Original Article

Study of the effects of controlled morphine administration for treatment of anxiety, depression and cognition impairment in morphine-addicted rats

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Abstract

Background: Morphine dependency usually results in undesired outcomes such as anxiety, depression, and cognitive alterations. In this study, morphine was used to manage morphine dependence-induced anxiety, depression, and learning and memory disturbances.

Materials and Methods: Forty rats were divided equally into five groups. Group 1 received saline for 21 days. Groups 2–5 were dependent by increasing administration of morphine (15–45 mg/kg) for 7 days. For the next 14 days, morphine was administered as the following regimen: Group 2: once daily; 45 mg/kg (positive controls), Group 3: the same dose with an increasing interval (6 h longer than the previous intervals each time), Group 4: the same dose with an irregular intervals (12, 24, 36 h intervals interchangeably), and Group 5: decreasing doses once daily (every time 2.5 mg/kg less than the former dosage). On days 22–26, elevated plus maze (EPM), open field test (OFT), forced swim test (FST), and tail suspension test (TST) were performed to investigate anxiety level and depression in animals. Between 17th and 21st days, Morris water maze (MWM) was used to evaluate the spatial learning and memory.

Results: Chronic morphine administration caused depression and anxiety as observed by FST, EPM, and TST and decreased motor activity in OFT and caused impairment in learning and memory performance in MWM. Treatment with our protocol as increasing interval, irregular interval, and decreasing dosage of morphine caused marked reduction in depression, anxiety, and improved cognition performance compared with positive control group; and attenuated motor deficits in morphine-dependent rats, remarkably.

Conclusions: Change in dosage regimens of morphine can reduce morphine-induced anxiety, depression, and cognitive impairments.

Key Words: Anxiety, depression and cognition impairment, morphine

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INTRODUCTION

Addiction is a complicated phenomenon with serious social, economic, and health consequences. Furthermore, morphine is a common drug of abuse which is widely used analgesic among patients with cancer and acute pain.[1] Effectiveness and safety of opioids in chronic pain have not been conclusive yet. Repeated exposure to morphine alters brain functions and neural mechanisms.[2] Studies have explored various long-term effects of exposure to opioids.[3] It has been demonstrated that opioid addiction causes disturbances in mood and promotes anxiety, depression, and cognitive impairments.[4,5] Frequent exposure to opioids also causes deficits in learning, memory, attention, reasoning, and impulse control.[6] One of the major problems in the long-term use of opioids such as morphine is drug dependence, which is characterized by withdrawal syndrome.[7,8] The exact mechanisms for tolerance, drug dependence, and withdrawal symptoms have not been precisely determined.[8,9] However, alterations in the neurobiology of dopaminergic neurons in the ventral tegmental area and nucleus accumbens (NAC) and noradrenergic neurons in the locus coeruleus (LC) are suggested to contribute to withdrawal symptoms. In opioid dependence, cessation of opioids diminishes the release of dopamine. In contrast, the release of norepinephrine by LC is observed to increase during withdrawal.[9,10] Another mechanism for opioid dependency operates via cAMP-dependent pathway. The resultant activation of PKC decreases opioid receptor activity and mRNA expression of proenkephalin, and increases the mRNA expression of gamma-interferon-inducible lysosomal thiol reductase (GILT-1).[10,11] Acute administration of drugs of abuse can cause changes in gene expression of various Fos family proteins which are transiently upregulated in NAC and dorsal striatum during addiction.[11] However, chronic drug exposure causes the accumulation of biochemically modified isoforms of ΔFosB in the same regions, but Fos family proteins remain unchanged.[11,12] Previous studies have documented that the rise in ΔFosB isoforms is responsible for drug tolerance, craving, and withdrawal syndrome.[13,14] Opioid dependency causes depression and anxiety, we have previously shown that naloxone-induced withdrawal in morphine-dependent rats stimulates hypothalamus-pituitary axis as evident by increased levels of cortisol, that eventually leads to higher anxiety levels.[15,16] Furthermore, previous studies have indicated that a major obstacle in the treatment of opioid dependence and withdrawal syndrome is the management of comorbid anxiety and depression.[6] Antidepressant and anxiolytic agents-as adjunctive therapy for treatment of opioid dependence have been ineffective to produce desired results.[6,16] Our previous studies showed that treatment of addicted animals by various dosage regimens such as increasing interval, irregular interval, and decreasing dosage manner can cause a decrease in severity of withdrawal signs in comparison with subjects under treatment of regular administration of morphine.[17,18] Actually, our results showed that regular standard administration of morphine in a constant pattern causes induction of dependency while the mentioned controlled regimen decreases morphine dependency and attenuate the severity of withdrawal syndrome.[17,18] Considering the fact that regular administration of morphine can cause morphine dependency-induced oxidative stress, inflammation, and apoptosis of hippocampus,[17,19] according to our mentioned previous studies, we decided to evaluate the various dosage regimen (increasing administration interval, irregular interval, and decreasing dosage) in morphine dependency-induced anxiety, depression, and cognitive impairments and compare these effect with standard treatment of morphine as regular administration. The authors designed this study to examine if changes in dosage regimens of morphine can reduce opioid-induced anxiety, depression, and cognitive impairments.

