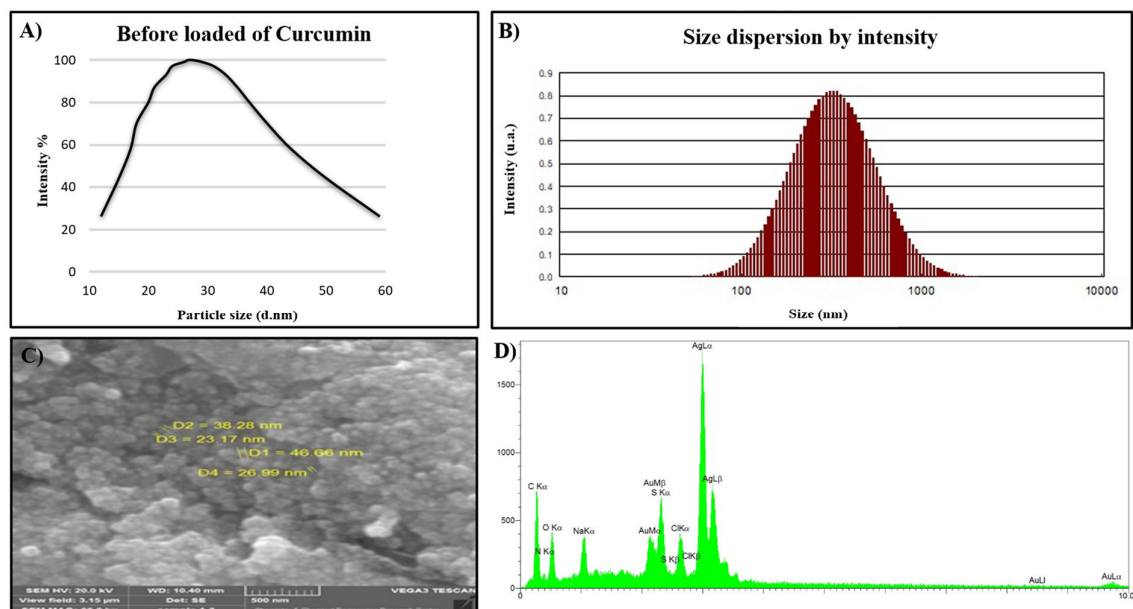


Figure 2. The sizes of the nanoparticles and curcumin-loaded nanoparticles were measured using DLS. (A) Size distribution of the synthesized silver nanoparticles (AgNPs), ranging from 10 to 60 nm. (B) Size distribution of the curcumin-loaded nanoparticles, with a particle size of approximately 320 nm. (C) Morphological characterization of the nanoparticles by SEM. (D) EDX analysis showing the elemental composition of the nanoparticles. The Au peaks observed in the EDX spectrum are attributed to the gold sputtering process used for SEM sample preparation and do not represent an intrinsic component or impurity of the nanoparticles

3.2 OLT and NORT tests

The analysis of covariance (ANCOVA), with weight as a covariate, was conducted using a repeated-measures ANCOVA (RMANCOVA) to analyze data from the object and novel-location recognition tests in the DI-OLT test at both the Pre-Trial and Post-Trial phases across all four groups. Notably, only the TNC50 group exhibited a significant increase in the DI-OLT index from Pre-Trial to Post-Trial, while the other three groups showed a decrease (Figure 3-A). Furthermore, this analysis indicated that the increase in the DI-NORT for TNC50 and TNC25 compared to the control group (Pre-Trial) was not statistically significant (Figure 3-B). This finding may highlight the beneficial effects of TNC50 on memory processes.

