Methadone and haloperidol combination effect on the acquisition and expression of morphine tolerance and dependence in male mice

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A B S T R A C T

Background and Objective: Today, opioids are used to control and relieve acute and chronic pain. However, the incidence of both tolerance and dependence phenomena for these drugs is a major problem. So, in this study, the combination effect of haloperidol and methadone on the acquisition and expression of morphine dependence and tolerance was examined.

Materials and Methods: Ninety-eight mice were randomly divided into groups of acquisition and expression. Each group was divided into seven subgroups, saline, morphine, methadone, haloperidol, haloperidol + methadone, methadone + haloperidol ratio of 2 to 1, methadone + haloperidol ratio of 1 to 2. All groups were addicted with gradually increasing doses of morphine for 7 consecutive days. All drugs in the acquisition group were injected 30 minutes before morphine injected for 7 days and in the expression group 30 minutes before morphine injected in the eight day (test day). Morphine tolerance was measured by tail immersion test for 30 minutes before and after administration of morphine in test day. To assess dependence, mice were administered with naloxone and withdrawal behaviors were observed for 30 minutes.

Results: Chronic morphine injections induced tolerance and dependence in mice. Percentage of MPE as a tolerance index was significantly increased in acquisition and expression groups in drugs combination methadone1+haloperidol2 than morphine ones. Also, in dependence group, a marked decrease was shown in withdrawal behaviors in the combination therapy groups.

Conclusion: Our results showed that probably methadone and haloperidol combination treatment, especially at a ratio of 1 to 2, could reduce tolerance and dependence more than single drug treatment in animal groups.

1. Introduction

Opioids such as morphine are currently used widely to control and relief acute and chronic pain cases. Morphine exerts its strong analgesic effect due to attachment to μ opioid receptor (1). But, long term prescription of opioids, results in resistance and dependence, and stopping using them, will result in symptoms such as inquietude, anxiety, aggression, and irritability, which are known together as withdrawal syndrome (2). According to such phenomena, high restriction has been made against using these compounds. Despite many researches have been made on this issue, the true mechanism of resistance, dependence, and withdrawal according to some of the studies,

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among mechanisms involved in resistance and dependence on opioids, neurotransmitter systems such as nitric oxide (3), glutamate (4), dopamine (5), and stimulatory amino acid receptors, especially NMDA (6), are significant. The role of NMDA glutamate receptors in opioid-related synaptic shape ability has been proven (6). According to previous studies, calcium entry into the cell increases by activation of these receptors. Obviously, higher intracellular calcium concentration can result in activating some of calcium-dependent second messengers, and many effects such as amplification of calcium calmodulin protein kinase II (CaMKII) activity (7), protein kinase C (PKC) positive feedback regulation (8), nitric oxide synthetase (NOS) activation, and finally, nitric oxide (NO) production (9). NO is a neural moderator derived from L-arginine by NOS enzyme. The NOS enzyme is activated by calcium calmodulin protein kinase II (CaMKII) (10). Many researches suggests NO interference in resistance and dependence to morphine. Evidence suggests cooperation of NO with other neurotransmitter systems such as glutamatergic system and NMDA to perform its role (11). Also, studies suggest a mutual relation between NO production and dopamine release (12,13). Methadone therapy is currently known as the most suitable opioid detoxification. Methadone is a unique industrial opioid which is also used instead of morphine for severe pain treatment (14,15), and is the agonist to opioid receptor, and antagonist to NMDA receptor (16). But unfortunately, some of the patients under treatment with methadone develop mental disorders such as anxiety or depression (17), and some of the patients do not respond to this treatment.

Haloperidol as anti-psychotic butirophenon medication is the antagonist to dopamine and has high tendency to D_2 dopamine receptors. This medication is used to treat various mental diseases such as schizophrenia, mania, and psychosis (18). Researches indicate that this medication is CaMKII inhibitor, and therefore, can be used to decrease opioid resistance and dependence (19). According to this, and regarding new research on effects of compound medications on diseases, this study addresses compound effect of methadone and haloperidol on acquisition and expression of morphine resistance and dependence.

2. Materials and Methods

In this experimental study, 98 NMRI mice (obtained from Razi Institute, Tehran) in the weight range of 20-25 g were used. Animals were kept in clear Plexiglas cages in groups of four, and were moved to animal house of the faculty few days before the experiment, in order to get adapted to the environment. The animal house was in suitable 12 hour day-night period situation and 30-40% humidity, and the temperature was fixed at 21±2°C. Also, all of the animals of every group had unlimited access to sufficient food and water, and each animal was assessed only once. In all phases, all morale principles about animals were respected.