MATERIALS AND METHODS

Animals

Forty male adult Balb/c mice (weighing 27–32 g) - about 8-week-old were obtained from Pasteur Institute of Iran (Tehran, Iran). For 2 weeks, the animals were housed at room temperature (21–23°C) with free access to standard food and water; and maintained on 12 h day-night cycle. Our experimental protocol was in accordance with guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996) and approved by the Research Council of the Iran University of Medical Sciences.[20] Animals were randomly divided into five groups (n = 8, each), and were treated according to the following protocol: All injections were performed at 8 a.m. All protocol of drug administration was performed based on our and other previous work.[1,15,17,18]

Experimental design

Forty-eight adult's rats randomly were divided as below diagram [Diagram 1]:

On days 22–26, standard behavioral methods including elevated plus maze (EPM), open field test (OFT), forced swim test (FST), and tail suspension test (TST) were used to investigate anxiety and depression level in the animals. In addition, Morris water maze (MWM) was used to evaluate spatial learning and memory

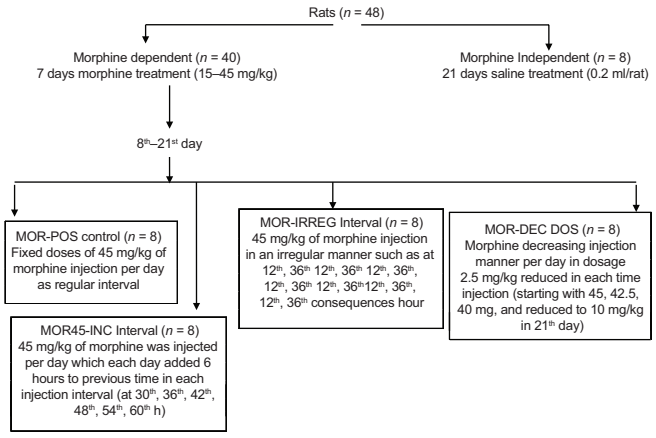

Diagram 1: Devision of animal in various experimental group

in animals between 17th and 21th day. All mentioned behavioral test were performed according to the following protocol.

Behavioral tests

Open field test

This assay was used to evaluate anxiety and locomotor activity in experimental animals. The base of OFT apparatus was divided into 16 equally spaced squares bordered with opaque and 70 cm high walls. The whole of apparatus was painted black except for the 6 mm broad white lines that divided the ground into 16 squares. This apparatus was illuminated using a 100W bulb which focused on the field from a height of approximately 110 cm. Except for the open field; the entire room was kept in dark during the experiment. To observe subsequent behaviors for evaluating anxiety and locomotor activity, each animal was brought to the center of the setup for about 5 min;

1. Ambulation distance: Total distance of the grid lines crossed by each rat
2. Center square entries: Number of times each rat enters the central red square lines with all four paws
3. Center square duration: The time spent by each rat in the central square
4. Rearing: Frequency with which each rat stands on their hind legs in the maze.[21-24]

Forced swim test

It is behavioral test used for the evaluation of depression-like behaviors in experimental model. The FST equipment consisted of a transparent Plexiglass cylinder (20 cm diameter × 60 cm height) filled with water up to the height of 30 cm from bottom. A day before the test, all rats were gently placed in the cylinder and were made to swim for a habituation period of 15 min. However, during experimentation, subjects were placed individually in filled glass cylinder for 5 min and the duration of swimming was recorded for each. The swimming activity was indicative of nondepressive behavior.[25-27]