The hippocampus has a high metabolic rate and a lipid-rich environment, making it particularly vulnerable to oxidative stress, which adversely affects its structure and function [7]. During insomnia, ROS are generated, leading to oxidative stress; the sleep process supports antioxidant activity, which helps brain cells reduce ROS levels [6]. Mitochondrial dysfunction and suppression of antioxidant systems, such as glutathione peroxidase (GPx) and superoxide dismutase (SOD), contribute to oxidative stress. Dysregulation of transcription factors such as NRF2, which regulates the expression of antioxidant genes like SOD1 and GST, is another consequence of insomnia [40, 41]. Oxidative stress can lead to neuronal death by impairing postsynaptic currents, reducing the proliferation of dentate progenitor cells, and hindering the maturation of interneurons [42]. Furthermore, oxidative stress disrupts GABAergic neurotransmission, which is essential for proper hippocampal function and memory processing [43].

In the PTIT-OLT index, the TNC50 group exhibited a significant difference compared to the control group. Additionally, the TNC25 group showed an increase in the index from Pre-Trial to Post-Trial, whereas the TCtrl

and TC50 groups demonstrated a decrease in the PTIT-OLT index (Figure 3-C). On the other hand, the PTIT-NORT analysis indicated that the TNC25 and TNC50 groups showed an upward trend; however, compared with the control group (Pre-Trial), these differences were not statistically significant. Similarly, the TCtrl and TC50 groups showed a decreasing trend (Figure 3-D).

The OLT assesses spatial learning and memory, which rely heavily on the hippocampus, whereas NORT emphasizes non-spatial learning [29]. Furthermore, NORT significantly involves various brain areas, including the perirhinal cortex, temporal association cortex, fusiform and parietal cortices, and the hippocampus, whereas OLT primarily focuses on the hippocampus [29,44,45]. It seems curcumin may enhance spatial memory and learning, particularly in the hippocampus, and also positively influences non-spatial learning and memory processes [46].