In this study, 98 mice were divided into acquaintance (chronic) and expression (acute) groups. Each group consists of seven sub-categories:

1. saline, 2. morphine, 3. methadone (10 mg/kg) 4. haloperidol, 5. methadone+haloperidol (5 mg/kg and 0.15 mg/kg, respectively), 6. 2 methadone+1 haloperidol (7 mg/kg and 0.1 mg/kg, respectively), 7. 1 methadone+2 haloperidol (3.5 mg/kg and 0.2 mg/kg, respectively).

Morphine was injected to acquaintance group for seven days, twice a day, and once in the eight day, in order to study acquaintance of tolerance and dependence. In this group, in each sub-category, all medications were injected 30 minutes before receiving morphine doses.

Morphine was injected to expression group for seven days, twice a day, and in the eight day, a single dosage of the medication was injected 30 minutes before receiving the last morphine dosage, in order to study expression of tolerance and dependence.

2.1. Tolerance and dependence circumstances

Morphine was injected to all mice with staircase dosages of mg/kg for seven days, twice a day (8 a.m., 4 p.m.), and once in the eight day (8 a.m.), according to the following program, in order to form tolerance and dependence:

First day: 10, second day: 20, third and fourth day: 40, fifth day: 60, sixth day: 80, seventh day: 100, and eighth day: 100.

2.2. Pain tolerance test

In this study, pain threshold of all mice was assessed in two phases (30 minutes after injection of the medication and 30 minutes after injection of morphine) by exposing their tails to hot water. One cm from the end of the tail was exposed to 56±0.5°C water and a stopwatch was immediately started, and stopped when the animal pull the tail out of water as a reflex. A 10 second limit was considered, in order to prevent tissue damages. If the animal did not show any reflex in 10 seconds, the tail was pulled out of water. The procedure was executed with 3 minute gaps, three times in each phase, and the average was calculated. Then, the averages were put into the following formula, maximum possible effect was given in percentages, and the final data was used for statistical analysis.

MPE % = [Delay before morphine injection (sec) - Delay after morphine injection (sec) / Delay before morphine injection (sec) - Stop time (sec)] × 100 %

2.3. Withdrawal syndrome induction and behaviors under study

Two hours after the last morphine injection in the test day, 5 mg/kg of naloxone was injected to each mouse in every group and then each animal was put into a clear box measuring 20×20×30 cm, in order to exhibit withdrawal symptoms, and then behavioral symptoms were observed and noted for 30 minutes. Mice show different behaviors during induction of withdrawal syndrome. In this study, we discussed jumping, standing, licking, and diarrhea. Among these symptoms, jumping is of a high importance, as in many researches, it is the only mentioned symptom.

2.4. Drugs

In this study, morphine sulphate (Tamad, Iran), methadone (Tamad, Iran), haloperidol (Minou, Iran), and naloxone (Tolid darou, Iran) were used. Morphine sulphate and methadone were dissolved in normal saline, and haloperidol was dissolved in methanol. All of the injections were made i.p. at a volume of 0.2 ml.

2.5. Statistical analysis

In this study, SigmaStat software (version 3.5) was used for statistical analysis. All data were reported as average ± variance. Statistical comparison between test groups was made by variance analysis test and then by Tukey post-test, and $p<0.05$ differentiation level was regarded significant. Non-parametric data analysis was done by Kruskal-Wallis test and the related post-test.

3. Results

3.1. The effect of methadone, haloperidol, and their compounds on acquisition of morphine dependence

As shown in figure 1, jumping (A) as an indicator of morphine withdrawal syndrome, in treatment groups of haloperidol, 2 methadone+1 haloperidol, and 1 methdone+2 haloridole, had an outbreak with averages of 30±6.25, 28±8.4, and 12±0.64, respectively, with a significant decrease as compared to morphine group with an average of 79.25±9.89. Frequency of diarrhea (B) in treatment groups of haloperidol, 2 methadone+1 haloperidol, and 1 methdone+2 haloridole, occurred with an average of 1.83±0.74, 2.25±0.9, and 2±0.36, respectively, with a significant decrease as compared to morphine group with an average of 5.16±0.4. Frequency of licking (C) in treatment groups of haloperidol, 1 haloperidol+1 methadone, 2 methadone+1 haloperidol, and 1 methdone+2 haloridule, decreased by 48±15, 49.42±16.74, 55.14±16.43, and 57.75±24, respectively, which was obviously significant, as compared to morphine group 138.28±17.71 ($p<0.05$). Frequency of standing (D) in treatment groups of haloperidol and 2 methadone+1 haloperidol with averages of 20±5.27 and 23±6.94 had an obvious decrease as compared to average of 54±6.86 of morphine group ($p<0.01$).