Elevated plus maze

EPM is a widely used assay to assess anxiety-related behaviors in rodents. EMP setting consists of a plus sign apparatus; a pair of oppositely placed open arms (55 cm × 15 cm), a pair of oppositely placed closed arms (55 cm × 15 cm), and an open central squared area (10 cm × 10 cm). All the arms were kept open from top and the closed arms had 40 cm elevated side walls. The entire apparatus was kept 50 cm above the ground using rods. One at a time, rats were placed in the center of the maze and the time spent by each rat in open arms and in closed arms was recorded. The subjects were freely allowed to explore the maze for 5 min. The preference for being in closed arms over the open ones was indicative of depressive behavior.[25,28]

Tail suspension test

In TST, a rat is suspended by its tail (50 cm above the ground) against a fixed metal rod such that the body faces downwards. Normally, the rat tries to escape from this stressful state by trying to climb up the metal rod. However, depressed ones give up and remain immobile. Therefore, we recorded the duration of immobility in a 5-min period which was indicative of depression-like behavior.[29]

Morris water maze

MWM apparatus was used to assess learning ability and memory in rats. It consisted of a circular galvanized black steel water tank (150 cm diameter × 85 cm height), settled in the center of a small room. This circular tank was divided into four quarters (North, East, West, and South) and filled with 1.6 ft deep water. An invisible metal platform (12 cm in diameter) was submerged approximately 1 cm below the surface of the water. An automated infrared tracking system (CCTV B/W camera, SBC-300 (P), Samsung Electronic Co., Ltd., Korea) was used to record the position of the animal in the tank. The camera was mounted 2.3 m above the surface of the water.[30]

Handling

In a 4-day training session, the tank was filled with water (room temperature 25 ± 2°C) and the platform was submerged in one of the quarter (North–East) approximately 25 cm from the wall of the tank. At the beginning of each trial, the rat was placed in the tank and the experimenter guided the rat to swim toward the platform placed in the specified quarter.[30]

Training procedure (learning assessment)

Some distinguished landmarks (such as pictures, windows, and doors) were placed in an extra maze present in the same room for spatial cues for learning of platform's position for animals. Each animal was trialed four times per day which they positioned in

each quarter randomize (North, East, West, and South) randomly. If the rats were unable to find the platform within 60 s, the trial was terminated automatically by the computer system. In this 4-day training procedure, two different activities were assessed:

1. Escape latency: Time taken by each rat to reach the platform
2. Traveled distance: Total distance traveled by each rat to find the platform.[30]

Probe testing (memory assessment)

On the 5th day (probe day), the experimenter removed the platform and animal was randomly placed into the water tank (in the quarter other than the North-East one) and the percentage of the rats that reached the North-East was calculated.[25,31]

Statistical analysis

The data were analyzed using Graph Pad PRISM version 6(San Diego, California USA) software, and average values in each experimental group were expressed as means ± standard error of mean (SEM). Differences between control and treatment groups were evaluated by one-way ANOVA. To evaluate the severity of behaviors, the differences among groups were compared using Tukey test. The value of $P < 0.05$ was considered statistically significant.

RESULTS

Assessment of open field test

OFT results revealed that the animals in negative control group more frequently entered the central square and also spent more time in the central region than morphine-dependent positive control group ($P < 0.05$). Furthermore, groups treated with increasing intervals, irregular intervals, and decreased the dosage of morphine showed more frequent in central square entries and also spent more time in the central region of the OFT as compared to positive control group ($P < 0.05$) [Table 1].

Negative control group as compared with the dependent positive control group showed more frequent ambulation and rearing and greater ambulation distance in OFT ($P < 0.001$). However, the groups treated with increasing intervals, irregular intervals, and decreased the dosage of morphine showed more frequent ambulation, and rearing and greater ambulation distance in OFT in comparison with positive control group ($P < 0.05$) [Table 1].

Assessment of forced swim test

Positive control group spent less time in swimming in the FST in compared to negative control group ($P < 0.05$). Furthermore, groups treated with increasing intervals, irregular intervals, and decreased the dosage of morphine spent more time swimming in FST than the positive control group ($P < 0.05$) [Figure 1].

Assessment of elevated plus maze

Positive control group spent less time in open arms in compared to negative control group in EPM ($P < 0.05$). However, animals treated with increasing intervals, irregular intervals, and decreased dosage spent more time in open arms in EPM in comparison to positive control group ($P < 0.05$) [Figure 2].

