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RESEARCH PAPER

Role of the cholinergic muscarinic receptors of the CA1 area in the memory impairment induced by iron oxide nanoparticle in adult male rats

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ABSTRACT

Objective(s): Nanoparticles of iron oxide (nFe_2O_3) are widely used in medicine and industry and could interfere with the brain processes associated with memory. The involvement of muscarinic cholinergic receptors in the process of memory formation has been confirmed. The present study aimed to investigate the possible interference of the cholinergic muscarinic receptors of the dorsal hippocampal CA1 area in the memory impairment induced by nFe_2O_3 in adult male rats.

Materials and Methods: In this study, we examined the possible involvement of the cholinergic muscarinic receptors of the dorsal hippocampal CA1 area in the memory impairment induced by nFe_2O_3. In total, 70 male rats were divided into 10 groups of saline (1 µl/rat)+saline (1 ml/kg; intraperitoneal [IP]), saline (1 µl/rat)+nFe_2O_3 (5 and 7.5 mg/kg; IP), pilocarpine (1 and 2 µg/rat)+saline (1 ml/kg), pilocarpine (1 and 2 µg/rat)+nFe_2O_3 (7.5 mg/kg; IP), scopolamine (1 and 2 µg/rat)+saline (1 ml/kg), and scopolamine (1 µg/rat)+nFe_2O_3 (5 mg/kg; IP).

Results: Pilocarpine and scopolamine were injected intra-CA1 after training and before the IP administration of nFe_2O_3. The latency to enter the dark compartment in the step-through apparatus and locomotor activity was performed on the animals in an open field at 24 hours and seven days after training. The results indicated that nFe_2O_3 (7.5 mg/kg) decreased memory retrieval ($P<0.01$), while pilocarpine (1 and 2 µg/rat) significantly increased the step-through latency compared to the animals receiving nFe_2O_3 only ($P<0.01$). In addition, scopolamine (2 µg/rat) significantly decreased the step-through latency compared to the controls ($P<0.05$), while scopolamine (1 µg/rat) with nFe_2O_3 (5 mg/kg) reduced the step-through latency compared to the controls ($P<0.01$).

Conclusion: According to the results, nFe_2O_3 could impair memory through decreasing the function of the cholinergic muscarinic receptors of the dorsal hippocampal CA1 area. It seems that the memory impairment caused by nFe_2O_3 might be due to the reduced activity of the cholinergic muscarinic receptors of the dorsal hippocampal CA1 area.

Keywords: *Cholinergic system, Hippocampus, Iron oxide, Memory, Nanoparticle*

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INTRODUCTION

Learning is defined as the biological process of new knowledge, where memory is involved in the retaining and reconstructing of the acquired knowledge over time [1]. Memory consists of various stages, including acquisition, consolidation, retention, and retrieval [2, 3].

The hippocampus, central amygdaloid nucleus, and ventromedial region of the striatum are involved in learning and memory [4]. The hippocampus plays a key role in the memory function [5]. The dentate gyrus, cornu ammonis (CA1-CA4), and subiculum are the subfields of the hippocampus [4, 6]. CA1 acts as a medium for neural plasticity processes, which are involved in the acquisition, storage, and retrieval of memory within the hippocampus. The neurons (cholinergic, GABAergic, peptidergic, and glutamatergic) from the medial septal-diagonal band of the Broca complex enter the hippocampal system. These inputs regulate the physiology of the hippocampal structure, which involved in the memory function [7].

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The central cholinergic system is critically involved in all the known memory processes. The release of endogenous acetylcholine (ACh) by cholinergic neurons is essential to the modulation of acquisition, encoding, consolidation, reconsolidation, extinction, retrieval, and expression [8, 9]. Furthermore, it is responsible for the regulation of neuronal excitability throughout the nervous system by acting on the cyst-loop ligand-gated nicotinic acetylcholine receptor channels (nAChRs) and G-protein-coupled muscarinic acetylcholine receptors (mAChRs) [10, 11].

Various neurological disorders (e.g., Alzheimer's disease) are associated with cognitive deficits that are caused by the dysfunction of the hippocampal cholinergic systems. The CA1 area of the dorsal hippocampus is a significant region in the brain, where mAChRs are expressed [10, 12, 13].

Iron is essential to many metabolic processes, including the biosynthesis of lipids and cholesterol and normal neuronal function. However, iron accumulation in cells leads to toxicity, and the high ability of this element to accept and donate electrons may lead to the formation of nitrogen and reactive oxygen species, causing oxidative damage to tissue components. Moreover, oxidative stress induced by iron deposition could lead to memory impairment and reduced motor activity, while playing a key role in the pathogenesis of several neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [14-16].

Recently, iron oxide (nFe_2O_3) has attracted the attention of researchers as a new source of the iron ions with superparamagnetic physicochemical characteristics and potential application in various biomedical and industrial fields, including biomedical brain imaging, cellular therapy, brain-targeted drug or gene delivery, and hyperthermia [17]. The accumulation of nFe_2O_3 results in the peroxidation of brain lipids, inducing damage to the membrane and reducing the levels of lactate dehydrogenase, which in turn leads to brain tissue and neuronal damage. In addition, degeneration of nFe_2O_3 nanoparticles in the pyramidal cells of the CA1, CA2, and CA3 areas of the hippocampus has been reported in histopathological studies [18].

