RESEARCH PAPER

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Coenzyme Q10 ameliorates anxiety and depression-like behavior associated with chronic opioid use and increases GDNF expression in the hippocampus of morphine-dependent rats

Mobina Gheibi[1], Somayeh Nazari[2], Fahimeh Mohseni[3], Pouria Salehi Sangani[4], Farzaneh Felehkari[1], Saba Niknamfar[2], Hamed Ghazvini[5], Seyedeh Masoumeh Seyedhosseini Tamijani[6], Hamid Kalalian-moghaddam[7], Hamid Jomehpour[8], Raheleh Rafaiee[7]*

[1] *Student Research Committee, Allied Medical School, Mazandaran University of Medical Sciences, Sari, Iran*
[2] *Student Research Committee, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[3] *Department of Addiction Studies, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran*
[4] *Student Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[5] *Department of Neuroscience, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[6] *Psychiatry and Behavioral Sciences Research Center, Addiction Institute, Mazandaran University of Medical Sciences, Sari, Iran*
[7] *Department of Physiology, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran*
[8] *Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
* *Email: rahelerafaie@gmail.com*

Opioid dependence is strongly associated with moderate to severe depression and anxiety. The primary objective of this investigation was to determine whether coenzyme Q10 (CoQ10) has the capacity to increase the level of glial cell line-derived neurotrophic factor (GDNF), with the aim of ameliorating anxiety- and depression-like behaviors in morphine (MOP)-dependent rats. In this study, 40 male Wistar rats were randomly divided into five experimental groups: Oil group, MOP+Oil group, MOP+Q10-100 group, MOP+Q10-200 group, and MOP+Q10-400 group. Rats received escalating doses of MOP (25 to 100 mg/kg, s.c.) once daily. After 21 days of drug dependency, CoQ10 was administered orally at doses of 100, 200, and 400 mg/kg once daily for four weeks. Behavioral assessments were conducted using the open field test, elevated plus maze, and forced swim test. GDNF expression in the hippocampus was evaluated using immunohistochemistry. Four weeks of CoQ10 treatment significantly improved anxiety- and depression-like behaviors induced by MOP administration. Furthermore, CoQ10 significantly increased GDNF expression in the hippocampus. Oral administration of CoQ10 at doses of 100, 200, and 400 mg/kg over four weeks significantly reduced depressive- and anxiety-related behaviors associated with prolonged MOP exposure. These behavioral improvements were accompanied by increased hippocampal GDNF expression.

Key words: coenzyme Q10, depression, anxiety, morphine, opioid-related disorders, addiction

INTRODUCTION

Drug use remains one of the most significant social problems worldwide, associated with numerous medical, psychiatric, familial, occupational, and legal consequences, imposing a substantial burden on society (Iyalomhe et al., 2022; Mohseni et al., 2022). According to the 2023 report by the United Nations Office on Drugs and Crime (UNODC), opioids are the

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most commonly used substances in 31% of countries, primarily in Europe and Asia. Globally, an estimated 60 million individuals aged 15–64 years engage in non-medical opioid use (53% men and 47% women), and opioids accounted for 70% of the 128,000 drug-related deaths reported in 2019 (Belfiore et al., 2024). Previous studies have demonstrated a high prevalence of psychiatric disorders among individuals with opioid use disorder (Farooqui et al., 2022). Furthermore, the co-occurrence of mental health problems is more common in people with opioid use disorder than in those with many other types of substance use disorders (Zaman et al., 2015). The prevalence of major depressive disorder in opioid-dependent individuals ranges from 27% to 61% (Rogers et al., 2021), compared to about 20% in the general population (Gutiérrez-Rojas et al., 2020), indicating a 1.3 to 3 times higher prevalence. Similarly, anxiety disorders are reported in 14% to 43% of opioid-dependent individuals (Rogers et al., 2021), while the prevalence in the general population is around 25% (Remes et al., 2016), suggesting that anxiety levels may be up to 1.5 times higher in some opioid user groups.

The hippocampus plays a crucial role in the pathophysiology of both depression and anxiety, mainly due to its involvement in stress regulation and its extensive connections with other brain regions. It suppresses the hypothalamic-pituitary-adrenal (HPA) axis *via* the paraventricular nucleus of the hypothalamus, effectively stopping the stress response. Disruption of this regulatory feedback can lead to hyperactivation of the HPA axis, elevated cortisol levels, and decreased expression of glial cell line-derived neurotrophic factor (GDNF) in the hippocampus, adversely affecting neuronal plasticity (Sapolsky, 2013; Lucassen et al., 2014). Moreover, impaired connectivity between the hippocampus and the prefrontal cortex—which is critical for emotional regulation—further contributes to depression and anxiety symptoms. These alterations are also linked to changes in neurotrophin signaling and neurotransmission (Arnsten, 2009; Duman & Aghajanian, 2012), underscoring the hippocampus's pivotal role in mood disorders.

