**Title:** Coenzyme Q10 suppressed Cue-related Reinstatement through Cognition Improvement and Hippocampal Increases BDNF Expression in Morphine-Dependent Male Rats

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## Abstract

**Background:** Opioid dependence significantly disrupts cognitive activities such as learning and memory, which may be the reason for a return to drug use. Morphine (MOR) can increase oxidative damage in the brain. We aim to investigate the effect of coenzyme Q10 (CoQ10) on cognitive impairment, cue-related reinstatement, and expression of BDNF in MOR-dependent rats.

**Methods:** In this study, 40 male Wistar rats (200-220g) were divided into 5 experimental groups (n=8) as follows: Oil group, MOR+Oil group, MOR+Q10-100 group, MOR+Q10-200 group, MOR+Q10-400 group. The rats were administered increasing doses of MOR (25 to 100mg/kg, s.c.) once daily. After 21 days of addiction, CoQ10 treatment is administered by gavage at doses of 100, 200 and 400 mg/kg once daily for one month. CoQ10 is dissolved in 1 cc of sesame oil and administered. Behavioral assessments were performed using a novel object recognition test, working memory in the Y-maze, social interaction, and conditioned place preference. Expression of BDNF was assessed in the hippocampus by immunohistochemistry.

**Results:** Treatment with CoQ10 at a dosage of 100, 200 and 400 mg/kg within 4 weeks resulted in a significant improvement in the NOR task ($P<0.01$, $P<0.001$), working memory in the Y-maze ($P<0.01$, $P<0.001$), social interaction ($P<0.001$), cue-related reinstatement in the CPP ($P<0.01$, $P<0.001$) and significantly increased expression of BDNF ($P<0.001$) in the hippocampus of male rats.

**Conclusions**: CoQ10 could improve cognitive impairment and reduce reinstatement in MOR-addicted male rats. Histologic examination confirmed the neuroprotective effects of CoQ10 in the hippocampus. CoQ10 could be a potential therapeutic agent for MOR-induced cognitive impairment and relapse.

**Keywords**: Coenzyme Q10, Cognitive Dysfunction, Recurrence, Morphine, Opioid Dependence

## Introduction

As we know, opioids prove to be the most efficient pain relievers for the treatment of various forms of pain.(Jaremko et al., 2014). But the use of opioids is limited by the fears of addiction and tolerance (Heshmatzad, Nasehi, & Vaseghi, 2021). One of the most powerful substances that is obtained from opium poppy (Papaver somniferum plant) is morphine (MOR), which can lead to relaxation and euphoria (Raehal & Bohn, 2005). Therefore, MOR and other opioids such as heroin have extremely high abuse potential (Ghamati et al., 2014). In recent decades, studies have considered opioid addiction to be a "relapsing chronic disease" that severely affects public health (Leshner, 1998). Of note, the persistence of opioid use disorder is known by the resumption of drug-seeking and drug-abusing behaviors that are activated by stimuli associated with drug use, even long after the withdrawal period (Han et al., 2015). Cue-related reinstatement in morphine-addicted rats refers to the phenomenon where environmental cues associated with drug use trigger a resurgence of drug-seeking behavior after a period of abstinence. Studies show that the insular cortex (IC) and the nucleus accumbens (NAc) are critical in mediating cue-induced reinstatement of morphine-seeking behavior. Activation of glutamatergic projections from the IC to the NAc core is essential for this reinstatement (Zhang et al., 2019).

Opioid addiction severely disrupts cognitive activities such as learning, memory, and mood processes, which can be the reason for returning to drug use. For example, a past study has revealed that chronic MOR significantly impairs working memory in rats (Sala et al., 1994). Also, a previous study has shown that the inhibitory memory of rats is significantly impaired by post-training administration of MOR (2.5, 5, and 7.5 mg/kg) (Tavassoli, Alinaghipour, & Ardjmand, 2017). Furthermore, it has been shown that long-term MOR addiction potently attenuates neurogenesis, disrupts memory performance, and changes emotional reactivity and anxiety levels in male rats (Famitafreshi, Karimian, & Marefati, 2015). Previous research has shown that rats exposed to long-term MOR exhibit deficits in spatial memory as assessed by the Morris water maze task (Brolin et al., 2018). In addition, reports are indicating that MOR has the potential to increase oxidative stress in the rodent brain. (Milanesi et al., 2023). As we know, oxidative stress can lead to a wide range of cognitive impairments (Kholghi, Alipour, Rezaie, Zarrindast, & Vaseghi, 2023; Mehrabanifar, Hesami-Tackallou, Vaseghi, & Nasehi, 2023).

