

# Attenuation of morphine withdrawal signs, blood cortisol and glucose level with forced exercise in comparison with clonidine

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**Abstract** **Background:** Morphine withdrawal usually results in undesired outcomes, despite partial benefits of alternative medication such as methadone, because of the lack of mental sedation during the withdrawal period, may not lead to the desired result. In this study, forced exercise by treadmill is used to manage morphine dependence in animal model.

**Materials and Methods:** Forty adult male mice were divided into 5 groups, from which 4 groups became dependent by increasing daily doses of morphine for 6 days (20-45 mg/kg, SC). Afterwards, the animals were treated for 21 days by either of the following protocol: Positive control (dependent) received once daily 45 mg/kg of morphine sulfate (SC) for 21 day, group under treatment by clonidine (0.4 mg/kg, SC) for 21 day group under treatment by forced exercise by treadmill for 21 day, group under treatment by combination of clonidine (0.4 mg/kg, SC) and forced exercise by treadmill for 21day and the negative control group(independent) received saline injection like other groups. Each of this administration was injected at 8 AM. Finally, in the test day (day 28), all animals received a single dose of naloxone (3 mg/kg, SC) at 8 AM and then were observed for withdrawal signs, and Total Withdrawal Score (TWS) was determined as described previously. After withdrawal sign evaluation for evaluation of stress level of dependent mice, blood cortisol and glucose level were measured in non-fasting situations well.

**Results:** This study showed that TWS significantly decreased in all treatment groups in comparison with positive control group ( $P < 0.001$ ). Moreover, blood cortisol and glucose level significantly decreased in group under treatment by clonidine (0.4 mg/kg) and group under treatment by combination of clonidine (0.4 mg/kg) and forced exercise by treadmill groups in comparison with control positive (dependent) ( $P < 0.05$ ).

**Conclusion:** This study suggested that forced exercise can be useful as adjunct therapy in dependent people and can ameliorate side effects and stress situation of withdrawal syndrome periods.

**Key Words:** Cortisol, drug dependence, glucose, morphine, withdrawal score

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## INTRODUCTION

The precise mechanism that describe tolerance, dependency, and withdrawal symptoms of morphine is not clear.<sup>[1]</sup> Drugs with long-lasting effect are used for pharmacotherapy of abstinence.<sup>[2]</sup> These alternative medications act like morphine with mild withdrawal syndrome.<sup>[3,4]</sup> Also in previous studies, the withdrawal

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syndrome was attenuated by a special group of drugs or receptors that act on major brain neurotransmitters.<sup>[5,6]</sup> Morphine withdrawal is characterized by a stressful condition and increased activity of hypothalamus — pituitary — adrenocortical (HPA) axis.<sup>[7]</sup> Naloxone-induced withdrawal syndrome can activate HPA axis. An increase in cortisol secretion and high glucose level are probably a kind of defense against stress and anxiety in withdrawal syndrome.<sup>[8]</sup> Previous studies indicated an increase in activity of cells secreting corticotropin releasing factor (CRF) leading to activation of adrenal gland.<sup>[9]</sup> These studies demonstrated that morphine dependency will increase the expression of CRF mRNA. An increase in cortisol and glucose level were also observed in users of cocaine.<sup>[10]</sup> Previous studies have also shown that exercise lowers stress and anxiety and releases endorphin secretion in brain during rehabilitation process.<sup>[11,12]</sup> It has also been shown that exercise can counteract withdrawal symptoms and physical activity can reduce the risk of drug addiction.<sup>[13]</sup> Exercise reduces the rewarding effects of drugs such as cocaine and morphine, by increase in synthesis and release of dopamine, stimulating neuroplasticity and promotes feelings of well-being.<sup>[14]</sup> Clinical approach to managing withdrawal syndrome is mostly based on detoxification and pharmacotherapy by long-acting opioids.<sup>[15]</sup> In the present study, the attenuation of the severity of withdrawal syndrome and decrease in stress level in experimental period was investigated by exercise, the cortisol and glucose level measured as important parameters in stress.

## MATERIALS AND METHODS

### Drugs

Morphine was purchased from Temad Company (Tehran, Iran) and clonidine was product of Tolid-e Darou Company (Tehran, Iran).

### Animals

Forty Male albino mice weighing 30 to 35 g were kept in temperature ( $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) and light controlled room under a 12-hour light and dark cycle. Food and water were available *ad libitum*. They were allowed to adapt to laboratory condition for at least 2 hours before testing and each animal was used only once. All experimental procedures followed the Guidelines on Ethical Standards for experiment on pain in animals and carried out according to a protocol approved by the local Animal Ethics Committee.