Assessment of tail suspension test

Mean immobility duration was significantly longer in the positive control group compared to that in the

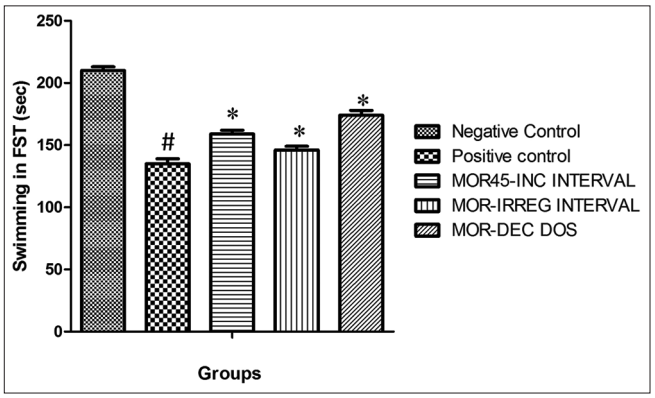

Figure 1: Swimming time (s) in forced swim test in negative control group, positive control group and dependent groups under treatment of various dosage regimen of morphine. All data are expressed as mean ± standard error of mean ($n = 8$). #$P < 0.05$ versus negative control groups, *$P < 0.05$ versus positive control group. MOR-POS control: Positive control group, MOR45-INC Interval: Group under retreatment by increasing interval, MOR-IRREG Interval: Group under treatment by irregular interval, MOR-DEC DOS: Group under treatment by decreasing doses

Table 1: Open field exploratory and anxiety like behavior in rat under treatment by various dosages of morphine

Group	Ambulation distance (cm)	Central square entries (number)	Time spent in central square (s)	Number of rearing
Negative control (morphine independent)	435±12	22±2	160±10	15±2
MOR-POS Control	350±16[a]	15±1.5[a]	105±8[a]	6±1[a]
MOR45-INC interval	370±19[b]	17±1[b]	129±11[b]	8±1[b]
MOR-IRREG interval	372±25[b]	17±1.2[b]	132±10[b]	7±1[b]
MOR-DEC DOS	410±25[b]	19±1[b]	151±12[b]	13±2[b]

[a]$P<0.05$ versus negative control groups, [b]$P<0.05$ versus positive control group. MOR-POS control: Positive control group, MOR45-INC interval: Group under retreatment by increasing interval, MOR-IRREG Interval: Group under treatment by irregular interval, MOR-DEC DOS: Group under treatment by decreasing doses

control group in TST ($P < 0.05$). Also that, groups treated with increasing intervals, irregular intervals and decreased the dosage of morphine remained immobile for a lesser duration compared to positive control group ($P < 0.05$) [Figure 3].

Escape latency and traveled distance during training days in the Morris water maze

The positive control group showed elevated escape latency and longer traveled distance during four days of training in the MWM compared to negative control group ($P < 0.05$) [Figures 4 and 5]. Groups treated with increasing intervals, irregular intervals, and decreased the dosage of morphine showed a significant decline in escape latency and, also traveled a lesser distance during four days of training in the MWM relative to positive control group ($P < 0.05$) [Figures 4 and 5].

Swimming speed during training days in the Morris water maze

Swimming speed did not differ significantly among any of the groups during training trials, suggesting that changes in dosage regimens had not led to any motor disturbances in animals [Figure 6].

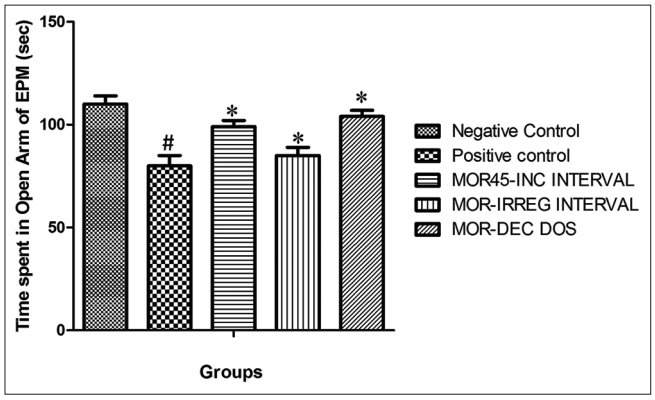

Figure 2: Time spent in open arms (s) in elevated plus maze test in negative, positive control, and dependent groups under treatment by various dosage regimen of morphine. All data is expressed as mean ± standard error of mean ($n = 8$). $^{\#}P < 0.05$ versus negative control groups, $^{*}P < 0.05$ versus positive control group. MOR-POS control: Positive control group. MOR45-INC Interval: Group under retreatment by increasing interval, MOR-IRREG Interval: Group under treatment by irregular interval, MOR-DEC DOS: Group under treatment by decreasing doses