Coenzyme Q10 (2, 3-dimethoxy-5-methyl-6-multiprenyl-1, 4-benzoquinone, CoQ10) is a fat-soluble vitamin-like substance and an essential electron carrier in the respiratory chain of the inner mitochondrial membrane for ATP metabolic processes. The nature of coQ10 in the rapid acquisition and loss of electrons causes the strong antioxidant properties of this compound. CoQ10 prevents lipid peroxidation and protein oxidation by preventing the generation of peroxyl radicals. To deal with oxidative stress, the cell is well equipped with an enzymatic antioxidant defense system. CoQ10 passes through the blood-brain barrier if taken orally or by intravenous injection. CoQ10 prevents tissue damage and causes exogenous preservation and survival of nerve cells. The medical literature highlights the recurring emphasis on the beneficial impact of CoQ10 on the process of learning and memory. The positive effects of CoQ10 on the learning and memory process are emphasized in the medical literature. A recent study has shown that CoQ10 significantly improves amyloid-beta (Aβ)-induced decline in discrimination index in the novel object recognition (NOR) test, learning, and spatial memory tested in the Morris water maze,

passive avoidance memory and learning, and long-term potentiation (LTP) deficit in the hippocampus of aged animals (Asadbegi et al., 2023). In another study, CoQ10 showed anti-inflammatory and anti-oxidant properties.

The change of brain-derived neurotrophic factor (BDNF) in morphine-addicted rats is a significant area of research, revealing complex interactions between substance use and neuroplasticity. Morphine dependence leads to a decrease in BDNF levels of cerebrospinal fluid (CSF) during active addiction, while withdrawal triggers an increase in BDNF levels (Rezamohammadi, Rahmani, Ghanbari, Khaleghian, & Miladi-Gorji, 2020). In the hippocampus, BDNF levels were found to enhance during morphine withdrawal, suggesting a compensatory mechanism following addiction (Fatahi et al., 2020). Increased BDNF expression in the ventral tegmental area (VTA) was associated with reduced behavioral sensitization to morphine, indicating a protective role against addiction (Deng et al., 2023). CoQ10 was observed to stimulate the production of BDNF, an important protein involved in neuroplasticity associated with learning and memory, and SOX2, a transcription factor critical for maintaining self-renewal, in the hippocampus of a rat model of Alzheimer's disease (M. Sheykhhasan et al., 2022). A study has shown that treatment with CoQ10 may lead to neuroprotection against the detrimental effects of Aβ on synaptic plasticity in the hippocampus of rats, by the improvement of antioxidant activity (H. Komaki et al., 2019). Furthermore, it has been shown that CoQ10 can alleviate cognitive impairments induced by intracerebroventricular injection of streptozotocin in experimental rodents (Ishrat et al., 2006). According to these findings, we aim to investigate the effect of CoQ10 on cognitive impairments, cue-related reinstatement, and expression of BDNF in MOR-dependent male rats.

## Material and Method

### Animals

In this study, 48 male Wistar rats (200-220g) were used. Each Plexiglas cage consisted of 4 rats, that were housed under a 12-h light cycle (lights beginning at 7:00 a.m.), constant humidity, and temperature (23±2°C). All the rats had free access to food and water and all the experiments were done during the light hours (8:00 a.m. to 3:00 p.m.). Also, the rats were bred at the Neuroscience Research Center, Mazandaran University of Medical Sciences, Sari, Iran. Our experimental protocol was designed under the National Institutes of Health Guide for the Care and Use of Animals Lab (Politis et al., 2011).

### Drugs

The drugs used in the study included MOR Sulfate (Darupakhsh-Iran), Coenzym $Q_{10}$ (303-98-0, Sigma-Aldrich; Merck, C9538, Germany), Naloxone hydrochloride (Caspian-Iran), Ketamine and xylazine (Alfasan Company, Holland).

### Experimental Protocol

This study included 6 experimental groups (n=8) as follows:
1) Oil group: received Sesame Oil for one month by oral gavage.
2) MOR+Oil group: received MOR for 3 weeks and normal saline by oral gavage during one month of withdrawal.

3) MOR+Q10-100 group: received MOR for 3 weeks and CoQ10 (100 mg/kg) for by oral gavage.
4) MOR+Q10-200 group: received MOR for 3 weeks and CoQ10 (200 mg/kg) for a month by oral gavage.
5) MOR+Q10-400 group: received MOR for 3 weeks and CoQ10 (400 mg/kg) for a month by oral gavage.
6) Q10-400 group: received CoQ10 (400 mg/kg) for a month by oral gavage.

MOR sulfate powder was dissolved in distilled water (DW). Rats were administered increasing doses of MOR (25 to 100mg/kg, s.c.) once a day, for 21 days. Days 1-5; 5mg (25mg/kg, s.c.), days 6-10; 10 mg (50mg/kg, s.c.), days 11-15; 15 mg (75mg/kg, s.c.), days 16-21; 20 mg (100mg/kg/s.c.). CoQ10 dissolved in Sesame oil and administered through gavage. The volume of oral gavage was 1 cc (Ebrahimi & Esmaeili-Mahani, 2020; Matthews, Yang, Browne, Baik, & Beal, 1998; Paul & Gueven, 2021; Rauscher, Sanders, & Watkins III, 2001; Shibani et al., 2019).

To confirm MOR dependence, on day 21, naloxone (2 mg/kg, i.p.) was injected 2h after MOR administration intraperitoneally and withdrawal symptoms were immediately observed (jumping, shaking the head like a dog, grinding teeth, chewing, standing on two legs, scratching the cage, digging) and recorded for 30 minutes (Fig 1, A). The frequency of defecation during the period of observing the signs of withdrawal (30 minutes), and also, the percentage of 24-hour weight loss of the rats are measured. After observing and recording the signs of withdrawal, the rats are returned to the cage. (Akbari & Mirzaei, 2013). After 21 days of addiction, CoQ10 treatment is administered orally with a gavage syringe number 24, at doses of 100, 200, and 400 mg/kg, once a day for one month (Fig 1, B). Then, behavioral tests including novel object recognition (NOR) test, Y-maze, social interaction (SI), and conditioned place preference (CPP) were performed in all animals of groups, except the animals of the morphine-naloxone group used for the confirmation addiction model. Four animals per group for immunofluorescent staining (IMF) were used.