### Morphine withdrawal syndrome protocol grouping protocol

#### Induction of morphine dependency

To induce morphine dependency, in 4 groups (of 5); the animals were injected morphine (20-45 mg/kg, SC)

subcutaneously with an increasing dosage for 6 days. Animals in control group received normal saline for 6 days.<sup>[16,17]</sup>

#### Treatments

From 7<sup>th</sup> day, positive control group (group 1) received morphine (45 mg/kg, SC) for maintenance of dependency for three weeks. Negative Control group (group 2) received normal saline for three weeks (0.2ml/mice, IP). Groups 3 received clonidine hydrochloride (0.4 mg/kg, SC) for three weeks. Groups 4 were treated by forced exercise (by following protocol) for three weeks. Group 5 received clonidine hydrochloride (0.4 mg/kg, SC) and was treated by forced exercise (by following protocol) for three weeks. Each of mentioned administration was injected at 8 AM.

#### Forced exercise protocol by treadmill

Mice were allowed to run on a motor-driven treadmill (Model T408E, Diagnostic and Research Instruments Co., Taoyuan, Taiwan). The animals in group 4 and 5 were trained on treadmill at an intensity of 80% of maximal oxygen consumption for 60 min/day for 5 days per week for 3 consecutive weeks. The training speed was 12 miles per minute (for first week) and reached 14 miles per minute (at third week) by the end of the experiments.<sup>[18,19]</sup>

#### Induction and Evaluation of withdrawal syndrome

In day 28, animals of all groups were injected a single dose of naloxone (3 mg/kg, SC) at 8 AM and their 14 behaviors (jumping, head shake, wet dog shake, for paw tremor, writhing, walking sniffing, sniffing, penile liking, rearing, chewing, body grooming, face wiping, swallowing, teeth chattering) were recorded by camera. After computation of data recorded, each of the behaviors divided to their weighing factor and a digit was obtained [Table 1]. The summation of these digits gives the Morphine Total Withdrawal Score (MTWS).<sup>[16,17]</sup>

#### Measuring blood cortisol ( $\mu\text{g}/\text{dl}$ ) and glucose level ( $\text{mg}/\text{dl}$ )

After the behavioral studies on the 28<sup>nd</sup> day, the mice were first anesthetized by diethyl ether and then killed

**Table 1: Weighing factors (WFs) of different withdrawal signs of morphine in the mouse**

Behavior	WF	Behavior	WF
Jumping	4	body grooming	10
head shake	5	face wiping	10
wet dog shake	5	swallowing	10
paw tremor	5	teeth chattering	10
Writhing	5	dysphoria	10
walking sniffing	5	rearing	20
Sniffing	5	Chewing	20
penile liking	5	–	–

by giotin. Whole blood was collected and their serum was separated and the level of serum cortisol was measured based on  $\mu\text{g/dl}$  and by ELISA method. The blood glucose level of animals were measured based on  $\text{mg/dl}$  by a manual glucometer.

### Statistical analysis

All data were analyzed by SPSS statistic software. The mean values were obtained in each experimental group and data expressed as means  $\pm$  standard error of the mean (SEM). The differences between groups were analyzed by unpaired Student's *t* test. Differences among groups receiving various protocols of treatments were first compared by one-way ANOVA and, then, group-by-group with a post hoc test of Bonferroni's *t* test. A value with  $P < 0.05$  was taken as statistically significant. Also, the difference between means measured by using the following ratio means of treated group- means of control group  $\times 100/\text{means of treated group}$  and result of changes report as percent in result section.

## RESULTS

### Morphine Total Withdrawal Score (MTWS)

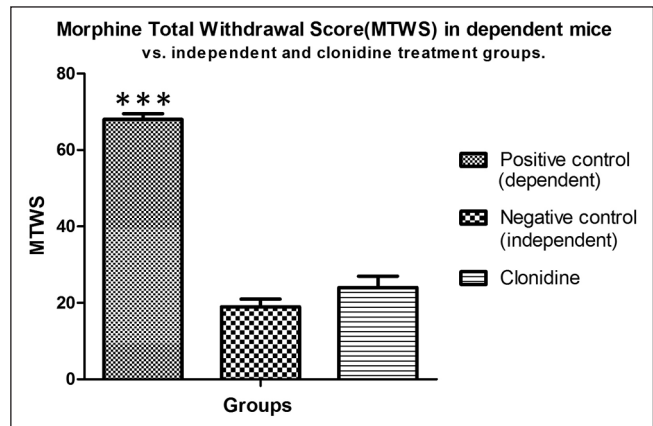
Our study showed that MTWS for negative control group that received normal saline during experimental period was  $19 \pm 2$  while for positive control group (dependent group) MTWS was  $68 \pm 1.5$  (72% higher than negative control) ( $P < 0.001$ ). Administration of clonidine caused 64% decrease in MTWS in comparison to positive control group ( $24 \pm 3$  compared to  $68 \pm 1.5$ ) ( $P < 0.001$ ) [Figure 1]. Also, forced exercise caused 67% decrease in MTWS ( $22 \pm 1$ ) in comparison to positive control group and in combination therapy with clonidine and Treadmill forced exercise the mean MTWS was  $20 \pm 3.5$  (i.e., 70% lower than positive control group) ( $P < 0.001$ ) [Figure 2].