Long-term opioid use disrupts various neurobiological pathways associated with mood regulation, including those involving neurotrophins. For example, preclinical studies have demonstrated that prolonged morphine (MOP) exposure significantly reduces central GDNF signaling (Leung et al., 2022). Additionally, clinical studies in humans have shown that individuals addicted to heroin exhibit lower serum GDNF levels compared to healthy controls. These reduced levels have been correlated with traits such as impulsivity, anxiety, and depressive symptoms (Kotan et al., 2018). Considering the well-documented capacity of GDNF to mitigate mood disorders, as supported by numerous studies (Hibi et al., 2009; Buhusi et al., 2017; Tsybko et al., 2017), and the strong association between chronic opioid exposure and mood disturbances in both animal models (Torkaman-Boutorabi et al., 2019; Mavrikaki et al., 2021) and humans (Liao et al., 2011; Larance et al., 2015), agents that enhance GDNF expression may represent promising therapeutic options for alleviating opioid-induced anxiety and depression.

Coenzyme Q10 (2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone), commonly abbreviated as CoQ10, is an endogenous antioxidant that plays a vital role in mitochondrial energy production (Kostrzewska et al., 2022). CoQ10 is located within the biological membranes of cells as well as in circulating lipoproteins (Mantle & Dybring, 2020). A deficiency of CoQ10 is associated with a decrease in mental and physical performance (de la Bella-Garzón et al., 2022; Ebrahimi et al., 2023). Research has shown that individuals with chronic fatigue and depression tend to have lower plasma concentrations of CoQ10 (Maes et al., 2009a; 2009b). Conversely, several studies suggest that CoQ10 supplementation can alleviate various psychopathological symptoms, including anxiety and depression. For instance, in elderly patients with bipolar disorder, high-dose CoQ10 supplementation (500 mg/day) significantly reduced depressive symptoms (Forester et al., 2012). Similarly, in women with polycystic ovary syndrome, CoQ10 intake resulted in significant reductions in depressive and anxiety symptoms, as measured by the Beck Depression Inventory and Beck Anxiety Inventory, compared to a placebo group (Karamali & Gholizadeh, 2022). Additionally, CoQ10 used as an adjunct therapy with pregabalin in fibromyalgia patients showed superior anxiolytic efficacy compared to pregabalin alone, further supporting the anxiolytic potential of CoQ10 (Sawaddiruk et al., 2019). Beyond human research, numerous animal studies have also demonstrated the antidepressant and anxiolytic properties of CoQ10 (Sharafi Chie et al., 2020).

Although the benefits of CoQ10 in various neuropsychiatric conditions are well documented, its potential therapeutic role in treating mood disorders resulting from prolonged MOP use remains largely unexplored. Considering the established association between long-term opioid exposure and psychiatric conditions such as anxiety and depression, along with the pivotal role of GDNF in mood regulation, this study aims to examine whether CoQ10 can alleviate opioid-induced anxiety- and depression-like behaviors by increasing enhancing GDNF levels in the hippocampus.

METHODS

Animals

This study was conducted on 40 adult male Wistar rats weighing approximately 180–220 grams. The rats were housed at the Animal Institute of Mazandaran University of Medical Sciences under controlled conditions: a temperature of 23 ± 2°C, a 12 h : 12 h light/dark cycle, constant humidity, and free access to food and water. All experimental procedures were carried out during the light phase between 8:00 a.m. and 3:00 p.m. The study protocol was designed and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Edition, 2011). The present study was completed under the Ethical Guidelines for Medical and Health Research established by the Ministry of Health and Medical Education and the Ministry of Science, Research and Technology, Iran. We obtained approval from the Ethics Review Committee of Mazandaran University of Medical Sciences, Iran (Registration no.: IR.MAZUMS.AEC.1403.123).

Drugs

The following drugs were used in this study: Coenzyme Q10 (CAS No. 303-98-0; Sigma–Aldrich/Merck, C9538, Germany), morphine sulfate (Darou Pakhsh, Iran), ketamine and xylazine (Alfasan, Netherlands), and naloxone hydrochloride (Caspian, Iran).

Experimental protocol

This study included five experimental groups (n=10) as follows: Sham group: Rats received normal saline for 21 days and sesame oil by oral gavage for one month. MOP group: Rats were administered morphine (MOP) for 21 days, followed by sesame oil *via* oral gavage one month after drug abstinence. MOP+Q10-100 group: Rats received MOP for 21 days, followed by CoQ10 (100 mg/kg) *via* oral gavage one month after drug withdrawal. MOP+Q10-200 group: Rats received MOP for 21 days, followed by CoQ10 (200 mg/kg) *via* oral gavage one month after drug withdrawal. MOP+Q10-400 group: Rats received MOP for 21 days, followed by CoQ10 (400 mg/kg) *via* oral gavage one month after drug withdrawal (Fig. 1).