Considering the growing application of nano-materials (especially nFe_2O_3) in industrial and consumable productions and the possibility of their unintentional entry into the human body and detrimental effects on various parts of the central nervous system, as well as the role of central cholinergic system in the process of memory formation, the present study aimed to investigate the possible interference of the cholinergic muscarinic receptors of the dorsal hippocampal CA1 area in the memory impairment induced by nFe_2O_3 in adult male rats.

MATERIALS AND METHODS

Animal care

This experimental study was conducted on male Wistar rats weighing 200±50 grams, which were purchased from the animal house at the Medical Science Department of Jondi Shapur University of Ahvaz, Iran. The rats were kept for more than a week in a room at the temperature of 24±1˚C within a controlled 12-hour light/dark cycle. They were accommodated in polypropylene cages (four rats per cage). With the exception of the short test periods, food and drinking water were available to the animals. Each study group contained 6-8 rats. The experiments were performed during the light phase, and each rat was used only once. The experiments were carried out at 09:0014:00. All the institutional guidelines for animal care and use were observed at Shahid Chamran University of Ahvaz.

Compounds

The components used in the study included nFe_2O_3 (USNANO Co., USA), pilocarpine (Sina Daru Co., Iran), and scopolamine (Sigma Co., Germany). In this study, nFe_2O_3 was prepared by sonication for 15 minutes in an ultrasonic bath. The resulting suspension was shaken for one minute before each injection [19]. Pilocarpine and scopolamine were dissolved in 0.9% saline.

For peripheral administration, nFe_2O_3 and saline were injected intraperitoneally (IP) at the measured concentrations (mg/kg) in the volume of one milliliter per kilogram. For central administration, pilocarpine (1 and 2 µg/rat), scopolamine (1 and 2 µg/rat) or saline (1 µl/rat) were injected into the intra-CA1 area of the dorsal hippocampus. The animals were divided into 10 groups of saline (1 µl/rat) + saline (1 ml/kg; IP), saline (1 µl/rat) + nFe_2O_3 (5 and 7.5 mg/kg; IP), pilocarpine (1 and 2 µg/rat) + saline (1 ml/kg), pilocarpine (1 and 2 µg/rat) + nFe_2O_3 (7.5 mg/kg; IP), scopolamine (1 and 2 µg/rat) + saline (1 ml/kg), and scopolamine (1 µg/rat) + nFe_2O_3 (5 mg/kg; IP) [20,22].

Animal surgery

The male Wistar rats were anesthetized via the IP injection of a solution containing ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) and kept in a stereotaxic frame in a flat-skull position. A midline cut was made in the skin of the skull, and the underlying periosteum was drawn back. A stainless steel guide cannula (22 gauges) was implanted in the dorsal hippocampus. The coordinates of the cannula implantation in the CA1 of the dorsal hippocampus were determined in accordance with the Paxinos and Watson rat brain atlas (anterocaudal: -2.6 mm, lateral: ±2 with respect to the bregma, vertical: 3.3 mm from the dura). In addition, two 22-gauge stainless steel guide cannulas were placed bilaterally and anchored to the skull with two jeweler screws and acrylic dental cement.

After the surgery, the rats were allowed to recover for seven days. Following that, the drug solutions were injected within one minute via in internal cannula (27 gauges), which was attached with a polyethylene tube to a Hamilton syringe (2 µl). The injection cannula was left in place for an additional one minute prior to the slow withdrawal. The left and right hippocampus was injected with 0.5 microliter of the solution on each side (1 µl/rat) within one minute [23].

Passive avoidance apparatus

The step-through instrument (learning box) used in this study was composed oftwo chambers; one chamber was light (white compartment), and the other was dark (black compartment). The diameters of both compartments were20×20×30 centimeters. At the center of the partition a guillotine door opening (diameters: 7×9 cm) was made between the two compartments. In addition, stainless steel grids (diameter: 2.5 mm) were placed at one-centimeter intervals (distance between the centers of the grids) on the floor of the dark compartment in order to produce a foot shock. Intermittent electric shocks (50 Hz, three seconds, 1mA intensity) were induced to the grids on the floor of the dark compartment using an insulated stimulator [24, 25].

Open field

The open field apparatus was used for the assessment of the locomotor activity in the rats, which was composed of a transparent Plexiglass square box (diameters: 40×40 cm) with high walls (30 cm). In this experiment, the number of the movements was recorded within five minute [26].

Behavioral procedures

In this study, the behavioral procedures were carried out in two phases of training and testing.