### Blood cortisol level

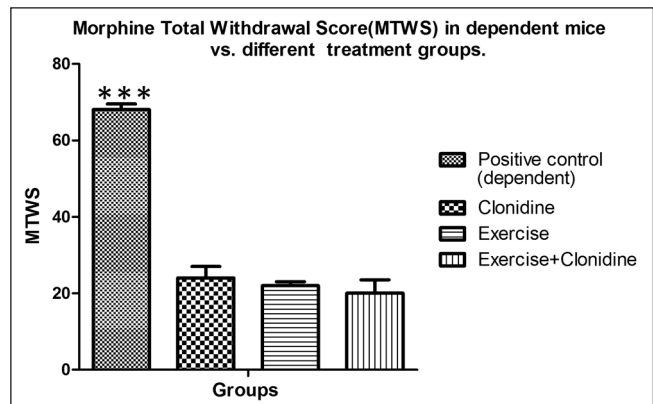
Blood cortisol level in the negative control group was  $6.1 \pm 1.6 \mu\text{g/dl}$  after injection of naloxone, while in the positive control group was significantly higher, about  $14 \pm 1.1 \mu\text{g/dl}$  ( $P < 0.05$ ). Administration of clonidine caused significant decrease in cortisol level (from  $14 \pm 1.1$  to  $7.9 \pm 1.1 \mu\text{g/dl}$  ( $P < 0.05$ ), i.e., 43%). Treatment of animals by forced exercise caused 28% decrease in cortisol level in comparison with positive control group and reached to  $10 \pm 1.3 \mu\text{g/dl}$ . In the last treatment group, combination therapy of clonidine and forced exercise caused 49% decrease in cortisol level and reached to  $7.1 \pm 2 \mu\text{g/dl}$  ( $P < 0.05$ ) [Figure 3].

### Blood glucose level

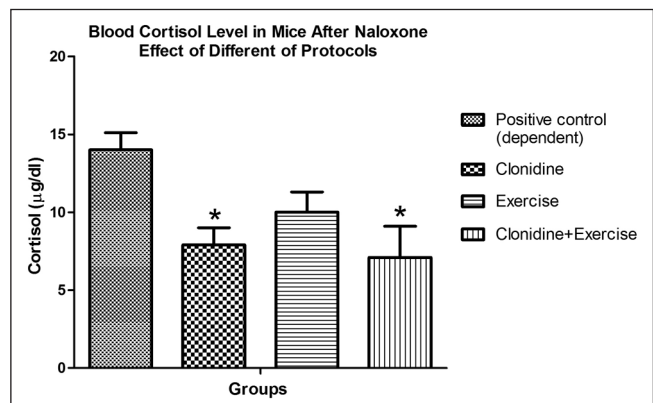
Blood glucose level in the negative control group was  $89 \pm 5 \text{ mg/dl}$  after injection of naloxone; while



**Figure 1:** Comparison of occurrence of the morphine withdrawal signs between the mice of the independent negative control group and dependent positive control group. \*\*\*: shows the significant difference ( $P < 0.001$ ) in comparison with the independent negative control group



**Figure 2:** The occurrence of the morphine withdrawal signs in the mice of the dependent groups under treatment by clonidine, under treatment by exercise, and under treatment by combination of clonidine and exercise, in comparison with the dependent positive control group. \*\*\*: shows the significant difference ( $P < 0.001$ ) in comparison with group under treatment by clonidine, exercise, and their combination



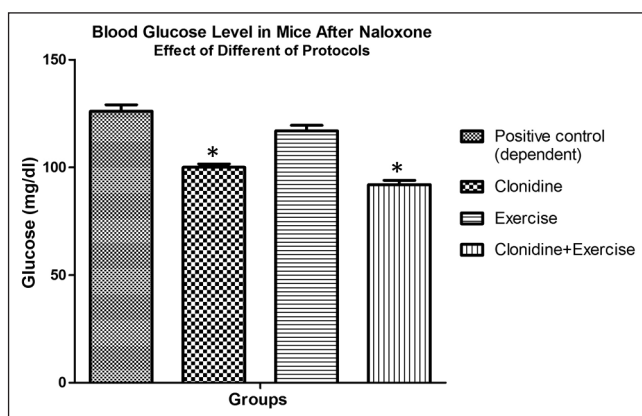
**Figure 3:** The blood cortisol levels in the mice of the dependent groups under treatment by clonidine, by forced exercise, and by combination of clonidine and forced exercise in comparison to the dependent positive control group. \*: shows significant difference ( $P < 0.05$ ) in comparison with the dependent positive control group