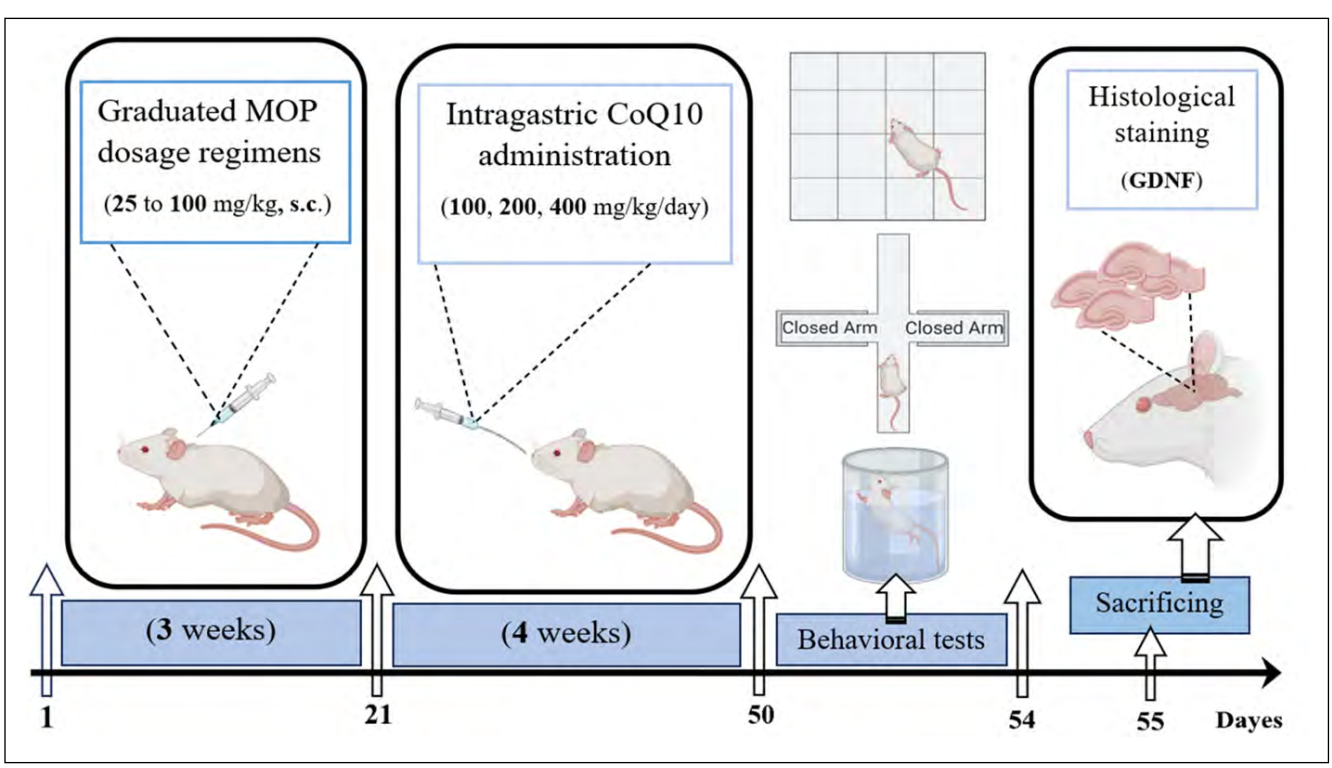

Fig. 1. The setup of the experiment. CoQ10, Coenzyme Q10; EPM, elevated plus maze; FST, forced swimming test; GDNF, glial cell line-derived neurotrophic factor; MOP, morphine; OFT, open field test (Created with BioRender.com).

It is important to note that the Sham group served as a baseline reference, as it underwent the same handling procedures as the experimental groups but without MOP or CoQ10 administration. This approach effectively controlled for stress-related confounding factors and minimized the need for a separate control group.

MOP sulfate powder was dissolved in distilled water. Rats were administered increasing doses of MOP (25 to 100 mg/kg, s.c.) once daily for 21 days (Table 1). CoQ10 was dissolved in sesame oil and administered by gavage. The volume of oral gavage was 1 mL.

To confirm MOP dependence, naloxone (2 mg/kg, i.p.) was injected intraperitoneally 2 hours after MOP administration on day 21. Withdrawal symptoms, including jumping, head shaking (similar to a dog), teeth grinding, chewing, standing on two legs, scratching at the cage, and digging, were observed and recorded over a 30-minute period. Additionally, the frequency of defecation during the 30-minute observation and the percentage of body weight loss over 24 hours were measured. After the withdrawal symptoms were observed and recorded, the rats were returned to their cages (Akbari & Mirzaei, 2013).

Following 21 days of MOP administration, CoQ10 treatment was initiated and given once daily by oral gavage (using a No. 24 syringe) for one month at doses of 100, 200, or 400 mg/kg. All groups subsequently underwent behavioral testing.

Open field test

The open field test (OFT) was used to assess locomotor activity in the rats. It consisted of a Plexiglas box (100 × 100 × 40 cm) with a black base divided into 16 equal squares. Each rat was initially placed at the center of the box. The number of square crossings, as well as the duration of rearing and grooming behaviors during a 5-minute session, were recorded and included in the statistical analysis (Seibenhener & Wooten, 2015; Rojas-Carvajal et al., 2018).