**Novel Object Recognition Test**

The investigation of the NOR test was performed in a cube-shaped box measuring 40×50×50 $cm^3$, in which different objects can be placed in a fixed position in two adjacent corners. This test consists of three phases: habituation, training, and testing. A- Habituation phase: In this phase, the rat is placed in the middle of the box without objects for ten minutes to fully investigate the arena. This phase of the test is repeated the next day in the same way. At the end of two consecutive days of the habituation phase, the habituation phase is completed.
B-Training phase: In this phase, two identical objects (A & A) are placed in the box and the animal is allowed to examine the objects for 10 minutes. Touching the object with the nose and placing the nose directly at a distance of less than two centimeters from the object is the criterion for testing by the animal. Leaning the animal against the objects or sitting on the objects is not considered part of the time for searching or examining the object. During the training phase, the time each animal spent examining each object was recorded.
C-Testing: A new object (A & B) replaced one of the previous objects in the box. In this phase, the animal has 10 minutes to examine the objects. It should be noted that the objects are exchanged for the next animal in each phase of the test to rule out the possibility of preference. After each

animal has left the box, the environment and objects in the box are cleaned with 70% alcohol. The time each animal spends with each object is recorded and the discrimination index (the time each animal spends examining the novel object (object B) minus the time spent examining the repeated object (object A), is divided by the total time spent examining both objects (Lissner, Wartchow, Toniazzo, Gonçalves, & Rodrigues, 2021).

**Y-maze**

Working memory was assessed using the Y-maze test. This test consisted of three identical arms, each 45 cm long and 10 cm wide and with a wall height of 30 cm. These arms were arranged at an angle of 120° to each other. The test was carried out in low light (5 lux). The performance of the rats during the test was recorded with a video camera attached above the device and the order of entries into the maze arms was recorded (arm entries, AE) was recorded manually. The percentage of spontaneous alternations (SA) was determined as follows: SA% = [SA / (number of AE - 2)] * 100. Only trials with eight or more arm movements were included in the data analysis (Shcherbakova et al., 2023).

**Social Interaction (SI)**

The SI test was carried out for 10 minutes in a box with dimensions of 43× 19 × 22 $cm^3$, which is divided into three parts (This space is unfamiliar to the animals and there is no habituation). Two wired cup-like cages large enough to accommodate the rat, were placed in two side chambers and in once an unfamiliar rat was placed. The experiment was carried out during the light phase with an intensity of 650 lux. An observer was carefully observed and recorded the parameters of the test. At the acclimatization stage, empty wire cages were placed in each part and the rat was placed in the middle part and remained there for five minutes to achieve habituation. The first stage of the test: is the aspect of social tendencies in this part of the behavioral test, the aim is to check the social tendencies of the rat. The assumption of the test is based on the fact that the natural subject animal has a greater desire to communicate with another animal than to interact and communicate with an empty chamber. At this stage, one of the control rats (stranger one) was placed in one of the chambers. The doors between the parts were removed so that the subject rat placed in the middle part could move between the areas. The following parameters were carefully checked and observed: a. the duration of direct (active) contact with a rat (a stranger) inside the chamber. In the second stage of the test, the level of the animal's desire to establish new social relationships and the type of its preference is checked. It is assumed that a healthy subject shows more desire and preference to communicate with a new animal. Therefore, the second animal (stranger 2) was placed in another empty chamber and the parameters stated in the previous stage were observed and checked, and the behavioral differences of the rat in interaction with stranger 1 and compared with stranger 2 were considered and observed. The duration of the two stages was 10 minutes (Kaidanovich-Beilin, Lipina, Vukobradovic, Roder, & Woodgett, 2011).

**Conditioned Place Preference (CPP)**

The CPP was tested in a three-part Plexiglas box (30×40×30 cm3). The CPP procedure comprised a pre-test followed by a 3-day conditioning phase and a test divided into three different phases.

Familiarization phase. Before making the addiction model (on the 1st day), each rat was placed in the neutral box for 15 minutes, where it had free access to all three rooms. The time spent in each room was measured and the preferred room was determined. Conditioning phase. From the 2nd to the 4th day, each rat received an injection of MOR and was placed and confined in the non-preferred room of the CPP device for 45 min while the doors were closed. After 6 hours, each rat received an injection of DW as a vehicle for MOR and was moved to the preferred room of the CPP. On the 3rd day, the protocol was the same as the 1st day in the morning to the afternoon and vice versa. Test. In this phase (on the fifth day), each rat was tested for the CPP under MOR-free conditions. The rat was placed in the CPP apparatus for 10 minutes and had freely movement to all three rooms. The time spent in each room was recorded. The difference between the time spent in the rooms paired with and without reward was considered as the CPP score (Jamali, Zarrabian, & Haghparast, 2021).