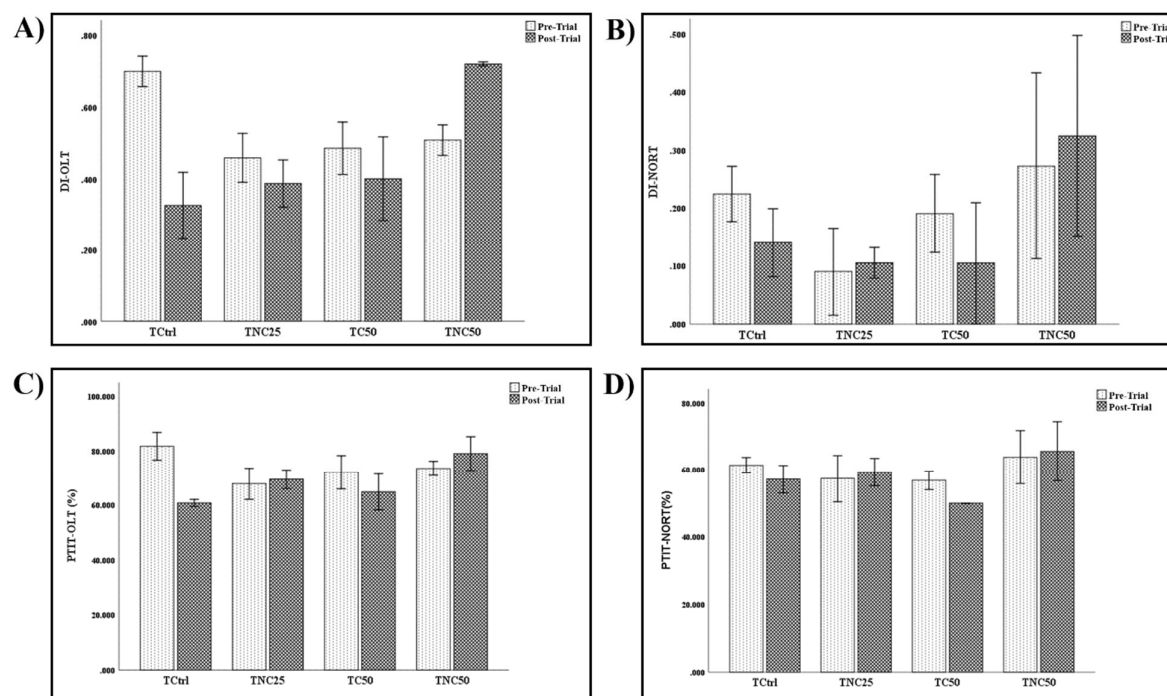


Figure 3. The results of the DI analysis and the PTIT index for the two tests—the Object Location Test (OLT) and the Novel Object Recognition Test (NORT)—were evaluated during the Pre-Trial and Post-Trial phases across all four tested groups (TCtrl, TNC25, TC50, and TNC50). Figure A presents the DI results for the OLT (DI-OLT), revealing a significant effect only in the TNC50 group. Figure B displays the DI results for the NORT (DI-NORT), indicating no significant differences between the Pre-Trial and Post-Trial phases in any of the four groups. Figure C displays the PTIT results for the OLT test (PTIT-OLT), revealing a statistically significant difference between the TNC50 and TNC25 groups. Figure D presents the PTIT results for the NORT test (PTIT-NORT), which show no significant differences among any of the tested groups during either the Pre-Trial or Post-Trial phases. Error bars represent ± 1 standard error (SE)

3.3 The ERAWM test

The nonparametric Kruskal-Wallis test was used to analyze the ERAWM test data statistically. The TNC50 and TNC25 groups showed significant differences in WMICE (Figure 4-A) and RME (Figure 4-B) compared to the TCtrl group. Since RME is associated with long-term memory and WMICE with short-term memory [30], these findings highlight curcumin's positive effects on memory processing [47, 48]. Mice subjected to stress but treated

with AgNPs-Curcumin showed reduced errors in both short- and long-term memory compared to the control group (TCtrl). The TC50 group showed no notable effects, suggesting that the nanocarrier plays a crucial role in curcumin efficacy. As documented, curcumin faces several intrinsic challenges, including poor stability in the bloodstream, low water solubility, rapid metabolism, limited bioavailability, and minimal ability to cross the BBB [24].

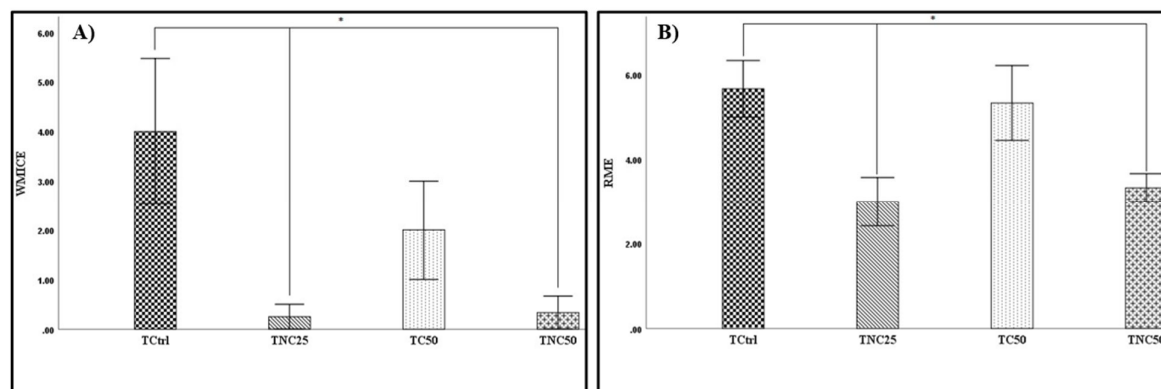


Figure 4. The results of the Elevated Radial Arm Water Maze (EARWM) test were analyzed using the nonparametric Kruskal-Wallis test, which evaluated two indices: RME and working memory incorrect errors (WMICE) across four test groups—TCtrl, TNC25, TC50, and TNC50. Figure A presents the EARWM results for the WMICE index, showing a statistically significant difference between the TNC50 and TNC25 groups compared to the control group. Similarly, Figure B shows the results for the RME index, indicating a statistically significant difference between the TNC50 and TNC25 groups compared with the control group. Error bars represent ± 1 standard error (SE)