Table 1. Details of increasing doses of MOP.

Days	Injected MOP (s.c.)	Dosage (mg/kg)
1-5	5 mg	25
6-10	10 mg	50
11-15	15 mg	75
16-21	20 mg	100

MOP – morphine; s.c. – subcutaneous.

Elevated plus maze

The elevated plus maze (EPM) was used to evaluate anxiety-like behaviors. The apparatus consisted of two open arms (10 × 50 cm) and two closed arms (40 × 50 × 10 cm) arranged in a plus shape and connected by a central square platform (10 × 10 cm). The maze was elevated 50 cm above the floor, providing a standard setting for anxiety assessment in rodents. Each rat was placed on the central square facing an open arm and observed for 5 minutes. The time spent in the open arms and the number of entries into the open arms were recorded for statistical analysis (Mohseni et al., 2020; Rezaeian et al., 2020).

Forced swimming test

The forced swimming test (FST) was used to assess depression-like behaviors. The apparatus consisted of a cylindrical tank (20 cm in diameter and 60 cm in height) filled with fresh water maintained at 34 ± 1°C to a depth of 45 cm. Each rat was placed individually in the tank for 8 minutes. The duration of immobility (defined as minimal movements necessary to keep the animal's head above water) during the test was recorded for statistical analysis (Yankelevitch-Yahav et al., 2015).

Immunohistochemistry

On day 55, after beginning of experiments, the animals (n=4) were anesthetized with xylazine (20 mg/kg) and ketamine (160 mg/kg) for histological examination. The rats were perfused *via* intracardiac injection of 0.9% saline followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, capitation was performed, and the brains were removed and postfixed overnight in 4% PFA.

After conventional paraffin embedding and serial sections (5 µm) according to the Paxinos atlas, immunohistochemical staining was performed after incubation with a primary anti-GDNF antibody (1:500, orb10704) overnight at 4°C and a fluorescein isothiocyanate-conjugated secondary antibody (orb688925) for 2 hours, after which the samples were imaged using an Olympus FluoView 1200 confocal microscope system (Olympus Corporation, Japan). Quantitative analysis of GDNF staining was conducted using three sections from each brain. ImageJ software was used for the quantification of immunofluorescence intensity, with the mean value from 25 randomly selected fields within each section calculated to obtain a single representative value for each rat. Statistical analysis of the quantified imaging data was performed using GraphPad Prism 6.

Statistical analysis

The results were analyzed with GraphPad Prism Version 6. The data are presented as mean (±standard deviation, SD). The Shapiro-Wilk test was utilized to ascertain the adherence of the data to a normal distribution pattern. The results of this test indicated that all data exhibited a normal distribution; consequently, the data were subjected to parametric analysis utilizing ANOVA. Comparisons of data between groups were analyzed using analysis of variance (ANOVA) and Tukey's *post hoc* test. P values less than 0.05 were considered to indicate statistical significance. *P<0.05, **P<0.01, and ***P<0.001 indicate statistical significance compared to the oil group, while #P<0.05, ##P<0.01, and ###P<0.001 indicate statistical significance compared to the MOP group.

RESULTS

The OFT results showed no significant difference in the number of lines crossing between the experimental groups (Fig. 2A). In addition, MOP-dependent rats showed significant differences (P=0.0019, P=0.0001) in the grooming and rearing indices compared with those of the oil group (Fig. 2B, C). Moreover, treatment with 400 mg/kg CoQ10 decreased grooming compared with that in MOP-dependent rats (Fig. 2B).

The EPM results revealed indicated that MOP-dependent rats spent significantly less time in the open arms ($F_{4,43}$=60.65, P<0.0001; Fig. 3A) and had fewer entries into the open arms (P<0.001; Fig. 3B) than the oil group. Additionally, treatment with CoQ10 at doses of 100, 200, and 400 mg/kg significantly increased both