**Immunohistochemistry Method**

To perform an immunohistochemical examination (n=4), rats were anesthetized with xylazine (20 mg/kg) and ketamine (160 mg/kg) and were stabilized by transcranial perfusion. Then, the head of the rats was separated and the brain was removed and kept in a 10% buffered formalin solution. 48 to 72 hours after immersing the brain in a 10% formalin solution, the samples were cut from the hippocampus, and tissue processing and preparation of paraffin blocks were performed. 5-micron thick slices were prepared from paraffin blocks by microtome. For immunohistochemical staining, slides were first deparaffinized, and then other steps were performed in the following order:
Deparaffinizing the slides in Gesylol solution, hydration in descending alcohols at 100, 90, 80, and 70 degrees respectively, incubation of the slices in Standard Sodium Citrate SSC 2x for 2 hours at 65 degrees, incubation in 2N HCL for 30 minutes at 37°C, soaking in one-tenth normal boric acid (PH=8.5) for 10 minutes, washing in PBS, incubation with primary antibody (BDNF) overnight at 4°C, immersion in three loads in PBS and each time for 10 minutes, incubation with secondary antibody-BDNF for 2 hours, hematoxylin staining for background staining (Counter staining), sticking the slide, observing with a light microscope, examining the slides with ImageE software, and determining the level of BDNF expression in different test groups and preparing images of the tissues by microscope (Sayyah et al., 2022).

**Statistical Analyses**

The behavioral and histological data were analyzed using GraphPad Prism software. One-way ANOVA, two-way ANOVA, and post hoc Tukey's were used to compare the differences among the experimental groups. Data are as mean±SD (standard deviation) and P-value <0.05 was considered statistically significant.

**Results**

**Effects of CoQ10 on Recognition Memory**

The MOR group showed a significant decrease in DI compared with the oil group (P<0.001, Fig. 2A). This finding shows the cognitive dysfunction that was observed in MOR-addicted rats. DI

was significantly increased in the MOR-Q10 (100), MOR-Q10 (200), and MOR-Q10 (400) groups of rats compared with the MOR group ($P<0.01$, $P<0.001$, Fig. 2A). In addition, Figure 2B indicated no significant difference in total time of exploration in comparison with the MOR group. MOR-addicted rats treated with CoQ10 explored more new objects and the DI increased, suggesting that CoQ10 has a neuroprotective impact on the recognition memory impairment caused by MOR.

**Effects of CoQ10 on Working Memory in the Y Maze**

The MOR group showed a significant ($P < 0.001$) decrease in percentage alternations in comparison to the oil group, which indicated a loss of memory in the MOR group. The CoQ10 treated groups showed a statistically significant ($P < 0.01$, $P < 0.001$, Fig. 3) increase in percentage alternations in comparison to MOR group.

**Effects of CoQ10 on Social Interaction**

The MOR group showed a significant ($P < 0.001$, Fig. 4A) decrease in time spent in social interaction (staying in rat room). The CoQ10 treated groups showed a statistically significant ($P < 0.001$, Fig. 4A) increase in time spent in social interactive behaviors. Figures 4B and 4C display that there was statistically significant ($P < 0.001$) interaction between MOR-Q10 (100) and MOR-Q10 (200) groups of rats regarding sociability interaction with trapped stranger2 (unfamiliar rat)) in comparison with trapped stranger1 (now-familiar rat).

**Effects of CoQ10 on Relapse in the CPP**

The rats in all groups that received MOR over three days of conditioning, showed a significant ($P < 0.001$, Fig. 5, CPP1) increase in time spent in MOR-paired room. This finding shows the place conditioning that occurred in MOR-addicted rats. After CoQ10 treatment in MOR-addicted rats, changes in the preference enhanced significantly increased in CoQ10 treated groups ($P < 0.01$, $P < 0.001$). The finding indicated the time spent in the MOR-paired chamber reduced significantly (between CPP1 and CPP2) and suggesting reduced relapse in CoQ10-treated groups.

**Effects of CoQ10 on BDNF Expression in Hippocampus**

The MOR-addicted group indicated a significantly lower BDNF expression level of the hippocampus in comparison with the Oil group ($P<0.05$, Fig. 6). The CoQ10 treated groups showed a statistically significant increase in BDNF expression level in comparison with the MOR-addicted group ($P<0.001$, Fig. 6).

**Discussion**

The present research aimed at determining the impacts of CoQ10 treatment on cognitive impairments and relapse induced by MOR use and BDNF expression was examined. Based on the findings, CoQ10 led to significant improvement of NOR, working memory, SI, cue-related reinstatement, and significantly increased expression of BDNF in the hippocampus of male rats. Our results show that CoQ10 may have a potential role in the treatment of cognitive deficits and relapse caused by MOR addiction. Our findings are in line with the multiple studies using laboratory animals that have reported the beneficial protective impacts of CoQ10 against various