3.2. The effect of methadone, haloperidol, and their compound on expressing morphine dependence

As shown in figure 2, jumping (A) in treatment groups of methadone and haloperidol with averages of 52.4±11.78 and 45±14.81, and in compound treatment groups of 1 methadone+1 haloperidol and 2 methadone+1 haloperidol with averages of 37±7.87 and 29.75±9.37, respectively, showed a significant decrease as compared to morphine group with an average of 106±16.76. Frequency of diarrhea (B) in treatment groups of

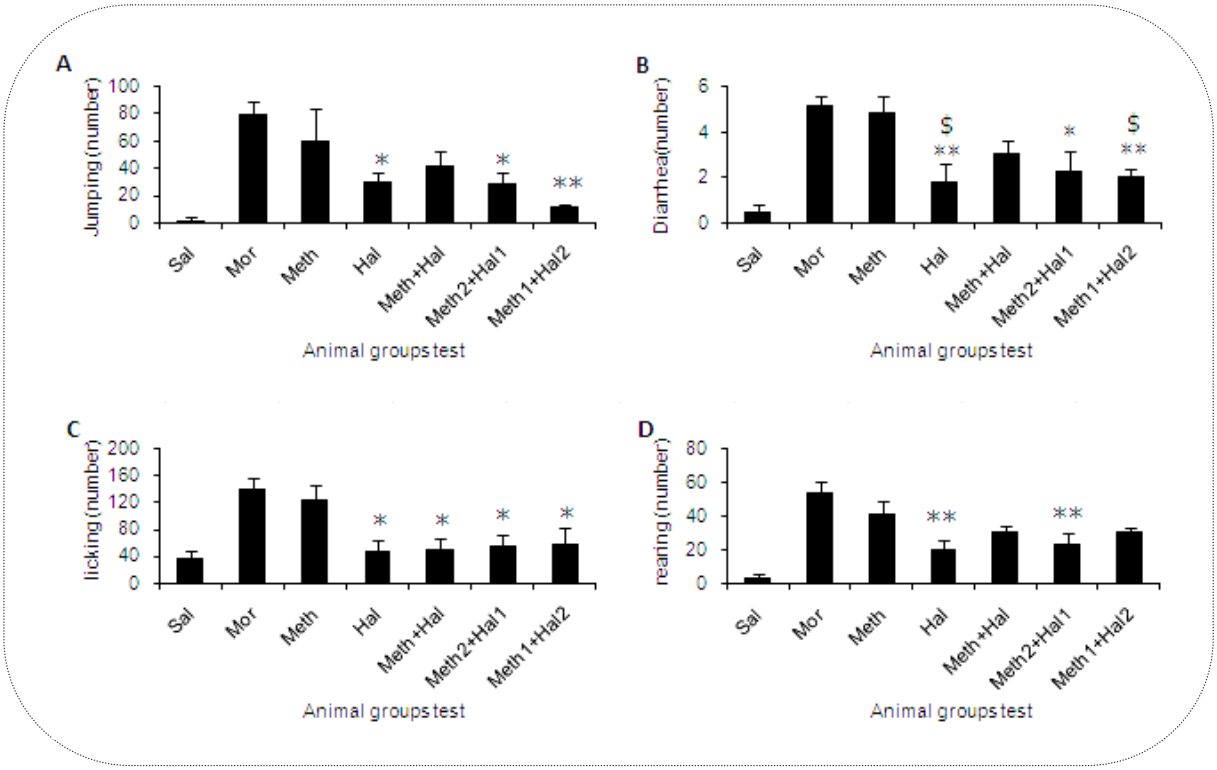

Figure 1. Effect of methadone, haloperidol, and their compound on acquisition of morphine dependence

A: jumping, **B:** diarrhea, **C:** licking, **D:** standing.