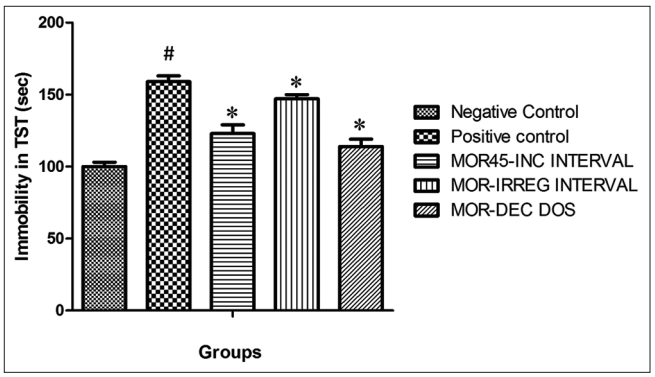

Figure 3: Time stayed in immobility (s) in tail suspension test in negative, positive control, and dependent groups under treatment of various dosage regimen of morphine. All data are expressed as mean ± standard error of mean ($n = 8$). $^{\#}P < 0.05$ versus negative control groups. $^{*}P < 0.05$ versus positive control group. MOR-POS Control: Positive control group, MOR45-INC Interval: Group under retreatment by increasing interval, MOR-IRREG Interval: Group under treatment by irregular interval, MOR-DEC DOS: Group under treatment by decreasing doses

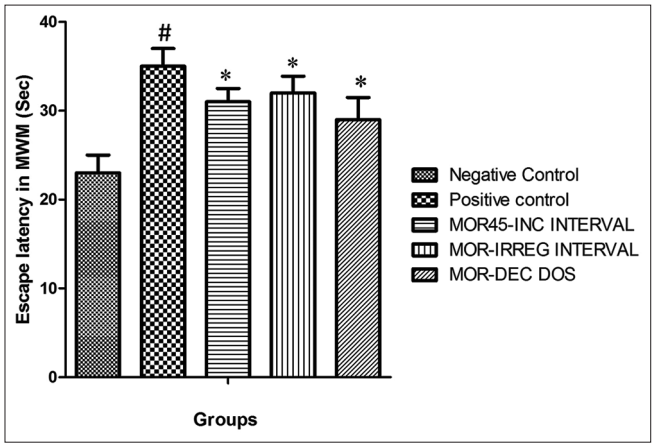

Figure 4: Average escape latency in negative, positive control, and dependent groups under treatment of various dosage regimen of morphine. All data are expressed as mean ± standard error of mean ($n = 8$). $^{\#}P < 0.05$ versus negative control groups. $^{*}P < 0.05$ versus positive control group. MOR-POS Control: Positive control group, MOR45-INC Interval: Group under retreatment by increasing interval, MOR-IRREG Interval: Group under treatment by irregular interval, MOR-DEC DOS: Group under treatment by decreasing doses

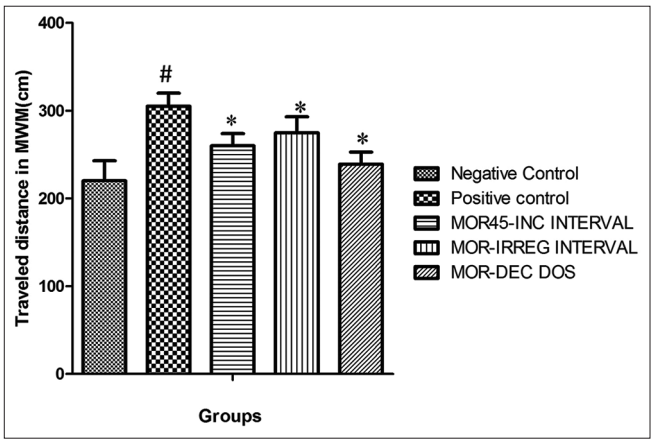

Figure 5: Average of traveled distance in in negative, positive control, and dependent groups under treatment of various dosage regimen of morphine. All data are expressed as mean ± standard error of mean ($n = 8$). $^{\#}P < 0.05$ versus negative control groups. $^{*}P < 0.05$ versus positive control group. MOR-POS Control: Positive control group, MOR45-INC Interval: Group under retreatment by increasing interval, MOR-IRREG Interval: Group under treatment by irregular interval, MOR-DEC DOS: Group under treatment by decreasing doses