CNS disorders including Parkinson's disease (Ghasemloo et al., 2021; Park et al., 2020), Alzheimer's disease (Hamidreza Komaki et al., 2019; Mohsen Sheykhhasan et al., 2022), aging-induced memory impairments (Hosseini et al., 2022), diabetes-induced memory impairments (Monsef, Shahidi, & Komaki, 2019; Omidi et al., 2019), DDVP-induced cognitive impairments and neurodegeneration (Binukumar et al., 2012). Our study is in line with studies that have shown that MOR impairs NOR memory. Craig et al. in a study in 2015 reported that performance on the NOR test of neonatal rats was compromised in the prolonged MOR administration group at a young age (Postnatal Days 27–31) (Craig & Bajic, 2015). Also, the results of a study performed by Paris et al. indicated significantly reduced NOR after i.c.v. administration of Kappa opioid receptor (KOR) agonists and pretreatment of antagonists of KOR prevented KOR-mediated dysfunction in NOR (Paris, Reilley, & McLaughlin, 2011). Moreover, in a study, Alipour et al. (2023) reported that paternal MOR use during adolescence can cause a dysfunction in the recognition of new objects and passive avoidance memory in male offspring (Alipour, Shojaei, Rezaei, Mirnajafi-Zadeh, & Azizi, 2023). Also, Ellis et al. in a study in 2020 showed paternal MOR addiction led to NOR memory deficit in the female offspring (Ellis et al., 2020). The findings of present study in agreement to multiple evidences indicated MOR have a negative impact on working memory (Amirteimoury, Fatemi, Hassanshahi, & Kaeidi, 2019; Bach et al., 2021; Bonk, 2011; Wang et al., 2013). Conversely, Miladi Gorji et al. in a study (2008) showed chronic use to MOR did not affect either retention or acquisition of spatial working memory (Miladi Gorji, Rashidy-Pour, & Fathollahi, 2008). Also, our data in agreement with previous research, indicated MOR administration in adult rats also reduced social interaction and lead to less time spent interacting with social peers (Šlamberová et al., 2016). Hughes et al. (2021) demonstrated that MOR reduced both social novelty preference and sociability behavior in adolescent rats (postnatal days 28–43) (Hughes et al., 2021). Our findings revealed the beneficial effects of CoQ10 treatment on cognitive impairments in MOR-addicted male rats. CoQ10 has been studied for its effects on memory and cognitive function in various contexts and the results were controversial. CoQ10 supplementation recommends CoQ10 as an adjunct to conventional treatment of migraine, Alzheimer's disease, multiple sclerosis, coronary heart disease, and cerebrovascular accidents (Rauchová, 2021). The obtained findings are consistent with the study of Omidi et al. (2019) on rats with streptozotocin-induced diabetes, high dose supplementation with CoQ10 improved spatial memory and learning, while low-dose supplementation did not have an effect (Omidi et al., 2019). Similarly, in a study on middle-aged rats by Monsef et al. (2019), CoQ10 supplementation improved memory, learning, and cognitive activities in both diabetic and healthy subjects, with higher doses showing better results (Monsef et al., 2019). In contrast, Maguire et al. (2021) reported that CoQ10 had no effect on working memory and attention in patients with schizophrenia and schizoaffective disorder (Maguire et al., 2021). It should be noted that CoQ10 is not approved by the FDA for the treatment of any diseases. It is consumed as a food additive, not as a drug (Arenas-Jal, Suñé-Negre, & García-Montoya, 2020). Moreover, Sumien et al. (2009) found that prolonged consumption of CoQ10 in a low dose has no discernable effect on motor and cognitive activities whereas intake at a higher dose exacerbates sensory and cognitive dysfunction in old mice (Sumien, Heinrich, Shetty, Sohal, & Forster, 2009).

The present results reveal that our findings support literature indicating the BDNF levels in the brain of MOR-addicted male rats altered. Fatahi et al. (2020) reported that BDNF levels increased

during delay-based decision-making in the hippocampus of MOR-addicted rats after withdrawal from MOR (Fatahi et al., 2020). Deng et al. (2022) showed that the BDNF-adenoviral vector administered in the VTA by injection, decreased MOR-induced CPP with changes in BDNF/TrkB/CREB levels in the VTA and NAc (Deng et al., 2022). Furthermore, a study conducted by Peregud et al. (2020) revealed that chronic MOR intoxication reduces the binding of HuD, an RNA-binding protein, to the long 3'-UTR of BDNF, and MOR withdrawal stimulates the expression of BDNF in the frontal cortex of rats (Peregud, Panchenko, & Gulyaeva, 2022).

The results of our research suggest that the administration of CoQ10 could increase the expression of BDNF in the hippocampus after chronic use of MOR in male rats. In line with our results, Vaselbehagh et al. (2021) found that CoQ10 treatment significantly increased the expression of BDNF in the CA1 area of the hippocampus in methadone-induced neurotoxicity (Vaselbehagh, Sadegh, Karami, Babaie, & Sakhaie, 2021). Also, Nagib et al. (2019) reported α-Tocopherol and/or CoQ10 on Phenytoin-induced cognitive dysfunction through a change in the BDNF-TrkB-CREB signaling pathway (Nagib et al., 2019). In addition, Abuelezz et al. (2023) showed a neuroprotective effect of CoQ10 against scopolamine-induced cognitive impairment by activating the PI3K/Akt/GSK-3β/CREB/BDNF/TrKB signaling pathway (Abuelezz & Hendawy, 2023). Moreover, the investigation carried out by Abuelezz and colleagues in the year 2023 exhibited the neuroprotective influence of CoQ10 in countering scopolamine-induced cognitive dysfunction through the stimulation of the PI3K/Akt/GSK-3β/CREB/BDNF/TrKB signaling pathway.