Sal: normal saline, Mor: morphine (addicted group), Meth: methadone, Hal: haloperidol.

Columns show Mean ± SEM (n=7).

* and ** show significant difference with morphine group (with P<0.05 and P<0.01, respectively).

$ shows a significant difference from methadone (P<0.05).

haloperidol, 1 methadone+1 haloperidol, and 1 methadone+2 haloperidol, decreased by 2±0.2, 1.6±0.8, and 1.57±0.64, respectively, which was significant as compared to morphine group with an average of 5.16±0.54.

Frequency of licking (C) as another sign of dependence in treatment groups with haloperidol and 1 methadone+2 haloperidol, with averages of 14.66±8/65 and 37±11.78, respectively, showed a significant decrease as compared to morphine group with an average of 91.33±11.95. Number of standings (D) in methadone and 1 methadone+2 haloperidol treatment groups, with averages of 22±4.6 and 23±2.4, respectively, decreased significantly as compared to morphine group with an average of 47.83±5. In addition to these two groups, haloperidol and 2 methadone+1 haloperidol treatment groups with averages of 16.14±2.07 and 18.6±5 also showed a significant decrease as compared to morphine group.

3.3. The effect of methadone, haloperidol, and their compound on acquisition of morphine tolerance

As shown in figure 3, the MPE percentage in 1 methadone+2 haloperidol with an average of 19.49±2.34 shows a significant increase as compared to morphine group with an average of 6.44±1.67 (p<0.001). In other words, it can be said that 1 methadone+2 haloperidol group has increased the analgesic response to morphine, and has caused a decrease in tolerance expression.

3.4. The effect of methadone, haloperidol, and their compound on expression of morphine tolerance

As shown in figure 4, none of the groups could cause a significant change in morphine tolerance