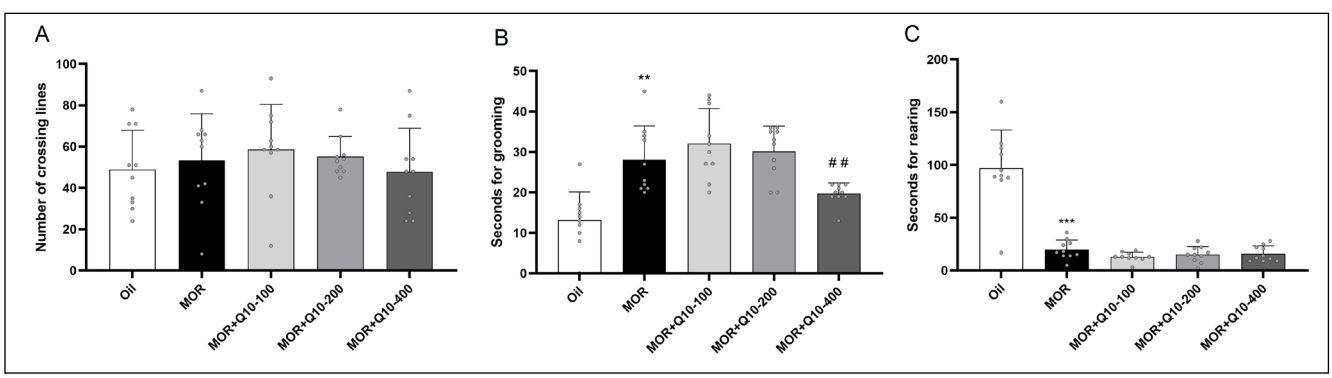

Fig. 2. Effects of CoQ10 treatment on locomotor activity and stereotypical behaviors following the MOP regimen. The total number of crosses moved (A), grooming (B), and rearing (C) were assessed in the open field test. The data are presented as mean ± SD. *denote comparisons *vs.* the oil group, and # indicate comparisons *vs.* the MOP group. MOP, morphine; Q10, Coenzyme Q10.

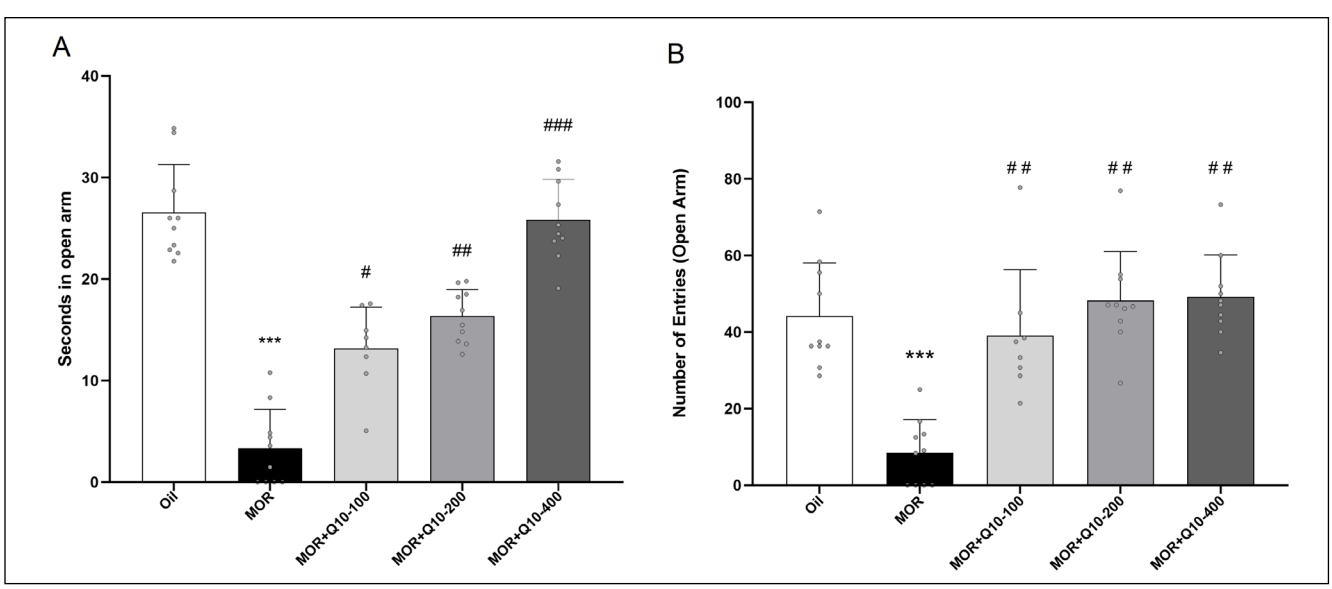

Fig. 3. Effects of CoQ10 treatment on anxiety behaviors following the MOP regimen. The time spent in the open arms (A) and the number of entries into the open arms (B) were assessed in the elevated plus maze. The data are presented as mean ± SD. *denote comparisons *vs.* the oil group, and # indicate comparisons *vs.* the MOP group. MOP, morphine; Q10, Coenzyme Q10.

the time spent and the number of entries into the open arms compared with MOP-dependent rats (P=0.0048, P=0.002, P=0.005, respectively; Fig. 3).

The FST results showed a significant increase in immobility time in MOP-dependent rats compared with the oil group (P<0.001; Fig. 4), indicating depressive-like behavior. Treatment with CoQ10 at doses of 200 and 400 mg/kg significantly reduced immobility time compared with the MOP-dependent group (P=0.0392, P=0.0012, respectively; Fig. 4).