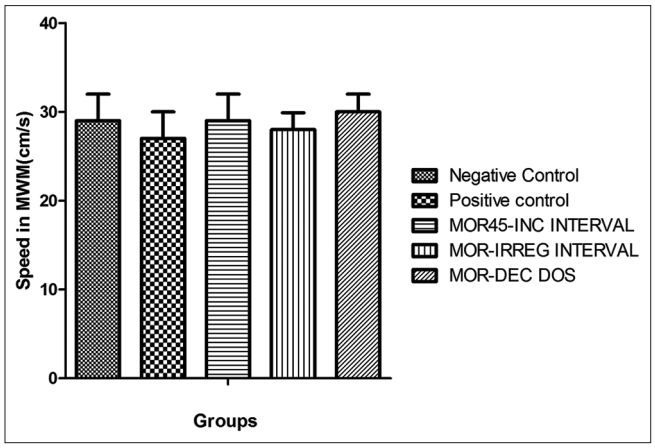

Figure 6: Average of swimming speed in negative, positive control, and dependent groups under treatment of various dosage regimen of morphine. All data are expressed as mean ± standard error of mean (n = 8). #P < 0.05 versus negative control groups. *P < 0.05 versus positive control group, MOR-POS Control: Positive control group, MOR45-INC Interval: Group under retreatment by increasing interval, MOR-IRREG Interval: Group under treatment by irregular interval, MOR-DEC DOS: Group under treatment by decreasing doses

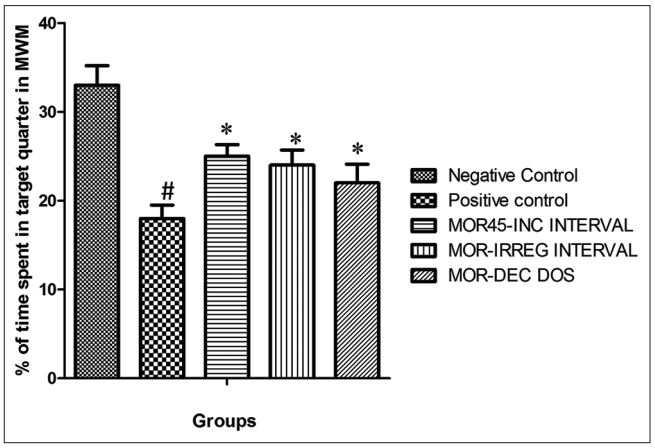

Figure 7: Percentages of time spent in target quarter in probe trial in negative, positive control, and dependent groups under treatment of various dosage regimen of morphine. All data are expressed as mean ± standard error of mean (n = 8). #P < 0.05 versus negative control groups. *P < 0.05 versus positive control group. MOR-POS Control: Positive control group, MOR45-INC Interval: Group under retreatment by increasing interval, MOR-IRREG Interval: Group under treatment by irregular interval, MOR-DEC DOS: Group under treatment by decreasing doses

Time spent in target quarter in probe trial

Animals in positive control group spent lesser time in target quarter in probe trial as compared to negative control group (P < 0.05). Also increasing intervals, irregular intervals, and decreased dosage of morphine groups stayed longer in target quarter relative to that of the positive control group (P < 0.05) [Figure 7].