CoQ10 transport across the blood-brain barrier (BBB) involves complex mechanisms primarily mediated by lipoprotein interactions. Studies indicate that CoQ10 is transported via scavenger receptors (SR-B1) and the receptor for advanced glycation end products (RAGE), facilitating its uptake into the brain. However, efflux mechanisms, particularly through low-density lipoprotein receptors (LDLR), limit net transport (Wainwright et al., 2020). While the primary focus is on CoQ10, its metabolites, particularly ubiquinol, may influence transport dynamics. Studies suggest that the redox state of CoQ10 affects its accumulation in brain tissues, indicating that metabolites could play a role in modulating transport efficiency (Watanabe et al., 2019).

In conclusion, CoQ10 treatment at 100, 200, and 400 mg/kg within 4 weeks could improve cognitive impairments and reduce cue-related reinstatement in MOR-addicted male rats. The histological assays supported the neuroprotective effects of CoQ10 in the hippocampus. CoQ10 may be a potential therapeutic agent for MOR-induced cognitive impairments and relapse.

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**Availability of data and material** All data generated or analyzed during this study are included in this published article.

**Ethics Approval** All procedures were performed under the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the ethics committee (IR.MAZUMS.REC.1401.029).

**Conflict of Interest** No potential conflict of interest was reported by the authors.

## Author's contribution

RR and SN, Conceptualization; Data curation; PS, FF, FG, RMN, MVK. Investigation; Methodology; HJ, SNF. Formal analysis; RR and NGH. Writing - review & editing. SMST, HGH. Funding acquisition; RR Supervision; RR. All the authors read and approved the manuscript.

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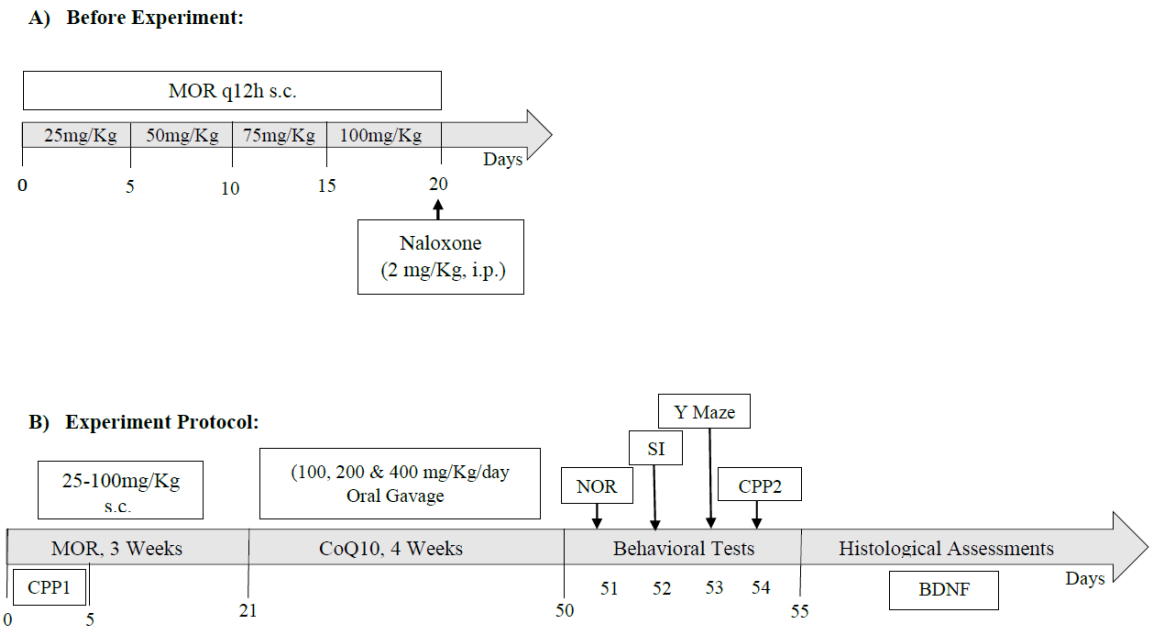


**Fig. 1**. Timeline of experimental procedures, behavioral and histological measures. Abbreviations: BDNF, Brain-derived neurotrophic factor; Coenzym Q10, CoQ10; MOR, morphine; NOR, Novel object recognition; SI, Social interaction; CPP, Conditioned place preference.