3.4 Catalase enzyme activity analysis

An ANCOVA was conducted with weight as a covariate, followed by Tukey's post hoc test to analyze CAT enzyme activity. CAT activity in blood plasma (PCA) was assessed, revealing that the TNC25 group differed significantly from the other groups (Figure 5-A). Similarly, CAT activity in hippocampal tissue homogenate (HCA) was significantly higher in the TNC50 group than in the other experimental groups (Figure 5B).

Antioxidant enzymes, including CAT, glutathione reductase (GR), SODs, and GPx, contribute to the reduction of ROS levels [22]. Several studies have indicated that curcumin can enhance antioxidant activity in the hippocampus under oxidative stress [47, 48]. The induction of antioxidant enzymes, including CAT, may contribute to this effect.

Accordingly, the reduction of ROS levels may help preserve hippocampal function and improve performance in the OLT. Curcumin exhibits antioxidant activity through several mechanisms. It can directly neutralize free radicals by donating hydrogen atoms from its phenolic groups. Curcumin may also modulate enzymes involved in oxidative and inflammatory processes, including cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS). In addition, curcumin can react with lipid peroxyl radicals and form relatively stable products through oxidative coupling and intramolecular reactions, thereby terminating lipid peroxidation chains [18, 19, 49].

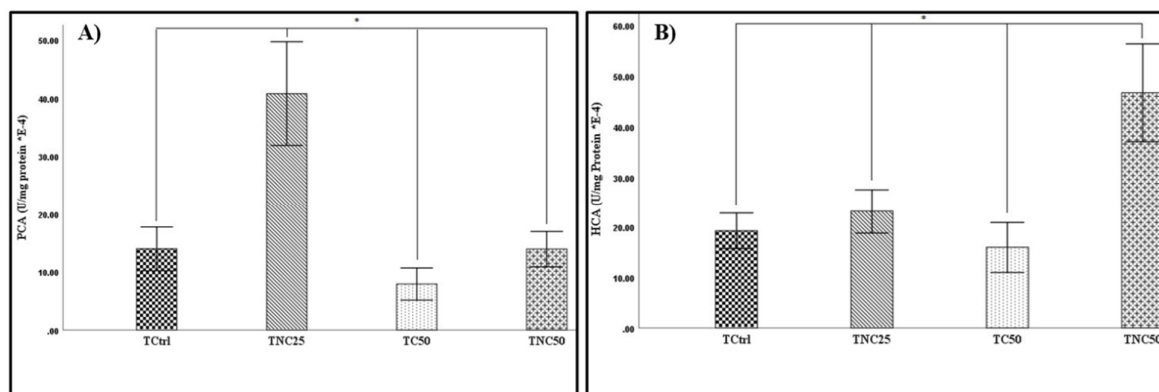


Figure 5. Analysis of CAT activity in blood plasma (PCA) and hippocampus (HCA) was conducted across all four experimental groups (TCtrl, TNC25, TC50, and TNC50). Figure A illustrates CAT activity in blood plasma, revealing a significant difference in the TNC25 group compared to the control group. Figure B presents CAT activity analysis in the hippocampus, showing a statistically significant difference in the TNC50 group compared with the control group. Error bars represent ± 1 standard error (SE)

3.5 Analysis of weight during the experiment

Examining the average weight on the first day of the study and comparing it with the weight on the day of dissection showed that there was no significant difference between the groups (Figure 6).