Training

At this stage, the rats were placed in the light compartment on the training day. After 10 seconds, the doors were opened and closed as soon as the rats entered the dark compartment and were transferred to the home cage. Afterwards, the latency of entry to the the dark compartment (step-through latency [STL]) was measured. The rats with longer STL than 100 seconds were excluded from the study [25]. After 30 seconds, each rat was placed in the light compartment, and after 10 seconds, the door was opened. The door was closed immediately after all the four paws of the animals were on the grid floor, and electrical AC shock was induced to the chamber. Finally, 20 seconds after receiving the shock, the rats were placed in their home cage temporarily.

After two minutes, the rats were placed in the light compartment again as in the prior trials. The acquisition stage of passive avoidance memory was successfully completed if the STL was longer than 120 seconds. This procedure was repeated once more on the animals that did not complete the test successfully; as such, each animal received a maximum of three electrical shocks [25].

Testing

Long-term memory retrieval of the animals was assessed 24 hours after the training. The rats were placed in the light chamber for 10 seconds, and the door was opened. During this phase, no electrical shocks were induced. In order to assess the memory retrieval [27], the STLs was recorded for 300 seconds. In addition, drug injections were performed after the training.

Confirmation of the injection sites

After the experiments, the rats were sacrificed by the microinjection of 1% chloroform (0.5 µl/side; intra-CA1). After decapitation, the brains of the rats were extracted and placed in 10% formalin solution for 10 days. Brain sections were studied in terms of the CA1 area using the Paxinos and Watson rat brain atlas (1997) in order to determine the cannula locations and injection

sites. Data on the rats receiving drugs outside the CA1 area were not considered [23].

Statistical analysis

Data analysis was performed using one-way analysis of variance (ANOVA) and Tukey's test in InStat software, and the results were expressed as mean and standard error of the mean (SEM) at the significance level of P<0.05.

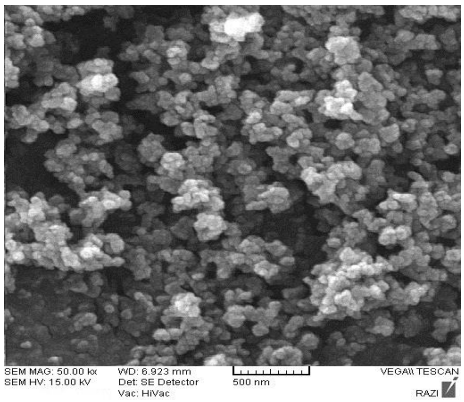

Fig 1. Scanning Electron Microscopy Image of nFe2O3 Dry Powder

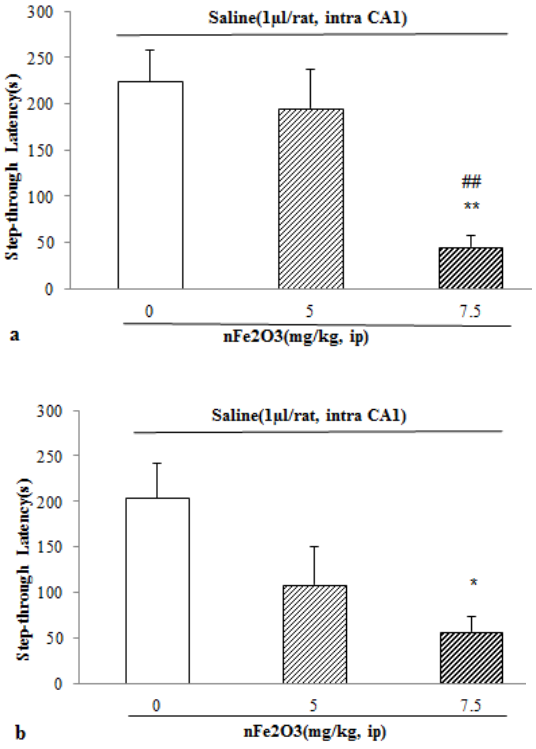

Fig 2. Effect of Saline + nFe2O3 on Memory Retrieval (vertical scale: step-through latency at a) 24 hours and b) after training; After training, the animals received nFe2O3 [5 and 7.5 mg/kg; IPwith saline [1 µg/rat; intra-CA1] and were tested after a) one day and b) seven days; Columns show mean±SEM [n=7]; **P<0.01 and *P<0.05 compared to control group##P<0.01 compared to nFe2O3 group [5 mg/kg]

RESULTS

Effect of saline + nFe_2O_3 on memory retrieval

Fig 2 shows the effect of nFe_2O_3 on the step-through latency 24 hours (Fig 2-a) and seven days (Fig 2-b) after training. According to the results of one-way ANOVA, nFe_2O_3 (7.5 mg/kg) significantly decreased the step-through latency compared to the control group (P<0.05; F[2:18]=9.163; F [2:18]=4.808).

Effect of the intra-CA1 microinjection of pilocarpine + saline on memory retrieval

Fig 3 depicts the effect of the intra-CA1 microinjection of pilocarpine on step-through latency 24 hours (Fig 3-a) and seven day (Fig 3-b) after training. The results of one-way ANOVA indicated no significant difference compared to the control group in this regard (F[2:18]=0.2384; F[2:18]=0.3483).