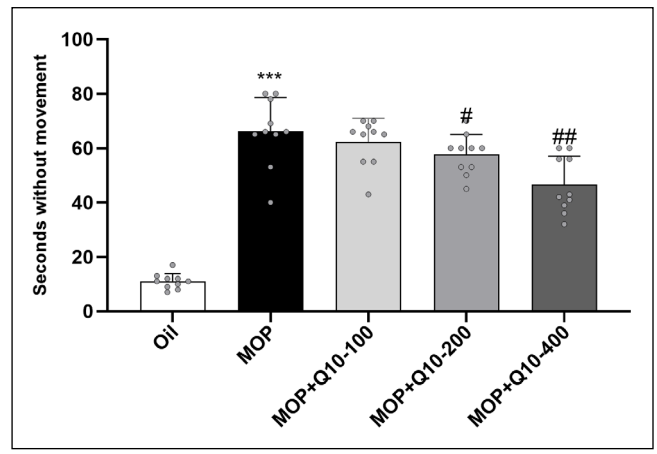

Fig. 4. Effects of CoQ10 treatment on depression following the MOP regimen. The time of immobility in the forced swim test was assessed. The data are presented as mean ± SD. *denote comparisons *vs.* the oil group, and # indicate comparisons *vs.* the MOP group. MOP, morphine; Q10, Coenzyme Q10.

GDNF expression in the hippocampus

Compared with the oil group, GDNF levels were significantly lower in the hippocampus of MOP-dependent rats ($F_{4,17}$=34.79, P=0.009; Fig. 5). Moreover, treatment with CoQ10 at doses of 100, 200, and 400 mg/kg significantly increased GDNF levels compared with the MOP-dependent group (P=0.021, P=0.009, P=0.0009, respectively).

DISCUSSION

The present study showed that CoQ10 administration effectively attenuated depression- and anxiety-like behaviors induced by chronic MOP exposure, with these behavioral improvements being associated with elevated hippocampal GDNF expression. Our findings are consistent with previous studies indicating that opioid administration contributes to the development of anxiety- and depression-like behaviors in animals subjected to chronic use (Torkaman-Boutorabi et al., 2019; Mavrikaki et al., 2021; Khani et al., 2022).

The mechanisms by which opioids exert their depressogenic and anxiogenic effects are not yet fully understood. However, certain rationales have been proposed. Opiates have been shown to impact the brain by

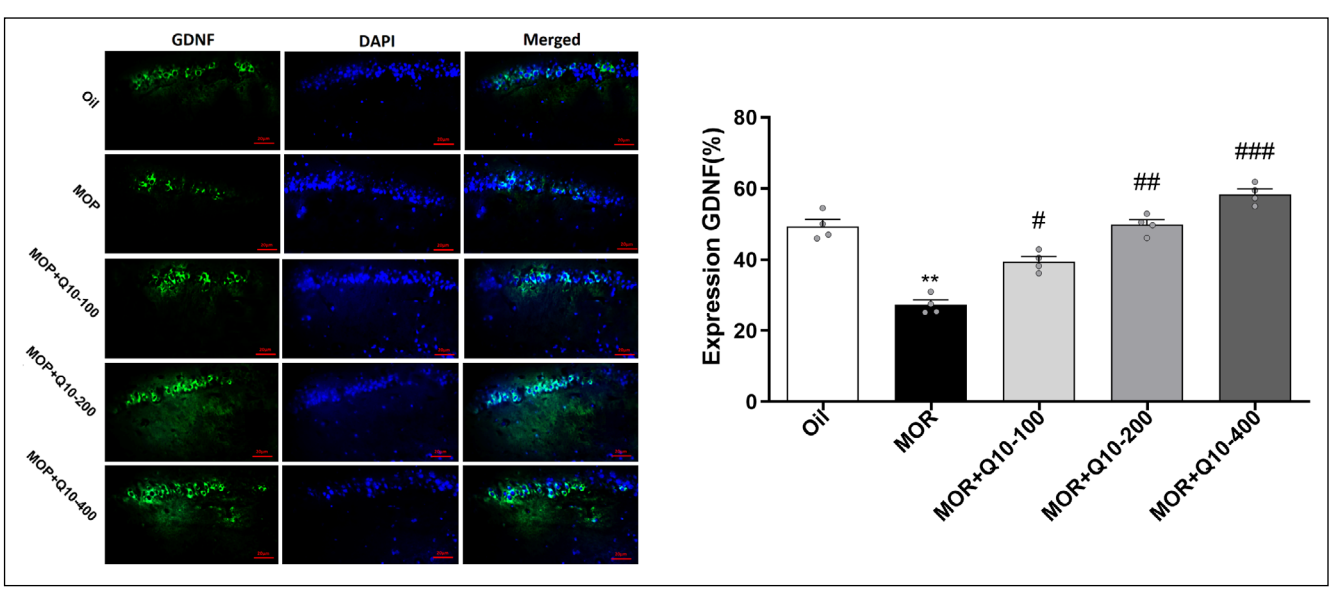

Fig. 5. Effect of CoQ10 treatment on GDNF expression in the hippocampus. Primary antibody against GDNF. Nuclei were stained with DAPI, and images from each group were merged (A). The percentage of positive reactions in each group (B). The data are presented as mean ± SD; *denote comparisons *vs.* the oil group, and # indicate comparisons *vs.* the MOP group. Scale bar: 20 μm. GDNF, glial cell-derived neurotrophic factor; MOP, morphine; Q10, coenzyme Q10.

influencing the HPA axis and the hippocampus, a brain structure associated with emotion and reward (Lutz & Kieffer, 2013). The hippocampus regulates HPA axis activity by modulating cortisol synthesis in response to stressful stimuli. Chronic MOP exposure markedly affects the hippocampus and leads to a reduction in dendritic spine density (Robinson et al., 2002), potentially compromising the hippocampal regulation of the HPA axis and ultimately resulting in depression- and anxiety-like manifestations (Semenkovich et al., 2014).