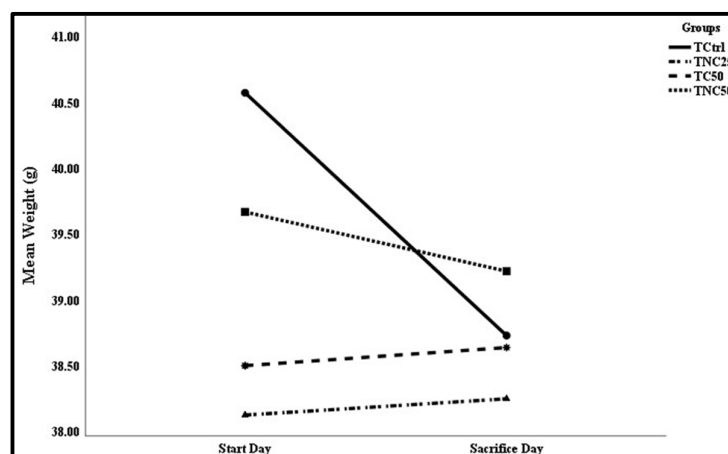


Figure 6. Analysis of the mean weight of mice on the first day of the study and on the day of dissection was conducted across all four groups tested (TCtrl, TNC25, TC50, and TNC50). The results showed that the TCtrl group experienced the greatest weight loss, losing 2 grams compared to the other groups; however, this difference was not statistically significant

One important limitation of the present study is the absence of a separate control group receiving AgNPs alone without curcumin loading. Therefore, the independent contribution of AgNPs to the observed behavioral and biochemical effects could not be directly determined. Although the free-curcumin group (TC50) did not show a significant improvement compared with the sleep-deprived control group, this finding alone does not establish that the observed effects of the curcumin-loaded AgNP formulation were specifically attributable to nanoparticle-mediated delivery.

Another limitation is the absence of a non-sleep-deprived control group. Consequently, the extent to which sleep deprivation altered the measured behavioral and biochemical parameters, and whether the treatments restored

these parameters toward non-sleep-deprived levels, could not be directly determined. Moreover, AgNPs may exert biological effects that depend on their physicochemical properties, concentration, and exposure conditions. Previous studies have reported potential toxicological effects of AgNPs, including oxidative stress, inflammation, genotoxicity, neurotoxicity, immunotoxicity, hepatotoxicity, nephrotoxicity, and reproductive toxicity [50]. Therefore, future studies should include both AgNP-only and non-sleep-deprived control groups to better distinguish the independent effects of AgNPs, the effects of curcumin loading, and the consequences of sleep deprivation.

4. Conclusion

This study demonstrated that AgNP-mediated delivery of curcumin produced greater behavioral and biochemical effects than curcumin administered without nanoparticles in mice subjected to sleep deprivation. Among the treatment conditions, AgNP-Curcumin at a dose of 50 mg/kg was associated with the greatest improvement in memory-related behavioral performance and increased CAT activity in hippocampal tissue and blood plasma. In contrast, curcumin administered without AgNPs did not produce a significant improvement under the conditions tested. These findings suggest that nanoparticle-based delivery may contribute to the biological activity of curcumin in the context of sleep deprivation-induced memory impairment. However, further studies are needed to clarify the underlying mechanisms, determine the pharmacokinetic and bioavailability profiles of AgNP-delivered curcumin, and evaluate the safety and efficacy of this approach using additional doses, experimental endpoints, and longer treatment periods.

Author Contributions

Amirhossein Reshadat: Investigation; **Seyfollah Ahmadi-Bootegaz:** Writing—original draft preparation, Theoretical concepts, Analysis and interpretation of results; **Toktam Hajjar:** Supervision, Writing—review and editing, Methodology and validation; **Nasrin Mollania:** Methodology and validation; **Javad Shakibaei-far:** Writing—original draft preparation; **Alireza Ghodsi:** formal analysis and software. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding this article.

Declaration of Generative AI and AI-assisted Technologies in the Manuscript Preparation Process

During the preparation of this manuscript, ChatGPT (OpenAI, USA) was used solely as a language support tool to enhance the fluency, grammar, and readability of the text. The tool was not used to generate data, conduct analysis, or create figures. All authors reviewed, edited, and approved the final content and take full responsibility for the manuscript.

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