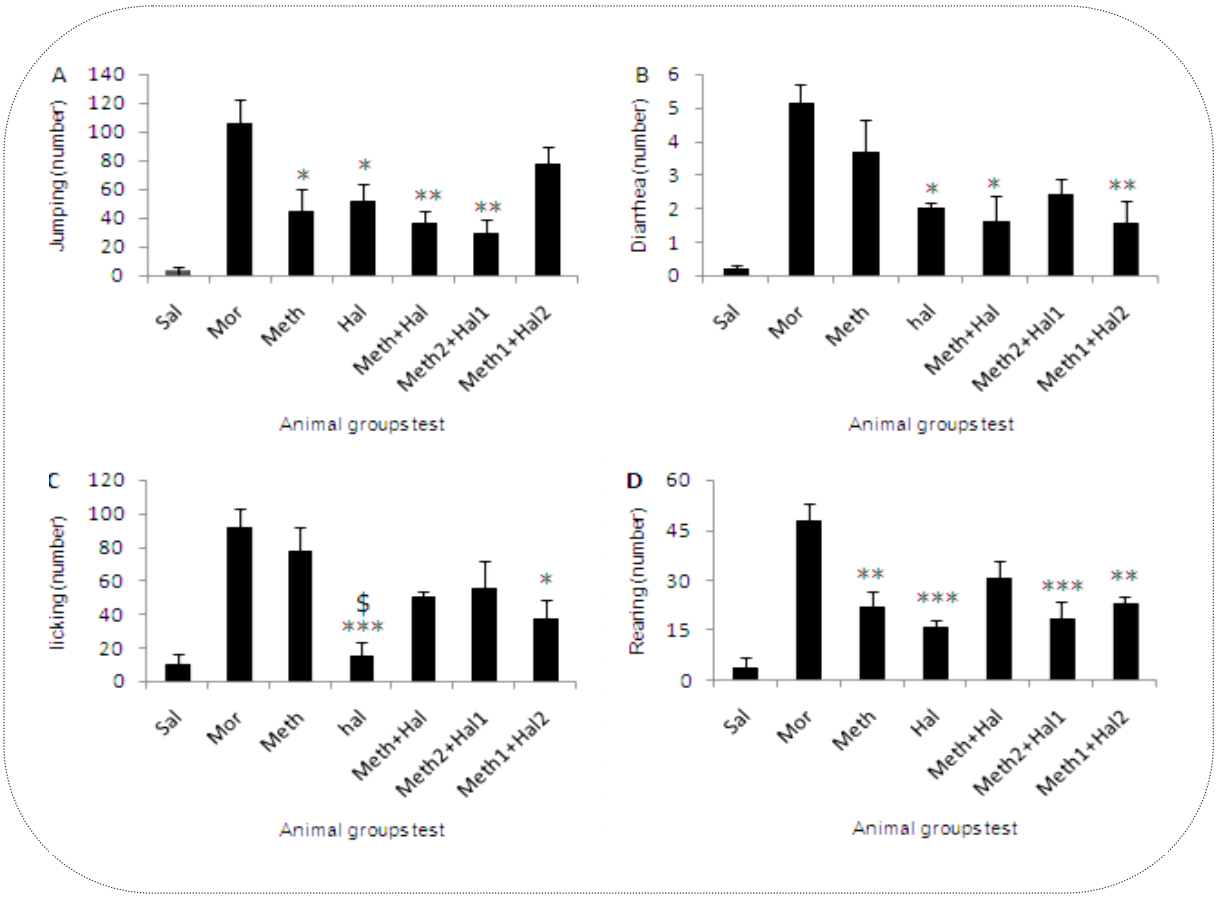

Figure 2. Effect of methadone, haloperidol, and their compound on expressing morphine dependence

A: jumping, **B:** diarrhea, **C:** licking, **D:** standing.

Sal: normal saline, Mor: morphine (addicted group), Meth: methadone, Hal: haloperidol.

Columns show Mean ± SEM (n=7).

* and ** show significant difference with morphine group (with P<0.05 and P<0.01, P<0.001, respectively).

$ shows a significant difference from methadone (P<0.05).

as compared to morphine group, except 1 methadone+2 haloperidol treatment group with an average of 17.92±3.33 could increase the MPE percentage as compared to morphine group with an average of 7.05±1.49, and decrease the tolerance expression significantly (p<0.05).

4. Discussion

The obtained results showed that morphine prescription for 7 days will cause tolerance against its analgesic effects, and stopping using morphine will cause symptoms of withdrawal syndrome, which indicates dependence on morphine. Prescription of pharmaceutical composition of methadone + haloperidol with a ratio of 1 to 2 in acquisition and expression groups can decrease tolerance to analgesic effects of morphine significantly. Therefore, it can be said that this pharmaceutical composition increased analgesic effect of morphine. Results of withdrawal syndrome symptoms showed that methadone in expression group only has been able to cause a significant decrease in jumping and standing on both feet as compared to morphine group, and has reduced none of the other behaviors significantly, but all of the symptoms of withdrawal syndrome reduced significantly in both acquisition and expression groups by haloperidol as compared to morphine.

Many studies have shown that using NMDA receptors antagonists prevent tolerance and dependence on morphine (20,21). Methadone is the antagonist to μ receptor and NMDA receptor, therefore, in addition to analgesic effects, can

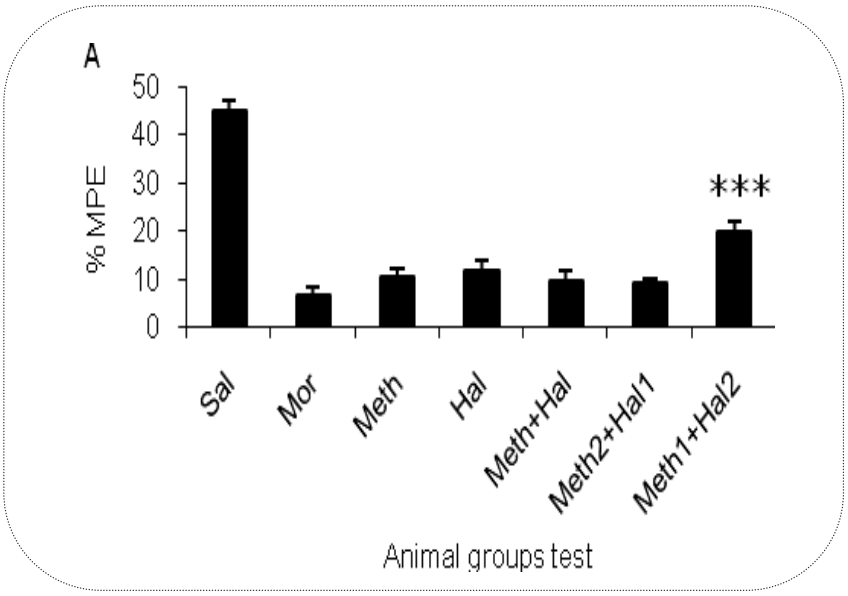

Figure 3. Effect of methadone, haloperidol, and their compound on acquisition of morphine tolerance

Sal: normal saline, Mor: morphine (addicted group), Meth: methadone, Hal: haloperidol.