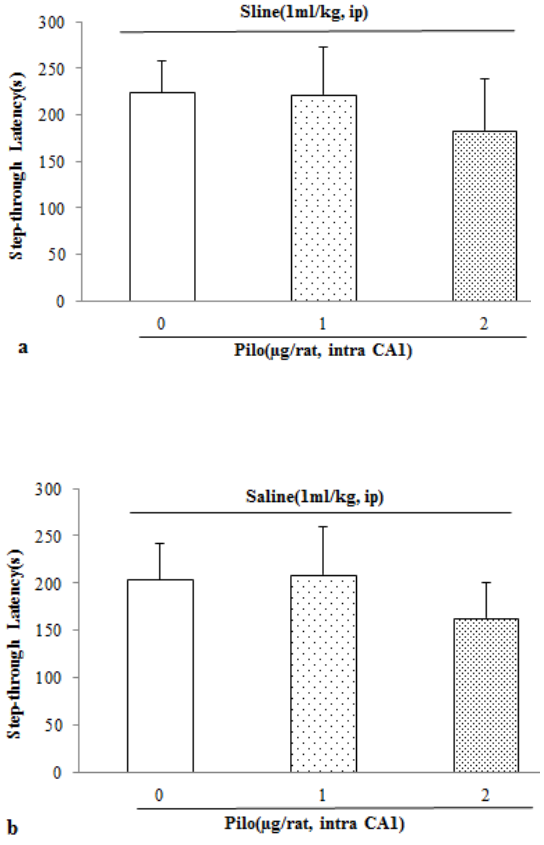

Fig 3. Effect of Pilocarpine on Memory Retrieval (vertical scale: step-through latency at a) 24 hours and b) seven days after training; After training, the animals received pilocarpine [1 and 2 µg/rat; intra-CA1]with saline [1 ml/kg; IP] and were tested after a) one day and b) seven days; Columns show mean±SEM [n=7]

Effect of the intra-CA1 microinjection of pilocarpine on the effect of nfe$_2$o$_3$ on memory retrieval

Fig 4 shows the effect of the intra-CA1 microinjection of pilocarpine on the effect of nFe_2O_3 on step-through latency 24 hours (Fig 4-a) and seven days (Fig 4-b) after training.The results of one-way ANOVA demonstrated that pilocarpine (1 and 2 µg/rat) significantly increased the step-through latency compared to the group receiving nFe_2O_3 alone (P<0.05; F[3:24]=5.692; F[3:24]=3.884).

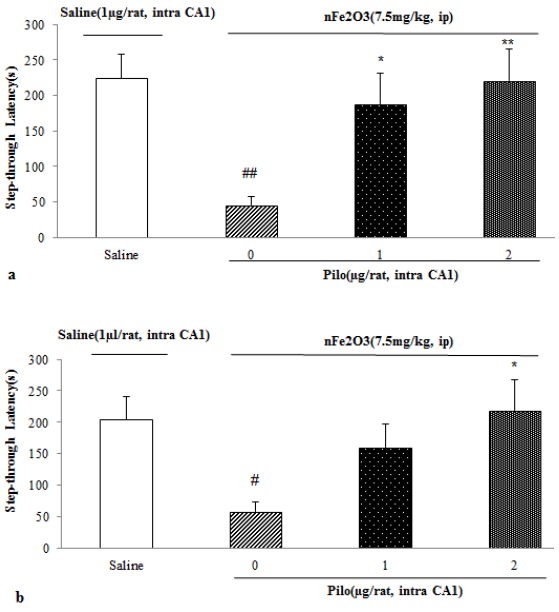

Fig 4. Effect of Intra-CA1 Microinjection of Pilocarpine on Effect of nFe2O3 on Memory Retrieval (vertical scale: step-through latency at a) 24 hours and b) seven days after training; After training, the animals received pilocarpine [1 and 2 µg/rat; intra-CA1] with Fe2O3 [7.5 ml/kg; IP] and were tested after a) one day and b) seven days; Columns show mean±SEM [n=7]; *P<0.05 and **P<0.01 compared to saline/Fe2O3 group and #P<0.05 and ##P<0.01 compared to control group

Effect of the intra-CA1 microinjection of scopolamine on memory retrieval

Fig 5 depicts the effect of scopolamine on step-through latency 24 hours (Fig 5-a) and seven days (Fig 5-b) after training.The results of one-way ANOVA indicated that scopolamine (2 µg/rat) significantly decreased the step-through latency compared to the control group (P<0.05; F[2:18]=2:18; F[2:18]=3.831).

Effect of the Intra-CA1 microinjection of an ineffective dose of scopolamine on the ineffective Dose of Fe_2O_3 nanoparticles on memory retrieval

Fig 6 shows the effect of the intra-CA1 microinjection of an ineffective dose of scopolamine on the ineffective dose of nFe_2O_3 on step-through latency 24 hours (Fig 6-a) and seven days (Fig 6-b) after training.