DISCUSSION

This study indicates that various dosage regimens of morphine can attenuate morphine-induced anxiety, depression, and cognition deficits. Our previous studies showed that increasing administration intervals, irregular interval, and decreasing dosage of morphine versus administration of morphine by fixed doses and regular interval, causes attenuation in withdrawal signs, and cortisol levels.[18] Another work, based on mentioned protocol, also showed that changes in method of administration of morphine causes a decrease of apoptosis and oxidative stress biomarkers,[17] however, despites this results the role of mentioned protocol in anxiety, stress, depressive-like behavior, and cognition performance remain unclear. In this study, we tried to evaluate the role of changes in the administration of morphine, as mentioned protocol, in morphine dependency-induced anxiety, depression, and cognition impairment. Our results showed that morphine dependency causes behavioral alterations in FST (swimming and immobility), EPM (open arm and close arm entry) and OFT (central area entry, stay duration in the central area, ambulation distance, and rearing number) tests. It also suppressed cognitive functions (spatial learning and memory). In contrast, changing dosage regimens of morphine in the manner of increasing intervals, irregular intervals, and decreasing dosage changed behavioral parameters in FST (swimming and immobility), EPM (open arm and close arm entry) and OFT (central area entry, stay duration in central area, ambulation distance, and rearing number). Changes in dosage of morphine also attenuate morphine-induced cognitive deficits as seen by enhanced learning and memory. The results of this study indicated that morphine dependence caused anxiety-like behaviors as indicated by reduced frequency of central square entries and less time spent in the central region of the OFT. In addition, morphine dependency decreased rearing activity and ambulation distance in OFT, which this decrease was significant in comparison with negative control group. These behaviors suggest disturbances in motor activity because in the negative control group, there is no significant changes in mentioned behavior.[32,33] On the other hand, changing various morphine regimens attenuated morphine-induced anxiety-like behaviors as observed by increased frequency of central square entries and time spent in the central region of the OFT. We have also demonstrated that changing morphine dosage regimens can increase ambulation distance and rearing and increase ambulation distance, which indicates that these protocols can reduce morphine, induced motor disturbances. Many previous studies demonstrated that drug addiction can cause disturbance in motor activity.[33,34] these studies indicates that long-term regular administration of opioid and other drug abuse cause disturbance

in motor activity in animal model; These studies suggested that long-term administration of morphine and amphetamine-like compound caused depletion of dopamine and noreadrenaline in reward system,[35,36] On the other way some previous studies showed that by regular administration of morphine the opioid receptor was downregulated and this down-regulation affected brain motor activity behavior.[37] We can argue our data with mentioned studies main concept, which by administration of morphine by our protocol as increasing interval, irregular interval, and decreasing dosage, the mentioned depletion in brain amines or down regulation of receptors less effected in comparison to group which received regular standard doses of morphine with regular interval of administration.[17,18]

In the present study, morphine-dependent rats showed depression like behavior in FST which was markedly improved by various dosage regimens of morphine. These results are in accordance with the previous studies which have indicated that morphine dependency can cause anxiety-like behavior and motor activity deficits.[32,38,39] The various dosage regimen of morphine seems to influence the expression of opioid receptors and thereby improving anxiety and depression-like behaviors.[13] As mentioned in previous studies one of the major problems associated with morphine addiction is depression, which this mood disorder is also the main cause of relapse to abuse of morphine and other drug abuse.[39,40] Some previous studies demonstrated that dysregulation in serotonin and noreadrenaline secretion in morphine-dependent subject responsible for addiction-induced depression, on the other way by administration of morphine as standard protocol, which lead to dependency, this dysregulation occurred.[40,41] Now, according to this concept, in our data by administration of morphine as increasing interval, irregular interval and decreasing dosage versus fixed and regular interval, to some extent this effect of morphine in serotonin and noreadrenaline secretion were inhibited and occurrence of depressive-like behavior in FST test was decreased.

Morphine dependency decreased the spent time in open arm in the EPM.[39] Previous study showed that the opioid dependency in adult rats, associated with the neural sensitization of anxiety-related behavior in EPM test in withdrawal syndrome period.[39,42] However various dosage regimens of morphine, as noted above in other behavioral assay, showed to diminish anxious and depressant effects related to morphine withdrawal. Numerous previous studies showed that addiction to opioid cause anxiety and stress disorder.[41] Our and other studies demonstrated that dependency to morphine and methamphetamine type stimulant cause increase of stress and anxiety biomarker.[30,43] These studies showed that in addiction and drug dependency phenomenon, reward system were involved and brain amines such as dopaimine, nordrenaline, and serotonin play a crucial role.[40,41] All data about drug dependency showed that regular administration of morphine by fixed and clear agenda cause disturbance of brain amine function in the modulation of stress and anxiety and recent data mention that morphine regular administration probably affected the expression of the gene responsible for stress and anxiety. However, it seems that in our study by administration of morphine as increasing interval, irregular interval and decreasing dosage versus fixed dose and regular interval, the mentioned affect of morphine dependency in the reward system, biologic brain amines and gene expression was diminished and anxiety-like behavior in EPM test less occurred. The data from FST showed that morphine dependence increased immobility time, and increasing intervals, irregular intervals and decreased the dosage of morphine, attenuated this increase. These results support previous findings that the morphine use develops a feeling of hopelessness, apathy, and anxiousness.[44] In FST and EPM, morphine dependence induced depression and anxiety like symptoms and results of OFT verified this effect. We demonstrated that morphine dependence impairs learning as observed by increased escape latency and traveled distance in MWM test. Our data also revealed that increasing intervals, irregular intervals and decreased the dosage of morphine can offset this learning impairment which confirms some parts of previous studies.[45,46] Our previous study showed that morphine dependency cause increase of cortisol in animal model, on the other way in similar other studies, we indicated that administration of morphine as increasing interval, irregular interval, and decreasing dosage versus fixed dose and regular interval cause decrease of cortisol level, as markers of stress, which confirm some parts of this study results.[18] Our mentioned studies results in both rat and mice showed that our protocol can be efficient in decrease of withdrawal syndrome and can decrease cortisol, as marker of anxiety, and oxidative stress and apoptosis factors.[17,18]