Our study revealed that prolonged MOP exposure significantly increased immobility time during the FST. The increase in immobility time serves as a reliable indicator of depression-like behavior in MOP-dependent rats, which was ameliorated by CoQ10 administration. It is important to note that, although the FST is widely recognized as a proxy for assessing depression-like behaviors in rodents, the findings should be interpreted as indicative of depression-like states rather than constituting a definitive diagnosis (Gencturk & Unal, 2024).

Prior research has provided evidence consistent with our findings regarding the impact of CoQ10 on reducing depression-like behaviors. For example, a study conducted by Andalib et al. (2019) demonstrated that four weeks of CoQ10 intake led to significant improvements in depressive symptoms, as assessed by the FST and the splash test in streptozotocin-induced depression-like animal models. In addition to supporting our findings on the antidepressant properties of CoQ10 (Andalib et al., 2019), our data also showed that CoQ10 exerts an anxiolytic effect. Specifically, MOP-dependent rats treated with CoQ10 spent more time in the open arms and exhibited more entries into the open arms of the EPM compared with MOP-dependent rats without CoQ10 administration, suggesting a reduction in anxiety.

In our study, locomotor activity in MOP-exposed animals treated with CoQ10 did not significantly differ from that observed in MOP-dependent rats in the OFT. However, animals treated with CoQ10 exhibited significantly less grooming behavior than MOP-treated rats. In line with previous studies, these findings suggest that the decreased grooming behavior observed in the CoQ10-treated group is due to reduced anxiety (Sinatra et al., 2003; Andalib et al., 2019). In other words, the animals receiving CoQ10 showed improved adaptability to an unfamiliar environment, indicating reduced anxiety and fear responses during exploration (Sinatra et al., 2003).

Taken together, the behavioral test results of the present investigation revealed a notable co-occurrence of anxiety and depression following prolonged MOP exposure, a phenomenon alleviated by the administration of CoQ10. Numerous animal studies have documented the therapeutic effects of CoQ10 in conditions where anxiety and depression manifest concurrently (Aboul-Fotouh, 2013; Fatemi et al., 2022). For example, chronic administration of CoQ10 improved the co-occurrence of anxiety- and depression-like behaviors in the FST and OFT in rats exposed to chronic restraint stress, an experimental model of depression (Aboul-Fotouh, 2013). In addition, Fatemi et al. (2022) indicated that global cerebral ischemia/reperfusion injury in rats increased anxiety- and depression-like behaviors. Chronic treatment with CoQ10 reduced stroke-induced anxiety, depression-like behaviors, and brain edema by enhancing antioxidant capacity and increasing neurotrophin levels (Fatemi et al., 2022). Increasing levels of neurotrophins have been demonstrated to improve different aspects of mood, whereas reducing these levels may result in impairments, as indicated by prior research (Mohseni et al., 2021).

In the present study, a decrease in the expression of the hippocampal neurotrophic factor GDNF following exposure to MOP resulted in the concurrent manifestation of anxiety- and depression-like behaviors. Consistent with our findings, Liu et al. (2012) reported a significant decrease in GDNF expression in the hippocampus of animals exhibiting depressive symptoms induced by chronic unpredictable stress. Furthermore, studies involving human subjects have suggested an association between reduced GDNF levels and mood-related neuropsychiatric disorders (Lin & Tseng, 2015; Shen et al., 2019). For example, a meta-analysis conducted by Lin and Tseng (2015), which included 526 patients and 502 control subjects from 12 original studies, revealed a significant reduction in plasma, serum, and mRNA expression levels of GDNF in patients diagnosed with depression compared to control subjects. Moreover, a study conducted in a Chinese Han population demonstrated that serum levels of GDNF were lower in individuals with generalized anxiety disorder than in healthy controls (Shen et al., 2019).

Several research studies have demonstrated the amelioration of anxiety- and depression-like behaviors through the direct administration of GDNF or its indirect upregulation facilitated by therapeutic medications (Wan et al., 2017; Liu et al., 2023). For instance, the administration of exogenous GDNF into the medial prefrontal cortex enhanced autonomous motor function and alleviated symptoms of depression and anxiety in animal models of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Liu et al., 2023). Additionally, intracranial GDNF delivery has been suggested to exert anxiolytic and antidepressant effects by upregulating regional D1 dopamine receptor protein levels, which subsequently activate the downstream adenylate cyclase/cyclic AMP/protein kinase A (AC/cAMP/PKA) signaling pathway (Liu et al., 2023).