The results of one-way ANOVA indicated that the scopolamine (1 µg/rat; intra-CA1) and nFe_2O_3 (5 mg/kg; IP) significantly decreased the step-through latency compared to the control group (P<0.05; F[3:24] =5.247; F[3:24] = 3.184).

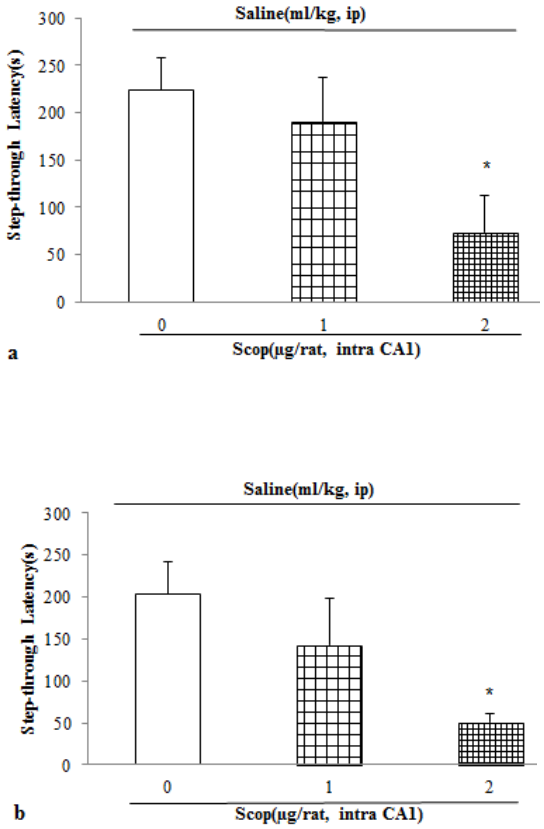

Fig 5. Effect of Scopolamine on Memory Retrieval (vertical scale: step-through latency at a) 24 hours and b) seven days after training; After training, the animals received scopolamine [1 and 2 µg/rat; intra-CA1]with saline [1 ml/kg; IP] and were tested after a) one day and b) seven days; Columns show mean±SEM [n=7]; *P<0.05 compared to control group

Effect of nFe_2O_3 on locomotor activity

Fig 7 depicts the effect of nFe_2O_3 on locomotor activity (number of crossing in the open field) 24 hours (Fig 7-a) and seven days (Fig 7-b) after training.

The results of one-way ANOVA showed no significant difference compared to the control group (F[2:18] =0.4030; F[2:18] =0.1226).

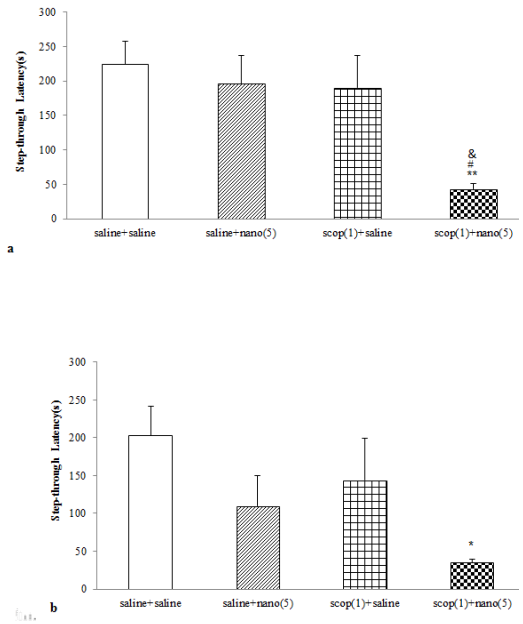

Fig 6. Effect of Ineffective Dose of Scopolamine with Ineffective Dose of nFe2O3 on Memory Retrieval (vertical scale: step-through latency at a) 24 hours and b) seven days after training; After training, the animals received scopolamine [1 µg/rat; intra-CA1] with nFe2O3 [5 mg/kg; IP] and were tested after a) one and b) seven days; Columns show mean±SEM [n=7]; **P<0.01 and *P<0.05 compared to control group, P<0.05 compared to nFe2O3 group, and #P<0.05 compared to saline/nFe2O3 group

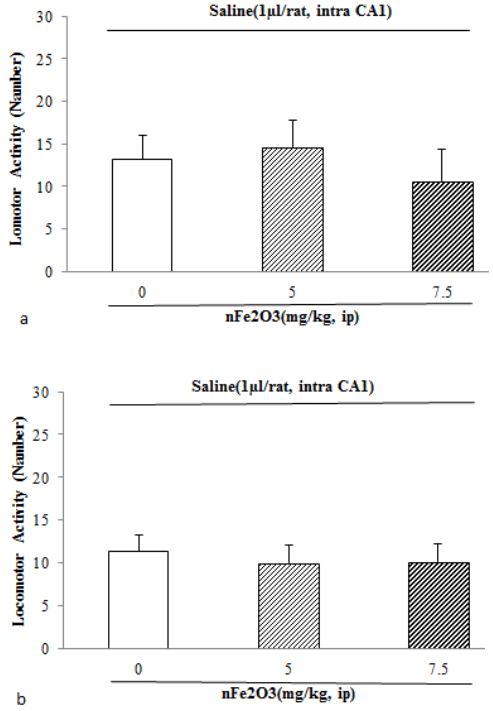

Fig 7. Effect of Scopolamine on Locomotor Activity (vertical scale: step-through latency at a) 24 hours and b) seven days after training). After training, the animals received nFe2O3 [5 and 7.5 mg/kg; IP] with saline [1 µl/rat; intra-CA1] and were tested after a) one day and b) seven days; Columns show mean±SEM [n=7]