in the positive control group was significantly higher ( $126 \pm 3$  mg/dl) ( $P < 0.05$ ). Clonidine administration caused significant decrease in blood glucose level (20%) and reached to  $100 \pm 1.6$  mg/dl ( $P < 0.05$ ). Treatment of animals by forced exercise with treadmill caused 7% decrease in glucose level in comparison with positive control group and became  $117 \pm 2.5$  mg/dl. In the last treatment group, combination therapy of clonidine and forced exercise caused 22% decrease in blood glucose level and reached to  $92 \pm 2$  mg/dl ( $P < 0.05$ ) [Figure 4].

## DISCUSSION

Many studies were performed in the field of the withdrawal syndrome, and treatment of drug dependency had been conducted by groups of medications such as naltrexone, dextrometorphane, methadone, tramadole, and buprenorphine.<sup>[20-22]</sup> The maintenance therapy of drug addiction is based on using or manipulating specific neurotransmitters reuptake, cerebral amino acids.<sup>[23,24]</sup> In this study, we have forced the animals to exercise with treadmill and combined this with clonidine, as standard treatment of dependency, and showed significant attenuation of withdrawal signs in comparison with positive control group (dependent without treatment). Also, dependent group under treatment by clonidine (0.4 mg/kg) alone showed significant decrease in signs and its severity. The present study shows that exercise can attenuate severity of withdrawal symptom. Recent studies showed that exercise can abolish these symptoms by attenuating of depression, reducing anxiety probably by increasing the endorphin release and other opioid like peptides.<sup>[25-28]</sup> Also, we can argue these findings by explaining the mechanism of exercise on reducing the rewarding effects of drugs such as cocaine and morphine since recent study has demonstrated that

exercise leads to an increase in the synthesis and release of dopamine.<sup>[29,30]</sup> Other studies showed that the chronic usage of opioids and its withdrawal syndrome or injection of naloxone can increase the activity of the CRF-secreting cells from Para-ventricular nuclei and finally activates adrenal cortex.<sup>[31]</sup> Morphine withdrawal syndrome increases the HPA axis activity, by changes in gene expression in selective neurons of the Para-ventricular nucleus.<sup>[32-34]</sup> The regulation of blood glucose level in intracerebroventricular (i.c.v.) administration of exogenous and endogenous opioid alone or during opioid withdrawal syndrome, evaluated in these studies, established that both morphine and  $\beta$ -endorphin administered (i.c.v.) acutely increase the blood glucose level.<sup>[35]</sup> The result of our study showed that morphine doses in the dependent positive control group caused a significant increase in blood cortisol level in comparison with the independent negative control group during the withdrawal syndrome period. Also, this result is arguable with the increasing level of stress in mice and consequently with increasing the cortisol secretion in the withdrawal period in mice. On the other hand, by applying the treatment protocols with clonidine, exercise, and exercise in combination with clonidine, a significant reduction in the blood cortisol level was reached, in comparison with the dependent positive control group, but only results of groups under treatment by clonidine or exercise in combination with clonidine was statistically significant ( $P < 0.05$ ). We conclude that these results of treatment protocols decreased stress level in animal in the withdrawal syndrome period and consequently cortisol level.<sup>[36]</sup> The present study shows that the blood glucose level in the dependent mice increased significantly in comparison with the independent negative control group, this increase correlates with cortisol level in dependent mice.<sup>[31]</sup> According to the previous studies, the blood glucose level in the opioid-dependent mice was significantly higher, because usage of opioids and injection of naloxone induce withdrawal signs and increase the blood glucose level.<sup>[31]</sup> Our data indicate that by treating the dependent animal with clonidine, exercise, and exercise in combination with clonidine, a significant reduction in the blood glucose level was revealed in comparison with the dependent positive control group, but in only results of groups under treatment by clonidine or exercise in combination with clonidine were statistically significant ( $P < 0.05$ ). We justify that this glucose level correlates with cortisol level that was mentioned in dependent group. Generally, our study indicates that there is significant difference in withdrawal syndrome, cortisol, and glucose levels in withdrawal syndrome, cortisol, and glucose levels between positive control group and the group treated by clonidine in combination with exercise.



**Figure 4:** Comparison of the blood glucose levels in the mice of the dependent groups under treatment by clonidine, by forced exercise, and by combination of clonidine and forced exercise in comparison with dependent positive control group. \*: a significant difference in comparison with the dependent positive control group. ( $P < 0.05$ )

## CONCLUSION

Our study shows that clonidine in combination with exercise forms a better protocol for attenuation of withdrawal syndrome and assist patients to get free from morphine dependency with less undesirable effects.

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