MWM test in probe days demonstrated that morphine dependence decreases time spent in target quarter where the platform was inserted. In comparison, increasing intervals, irregular intervals, and decreased dosage of morphine increased the percentage of time spent in target quarter. This is also conforming to previous studies where morphine use was found to impair learning and cognition.[45,47] Cognition

impairment and disturbance in learning and memory is one of the adverse consequences of drug dependency.[48] The previous study demonstrated that morphine, amphetamine, and other drug abuse cause disturbance in cognition in animal and human subject.[48] Some molecular studies showed that drug abuse cause disturbance of function of molecules involved in memory pathway in the brain.[48,49] These studies indicate that fixed daily dose of abused drug can disturb the function of mentioned molecule in both gene and protein level.[48,49] By conceptualization of this data, with our results, we can suggest that by increasing interval, irregular interval, and decreasing dosage, mentioned molecules were affected less than condition which morphine administrated as fixed doses and regular interval and thus cognition behavior, learning, and memory was improved in MWM, on the other word our protocol diminish morphine effects on cognition impairment.

We can discuss these results with the genetically basis that various responses are observed after chronic administration of drugs of abuse. Previous studies showed that This biochemically modified isoforms of ΔFosB accumulate within the some part of brain regions after repeated drug exposure while other Fos family members remain unchanged.[50,51] ΔFosB isoforms accumulate with chronic drug exposure because of their extraordinarily long half-lives and therefore persist in the neurons for at least several weeks even after cessation of drug administration.[50,51] Previous studies have demonstrated that ΔFosB isoforms are responsible for drug tolerance, craving, and withdrawal syndrome induced anxiety and depression.[52-54] Interestingly, Alterations in Fos gene expression are also implicated in mediating certain destructive effects such as apoptosis and oxidative stress in cells.[55] Based on mentioned result and studies we can discuss that by altering dosage regimen, such as our protocol, probably the ΔFosB isoforms, which involved in addiction behavior, were changed and addiction relative behavior such as anxiety, depression, and cognition impairment were modulated.

We can relate these findings to our study in a way that, varying dosage regimens of morphine as by increasing dosage intervals, decreasing dosage and irregular administration intervals, the ΔfosB gene expression during chronic administration can be altered and this can modulate the mentioned behaviors in a withdrawal syndrome. Furthermore, previous studies have demonstrated that repeated cocaine and opioid administration induces changes in the expression of proteins like regulators of G-protein signaling protein 4, Ca^{2+}/calmodulin-dependent protein kinase II, and cyclin D2.[54,56] Hence, our treatment protocol can interact with these proteins and alter some transcription factors, which ultimately can affect animal behaviors like those observed in withdrawal syndrome.

CONCLUSIONS

Our study suggests that various dosage regimens of morphine-like increasing intervals, irregular intervals, and decreased dosages can be beneficial in reversing morphine-induced depression and anxiety-like behavior, and cognitive impairment. This study opens new insights for managing opioid dependence side effects such as anxiety, depression, and cognition impairment in an animal model, also using mentioned protocol in the human abuser, which will be indicated in next studies, its efficacy for managing of dependency-induced anxiety, depression, and cognition impairment can be confirmed.

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Conflicts of interest
There are no conflicts of interest.

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