Effect of the intra-CA1 microinjection of pilocarpine on locomotor activity

Fig 8 shows the effect of pilocarpine on locomotor activity (number of crossing in the open field) 24 hours (Fig 8-a) and seven days (Fig 8-b) after training.

The results of one-way ANOVA indicated no significant difference compared to the control group (F[2:18]=0.2912; F[2:18]=0.8922).

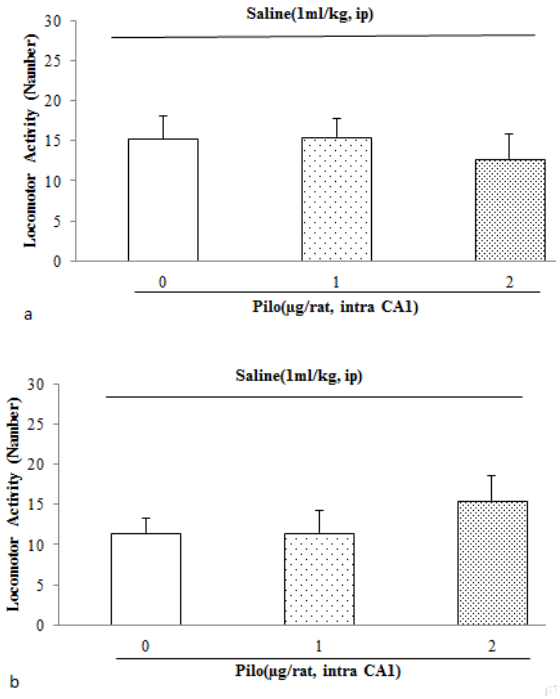

Fig 8. Effect of Pilocarpine on Locomotor Activity (vertical scale: step-through latency at a) 24 hours and b) seven days after training). After training, the animals received pilocarpine [1 and 2 µg/rat; intra-CA1]with saline [1 ml/kg; IP] and were tested after a) one day and b) seven days; Columns show mean±SEM [n=7]

Effect of the intra-CA1 microinjection of pilocarpine on the effect of nfe_2o_3 on locomotor activity

Fig 9 shows the effect of the intra-CA1 microinjection of pilocarpine on the effect of nFe_2O_3 on locomotor activity (number of crossing in the open field) 24 hours (Fig 9-a) and seven days (Fig 9-b) after training.

The results of one-way ANOVA showed no significant difference compared to the control group (F[3:24]=0.1403; F[3:3]=0.5445).

Effect of the intra-CA1 microinjection of scopolamine on locomotor activity

Fig 10 depicts the effect of the intra-CA1 microinjection of scopolamine on locomotor activity (number of crossing in the open field) 24

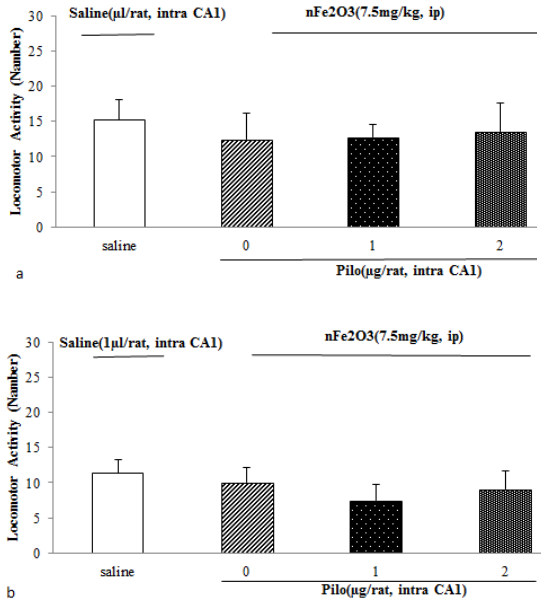

Fig 9. Effect of Intra-CA1 Microinjection of Pilocarpine on Effect of nFe2O3 on Locomotor Activity (vertical scale: step-through latency at a) 24 hours and b) seven days after training). After training, the animals received pilocarpine [1 and 2 µg/rat; intra-CA1] with Fe2O3 [7.5 ml/kg; IP] and were tested after a) one day and b) seven days; Columns show mean±SEM [n=7]