Columns show Mean ± SEM (n=7).

*** shows significant difference with morphine group (with P<0.001).

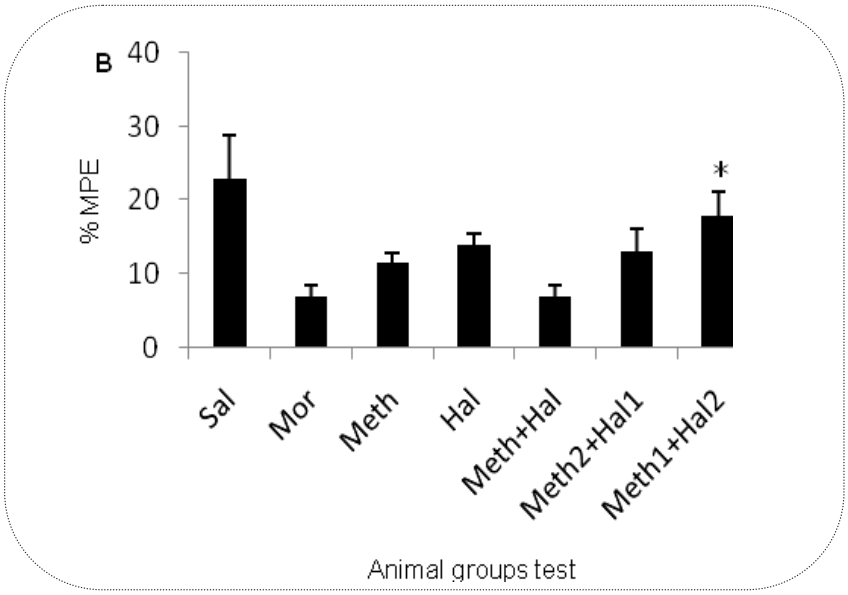

Figure 4. Effect of methadone, haloperidol, and their compound on expression of morphine tolerance

Sal: normal saline, Mor: morphine (addicted group), Meth: methadone, Hal: haloperidol.

Columns show Mean ± SEM (n=7).

* shows significant difference with morphine group (with P<0.05).

prevent tolerance and dependence on morphine (16). A research by Whistler et al showed that prescription of morphine alongside with low dosages of methadone can increase analgesic effect of morphine in treatment of chronic pains. Also, patients in need of long term opioid usage, are able to use a composition of methadone with opioids in order to reduce dependence (1). As shown before, long term treatment using morphine can cause CaMKII activity in the body. Also, it has been proven that spinal and supra-spinal inhibition of CaMKII not only causes prevention, but also inverts tolerance to analgesia and physical dependence on opioids in some rodents (22,23). A study performed by Young et al has shown that haloperidol as an antipsychotic medication can reduce tolerance and dependence on opioids by inhibition of CaMKII activity (19). Also, as mentioned above, haloperidol is mostly known as D_2 dopamine receptors antagonist. Previous studies indicate the ability of dopamine receptor antagonists in movement inhibition (24), conditioned place preference (25), and morphine self-prescription (26) in mice. But what is certain, is that the combination of methadone and haloperidol has been more effective in reducing tolerance and dependence on morphine than the effect of each medication. Perhaps the reason for such phenomenon is that according to experts, CaMKII can phosphorylase NMDA receptor, which can cause an increase in NMDA receptor activity and calcium penetration through the channels. This positive feedback between CaMKII and NMDA receptor can be a CaMKII and NMDA receptor activity booster in tolerance and dependence on opioids (19); which explains reduction of tolerance and dependence due to CaMKII activity inhibition by haloperidol and NMDA receptor inhibition by methadone in this study. However, the results of this study showed that prescription of pharmaceutical combination of methadone and haloperidol can reduce acute or chronic acquisition and expression of tolerance and dependence on morphine. Since the usage of methadone as a common medication for addicted people today, and also according to antipsychotic effects of haloperidol as an effective medication in mental disorders, it is suggested to use a combination of above-mentioned medications as a more effective way of prevention and treatment of tolerance and dependence on opioids such as morphine. Further research is necessary in order to be considered in future studies.

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