Interestingly, GDNF has also been shown to exert a protective effect against methamphetamine-induced neurotoxic degradation of dopaminergic neurons, a finding particularly relevant given the high prevalence of anxiety disorders among methamphetamine-dependent adults (Cass et al., 2006). Moreover, GDNF is recognized as a critical factor in the growth, viability, and maintenance of not only dopaminergic but also serotonergic neurons (Ducray et al., 2006; Marshall, 2023). Acute central administration of GDNF in mouse strains genetically predisposed to depressive-like behavior has been shown to induce an anxiolytic response, as evaluated by the EPM and dark/light box tests. This effect is mediated through the upregulation of key genes involved in the serotonin (5-HT) system, such as Tph2, 5-HT1A, and 5-HT2A receptor genes (Naumenko et al., 2014). Moreover, in cell culture, exposure to GDNF significantly augmented the soma size of serotonergic neurons and increased both the quantity of primary neurites/neurons and the length of neurites/neurons (Ducray et al., 2006). Taken together, these studies suggest that GDNF exerts its anxiolytic and antidepressant effects, at least in part, by regulating the function of mood-related neurotransmitters, namely serotonin and dopamine (Naumenko et al., 2014; Liu et al., 2023). Conversely, prolonged opioid exposure has been shown to disrupt endogenous serotonin and dopamine pathways and their associated signaling mechanisms (Yeh et al., 2012; Haleem et al., 2018) as well as GDNF (Kotan et al., 2018; Leung et al., 2022). In light of these findings, we selected GDNF for measurement over other neurotrophic factors, given its specific involvement in modulating the dopaminergic and serotonergic systems, which are critical to the neurobiological mechanisms underlying morphine dependence.

Therefore, the observed association between increased GDNF expression and improved mood-related behaviors suggests that targeting GDNF pathways may represent a potential strategy for ameliorating MOP-induced depression-like behavior and anxiety.

CoQ10 modulates the expression of GDNF through several potential molecular mechanisms. First, CoQ10 reduces oxidative stress by scavenging reactive oxygen species, thereby activating the nuclear factor erythroid 2-related factor 2/antioxidant response element (Nrf2/ARE) pathway, which plays a pivotal role in maintaining cellular redox homeostasis. Activation of Nrf2 upregulates the expression of various neuroprotective genes, including GDNF, by enhancing transcriptional activity *via* ARE-binding sites (Gutierrez-Mariscal et al., 2020; Zgorzynska et al., 2021; Samimi et al., 2024). Second, CoQ10 improves mitochondrial ATP production by enhancing electron transport chain efficiency and mitigating mitochondrial dysfunction. Optimal mitochondrial function facilitates the activation of intracellular signaling pathways, such as the cAMP response element-binding protein (CREB) pathway. CREB, a transcription factor, directly regulates GDNF gene expression. By promoting mitochondrial health and sustaining adequate ATP levels, CoQ10 supports the activation of CREB and subsequent upregulation of GDNF transcription (Liu et al., 2020; Zgorzynska et al., 2021; Gherardi et al., 2022). These interconnected mechanisms highlight the role of CoQ10 in regulating GDNF expression through its mitochondrial-enhancing and antioxidant properties, alongside modulation of key intracellular signaling pathways.

The present study identified CoQ10 as a promising therapeutic agent capable of attenuating depression- and anxiety-like behaviors induced by chronic MOP exposure, effects that were accompanied by increased hippocampal GDNF levels. However, the precise molecular mechanisms and the specific neurotransmitter systems modulated by GDNF remain to be fully elucidated. Future investigations exploring the influence of CoQ10-induced GDNF upregulation on neurotransmitters critical to addiction and emotional regulation could provide deeper insights into the neurobiological underpinnings of opioid-induced mood disorders. Moreover, assessing the involvement of additional neurotrophic factors—such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), and ciliary neurotrophic factor (CNTF)—in future studies would offer a more comprehensive understanding of the neurotrophic networks implicated in morphine dependence.

Limitations

A limitation of our study is the small experimental group size (n=4) for the immunohistochemical analysis, which was guided by the ethical principles of the 3Rs (Replacement, Reduction, and Refinement) to minimize animal use. To mitigate this limitation and enhance statistical power, three brain tissue replicates were collected from each animal. Although the small sample size may restrict the generalizability of the findings, the observed effects in the current study were highly consistent and robust, supported by validated methodologies and previous studies employing similar sample sizes.

CONCLUSION

Administration of CoQ10 at doses of 100, 200, and 400 mg/kg over 4 weeks significantly reduced depres-

sive- and anxiety-related behaviors associated with prolonged MOP consumption. This behavioral improvement was accompanied by increased GDNF expression in the hippocampus. While these findings are promising, they represent correlational data, and further evaluation studies are warranted to validate the efficacy of CoQ10 and to explore the underlying mechanisms and causal relationships in greater depth.

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