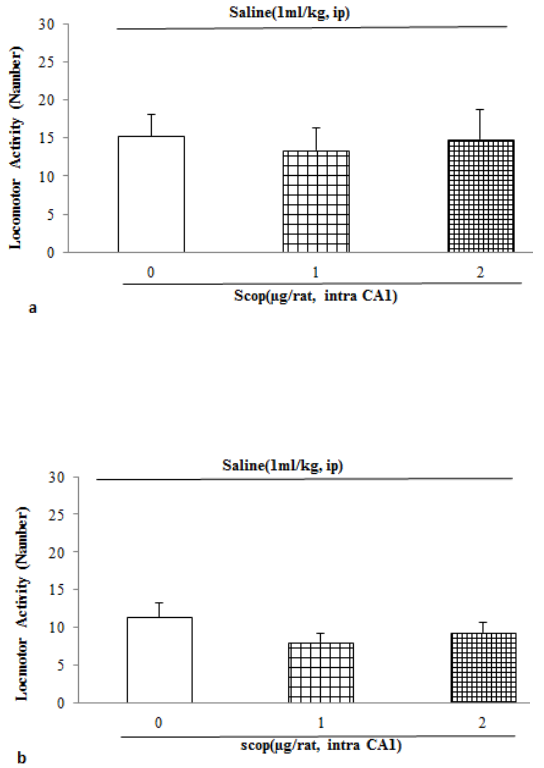

Fig 10. Effect of Scopolamine on Locomotor Activity (vertical scale: step-through latency at a) 24 hours and b) seven days after training). After training, the animals received scopolamine [1 and 2 µg/rat; intra-CA1] with saline [1 ml/kg; IP] and were tested after a) one day and b) seven days; Columns show mean±SEM [n=7]; *P<0.05 compared to control group

hours (Fig 10-a) and seven days (Fig 10-b) after training.

The results of one-way ANOVA indicated no significant difference compared to the control group (F[2:18] =0.07935; F[2:18] =1.118).

Effect of the intra-CA1 microinjection of scopolamine on the effect of nfe$_2$o$_3$ on locomotor activity

Fig 11 shows the effect of the intra-CA1 microinjection of scopolamine on the effect of nFe_2O_3 on locomotor activity (number of crossing in the open field) 24 hours (Fig 11-a) and seven days (Fig 11-b) after training.

The results of one-way ANOVA showed no significant difference compared to the control group (F[3:24] =0.2728; F 3:24] =0.2728).

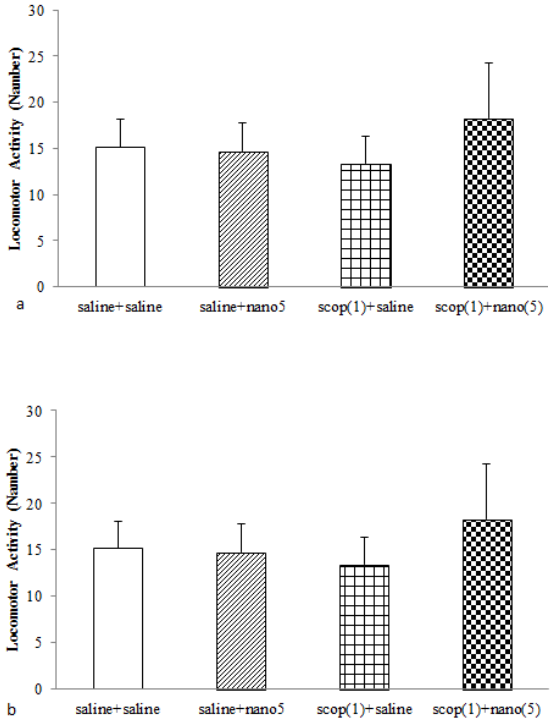

Fig 11. Effect of Ineffective Dose of Scopolamine with Ineffective Dose of nFe2O3 on Locomotor Activity (vertical scale: step-through latency at a) 24 hours and b) seven days after training). After training, the animals received scopolamine [1 µg/rat; intra CA1] with nFe2O3 [5 mg/kg; IP] and were tested after a) one day and b) seven days; Columns show mean±SEM [n=7]

DISCUSSION

According to the results of the present study, the IP injection of nFe_2O_3 after training decreased memory retrieval in the passive avoidance memory. Previous studies have indicated that iron therapy in the neonatal period degenerates the

seminal cognitive memory in rats. Furthermore, other findings in this regard have denoted that iron therapy in the neonatal period results in lipid peroxidation and protein carboniferous in the hippocampus, cortex, and substantia nigra, thereby leading to cognitive weakness [28]. According to Rivet et al. (2011), the exposure of neurons to nFe_2O_3 causes cell damage and neuronal death [29].

According to the findings of Sun et al. (2013), nFe_2O_3 accumulates in neurons, astrocytes, and capillary endothelial cells, thereby reducing the efficacy of neurons and astrocytes [30]. On the same note, Wu et al. (2013) stated that nFe_2O_3 may contribute to cell cytotoxicity and the development of neurodegenerative diseases (e.g., Alzheimer's disease and Parkinson's disease). In addition, nFe_2O_3 plays a key role in reducing the viability of neurons, triggering oxidative stress, and activating JNK and *p53*, which are involved in the pathway of cell cycle and apoptosis. Other findings have indicated that nFe_2O_3 could be transferred via the olfactory nerve tract to the brain, causing oxidative stress and ultra-structural alterations in the olfactory bulb nerve cells [31].