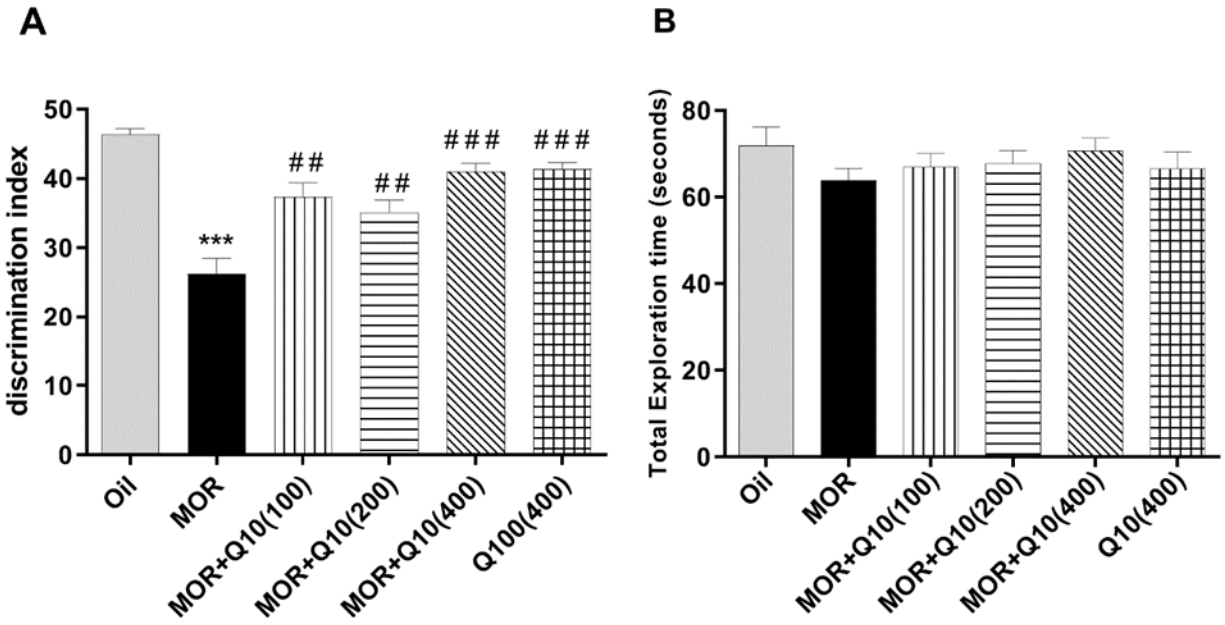


**Fig. 2**. Effect of CoQ10 treatment on novel object recognition test following MOR addiction. Discrimination index (A), Total exploration time (B). Total exploration time shows the total time exploring for the two objects during the test phase. The discrimination index indicates the difference in exploration time, measured as (new object - known object)/ (exploration time new object + known object). n = 8, Data are mean±SD; (***P<0.001 vs. oil group, $^{\#}$ $^{\#}$P<0.01, $^{\#}$ $^{\#}$ $^{\#}$P<0.001 vs. MOR group). MOR, Morphine; Q10, CoQ10.

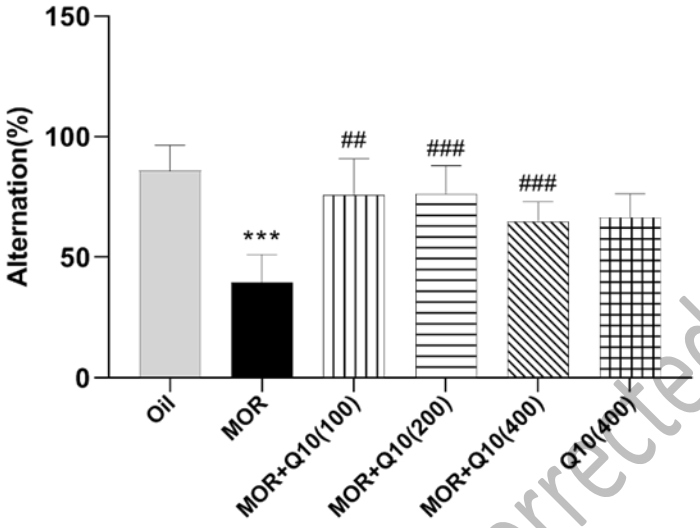


**Fig. 3**. Effect of CoQ10 treatment on working memory following MOR addiction in the Y-Maze Task. Percentage of alternations in the task. n = 8, Data are mean±SD; (***P<0.001 vs. oil group, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. MOR group). MOR, Morphine; Q10, CoQ10.

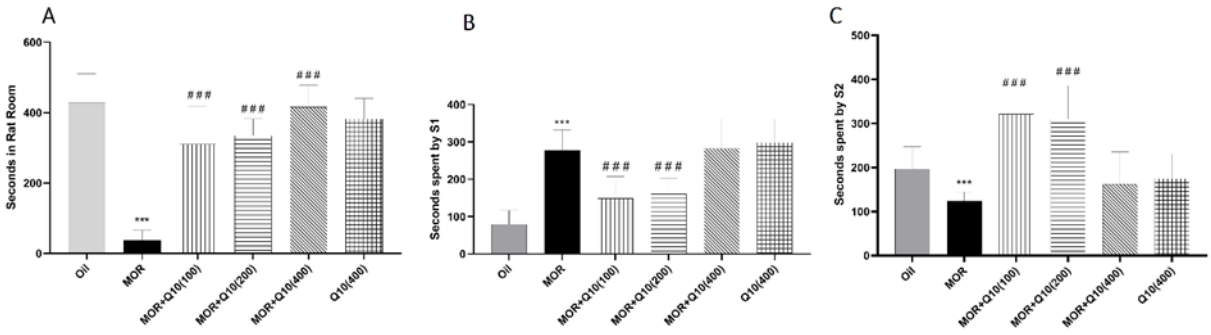


**Fig. 4**. Effect of CoQ10 treatment on social interaction behaviors following MOR addiction. The duration of time spent inside the chamber with a stranger, (A). The duration of direct (active) contact with the stranger 1, (B). The duration of direct (active) contact with the stranger 2, (C). n = 8, Data are mean±SD; (***P<0.001 vs. oil group, $^{\#\#\#}$P<0.001 vs. MOR group). MOR, Morphine; Q10, CoQ10.