The results of the present study indicated the involvement of the cholinergic muscarinic receptors in the effects of nFe_2O_3 on memory retrieval. Furthermore, our findings showed that the intra-CA1 microinjection of pilocarpine (agonist of the muscarinic cholinergic receptors) had no effects on the memoryfunction when used alone; whileit reduced the destructive effects of these substances on memory in combination with nFe_2O_3. Consistently, Roesler et al. reported that the acute injection of the muscarinic cholinergic receptor agonist after training could improves memory impairment through the elevated levels of iron in the neonatal period of rats [32]. The cholinergic input to the neocortex, dorsal hippocampus, and basolateral amygdala in these brain regions is essential to neural function and synaptic plasticity [33].

Pharmacological inhibition or the genetic ablation of muscarinic receptors disrupts hippocampus-dependent memory [34-36]. On the contrary, enhancing endogenous acetylcholine with acetylcholinesterase inhibitors in Alzheimer's disease or the activation of M1 muscarinic receptors in cognitively impaired humans have been reported to improve the memory function [37]. Given the importance of synaptic plasticity in memory processes, the release of acetylcholine has been suggested to improve learning through modulating the induction and expression of synaptic plasticity. Undoubtedly, the induction of hippocampal synaptic plasticity during learning requires muscarinic receptors [38]. In this regard, the findings of Sarlak et al. (2015) indicated that acetylcholine agonists (i.e., nicotine and pilocarpine) could enhance learning and memory, whereas the antagonists of acetylcholine (i.e., succinylcholine and scopolamine) decrease learning and memory [13]. According to a study by Bymaster et al. (2003), pilocarpine manifests its effect through increasing phosphoinositide hydrolysis [39]. The cholinergic system is closely connected with the GABAergic and glutamatergic neurons, and the terminal of the cholinergic neurons in the hippocampus, neocortex, and amygdala often contribute to the formation of GABAergic fibers [25]. According to the research by Khakpai et al. (2012), the injection of ineffective scopolamine along with D-AP7 (NMDA receptor antagonists) into the CA1 area decreased the memory function, indicating the reciprocal interaction of the cholinergic and glutamatergic systems [40]. In the present study, the injection of an ineffective dose of scopolamine (intra-CA1) after the IP injection of an ineffective dose of nFe_2O_3 was observed to reduce long-term memory retrieval on the test day.

Oxidative stress and inflammation are among the main causes of age-related cognitive impairment. Muscular cholinergic receptors are involved in antioxidant and anti-apoptotic responses, and the agonist of muscarinic receptors mediates the anti-epitope responses by activating the antioxidant defense mechanism, while the inhibition of the muscarinic receptors reduces the expression of the E2 factor (a transcription factor for regulating the expression of the proteins that activate the antioxidant cell responses) [41].

Disorders of the cholinergic system may be caused by ageing, and the injection of muscarinic receptor antagonist decreases the expression of antioxidant enzymes (e.g., catalase and superoxide dismutase) by reducing the expression of the transcription factor Nrf2 [41]. In this regard, Tota et al. (2012) reported that the IP injection of scopolamine could decrease the blood-brain flow and acetylcholine levels, while increasing the levels of acetylcholinesterase (AChE) and malondialdehyde (MDA) due to lipid peroxidation,

thereby playing a role in memory impairment [42]. The findings of Ghasemi et al. (2018) indicated that the IP injection of scopolamine increased the AChE and MDA levels in the hippocampal and cortical tissues, while decreasing superoxide dismutase and catalase activities in the brain [43].

In the study by Khakpai et al. (2012), the injection of an ineffective dose of scopolamine with D-AP7 (NMDA receptor antagonist) into the CA1 area was reported to cause memory impairment, indicating the interaction between the cholinergic and glutamatergic systems [40]. Normal memory function and cognition involve a complex interaction between dopamine and acetylcholine [44]. The intra-injection of scopolamine has been reported to reduce the release of dopamine in the hippocampus and protuberance cortex, thereby resulting in forgetfulness in the passive avoidance memory [44].

In another research, Azami et al. (2010) stated that the intra-hippocampal injection of scopolamine after training and at the pretest caused the impairment of the memory retrieval process, while the injection of various concentrations of the α-noradrenergic agonist (phenylephrine) and 2α-noradrenergic agonist (clonidine) into the CA1 area was reported to improve the memory impairment caused by scopolamine injection after training; this finding confirms the association of the cholinergic and adrenergic systems in the memory process [44].

CONCLUSION

According to the resultsnFe_2O_3 decreased the memory function through the reduction of the activities of the muscarinic cholinergic systems in the CA1 area of the dorsal hippocampus. Such effect of nFe_2O_3 might be directly and or indirectly exerted through the mediation of other neurotransmitter systems.

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