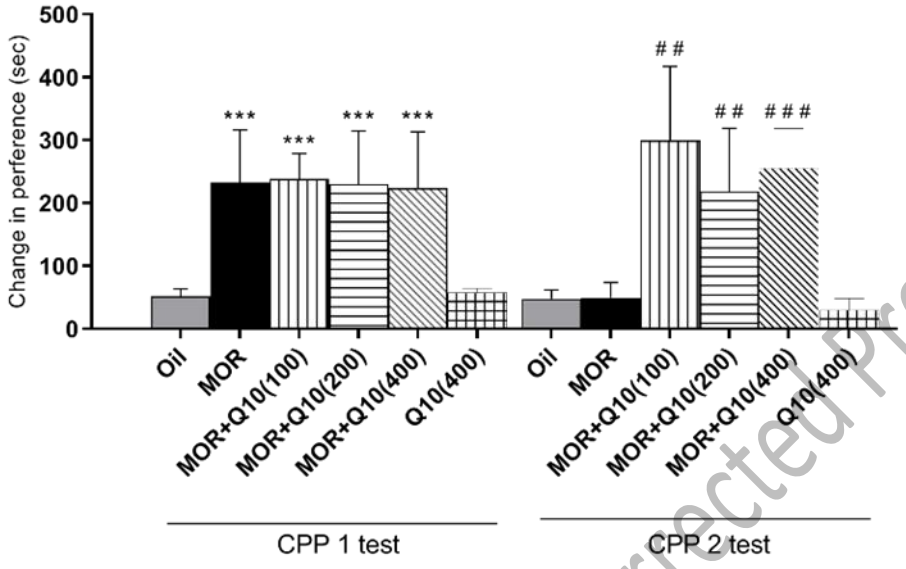


**Fig .5**. Effect of CoQ10 treatment on conditioned place preferences following MOR addiction in the CPP. n = 8, Data are mean±SD; (***P<0.001 vs. oil group, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. MOR group). MOR, Morphine; Q10, CoQ10.

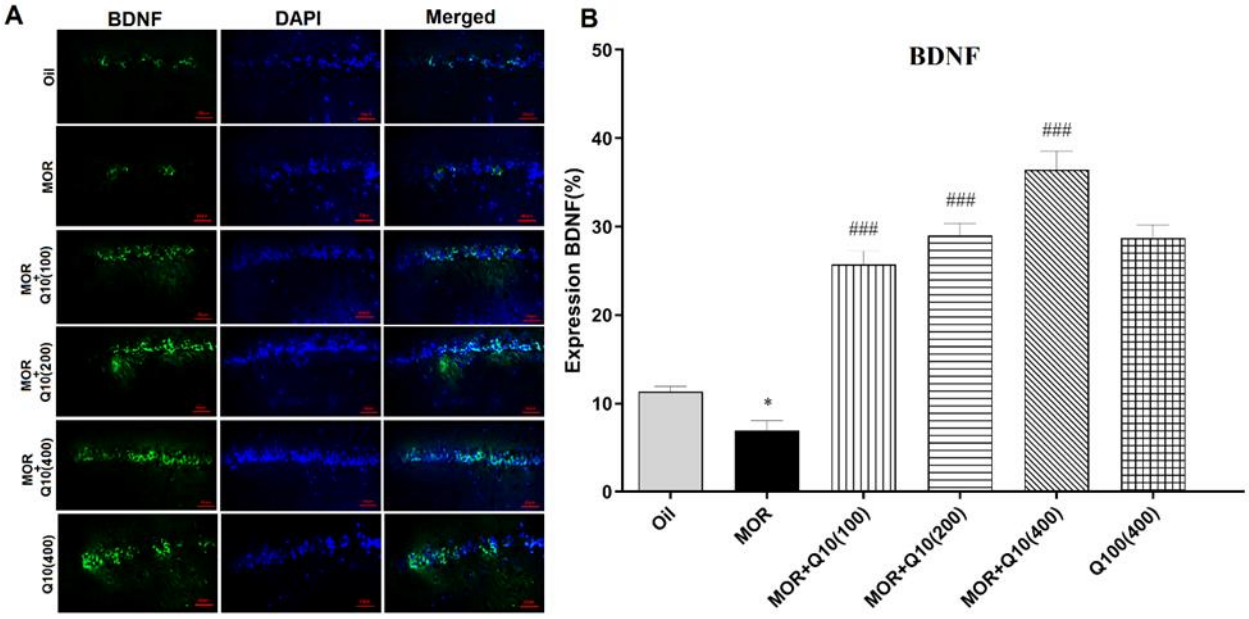


**Fig .6**. Effect of CoQ10 treatment on BDNF expression in hippocampus. Primary antibody to BDNF, Nuclei staining by DAPI and merge of them from each group (A). The percentage of positive reaction in each group (B). Data are mean±SD, $^{*}P<0.05$ vs. oil group, $^{###}P<0.001$ vs. MOR group); Scale bar: 20µm; n=4; BDNF, brain-derived neurotrophic factor; MOR, Morphine; Q10, CoQ10.