Preventive Effects of Forced Exercise against Alcohol-induced Physical Dependency and Reduction of Pain Perception Threshold

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ABSTRACT

Background: Treatment of postabstinence syndrome of alcohol is one of the major strategies of alcoholism treatment. Exercise can be modulated major brain pathways such as a reward system and pain perception centers. The aim of this study was to evaluation the effects of forced exercise in the management of alcohol dependence and pain perception alteration which induced by alcoholism.

Methods: 72 adult male rats were divided into 2 major groups: (1) 40 of them was divided into groups of positive control (alcohol dependent) negative control and alcohol dependent groups under treatment by forced exercise, diazepam (0.4 mg/kg) and forced exercise in combination with diazepam and alcohol withdrawal signs, and blood cortisols, were measured in this groups. (2) 32 rats were divided into control, alcohol dependent (without treatment), and alcohol-dependent groups under treatment by forced exercise or indometacin (5 mg/kg) and then pain perception was assessed by using writhing test, tail-flick and hot plate test.

Results: Forced exercise, diazepam, and their combinations significantly attenuates withdrawal syndrome to 20 ± 2, 22 ± 1.3 and 16 ± 2 and blood cortisol level to 6.8 ± 1.3,7.9 ± 1.2 and 5.8 ± 1.1, respectively, in comparison with the positive control group ($P < 0.05$ and $P < 0.001$). In alcohol dependent animal under treatment by forced exercise, pain response significantly inhibited with 37%, 57% and 38% decreases in writhing test, hot plate, and tail-flick test, respectively, in comparison with alcohol dependent (without treatment) group ($P < 0.05$).

Conclusions: This study suggested that forced exercise can be useful as adjunct therapy in alcoholism patient and also can be effective in modulation of pain threshold reduction that was induced by alcohol dependency.

Keywords: Alcohol, forced exercise, pain, withdrawal syndrome

Original Article

INTRODUCTION

Alcohol dependence is a substance related disorder in which an individual is addicted to alcohol either physically or

mentally.[1] The exact mechanism that describes dependency and withdrawal symptoms of alcohol is not clear.[2] A variety of techniques exist for managing alcohol withdrawal, some that involve the pharmacotherapy with sedatives and some that do not. Drugs with a sedative effect such as diazepam, chlordiazepoxide, topiramate, and nalteroxone are used for pharmacotherapy of alcohol withdrawal syndrome. These alternative medications act for reduction of withdrawal syndrome severity.[3-6] Alcohol withdrawal is characterized by a stressful condition and increased activity adrenal gland and cortisol level.[7,8] Previous studies demonstrated that alcohol dependency will increase the expression of corticotropin releasing factor (CRF) mRNA leading to activation of the adrenal gland that cause increasing cortisol level as a stress hormone.[7] Previous data demonstrate that CRF receptors play an important role in the management of ethanol self-administration and attenuation of withdrawal syndrome in dependent rats. These data showed that CRF antagonists and attenuation of cortisol level can be useful as new pharmacotherapeutic targets for the treatment of alcoholism in humans.[9] Also, these study indicate that there was an increase in the basal serum cortisol level in the alcoholic subjects compared to the controls.[10,11] This study approved that serum cortisol level can be used as a marker of alcohol withdrawal syndrome severity.[11] On the other way, pain transmission and alcohol's reinforcing effects share overlapping neural substrates giving rise to the possibility that alcohol use states significantly affect pain patterns and promote the development of dependence and addiction.[12] Previous study demonstrates that long-term alcohol intoxication and alcohol dependence induce pain symptoms and may exacerbate chronic pain arising from other sources. Neuroanatomical regions involved in pain transmission have been shown to play an important role in the development of alcohol dependence and addiction.[12,13] The likelihood that alcohol dependency and pain transmission share overlapping neural substrates suggests that alcohol reinforcement would alter the pharmacology and neurochemistry of pain.[13] Exercise plays an important role in the prevention and treatment of addictive disorders. Exercise may benefit drug dependent patients attempting recovery from substance problems through a number of different mechanisms of action.[14] Recent study has also shown that exercise alleviate mood disturbance and morphine and nicotine withdrawal symptoms.[15,16] It has also been shown that exercise can counteract withdrawal symptoms, and physical exercise can reduce the risk of some type of drug addiction.[17] Studies in recent years have found exercise has been consistently associated with reductions in depressive symptoms, and thus exercise may reduce the risk for relapse of addictive disorder by reducing depressive symptom.[17] Recent studies have demonstrated the acute effects of exercise on decreased craving to nicotine and morphine consumption and their withdrawal syndrome.[15] In the present study, the effects of forced exercise on alcohol dependency, stress level of the withdrawal period, by measurement of cortisol, and alteration of pain perception in alcohol dependent animals was investigated.

METHODS

Seventy adult male Wistar rats (180-210 g) were purchased from Iran Razi Institute (Tehran, Iran). All the rats were maintained at standard condition (22°C ± 2°C and 12/12 h dark and light cycle), having free access to food and water. The protocol was approved as an undergraduate research and was projected by the Research Council of the Tehran University of medical science.

Drugs

Alcohol, Diazepam, and indomethacin were purchased from Sigma–Aldrich Inc (St Louis MO, USA).

Experimental design for alcohol withdrawal syndrome

40 animals were divided randomly into 5 groups:

- Group I, as a negative control (independent) received normal saline (0.2 ml/rat. ip, once daily) for 21 days
- Group II, as a positive control (dependent) received alcohol (2 g/kg/day. By gavage, once daily) for 21 days
- Group III, IV, and V as treatment groups, respectively, received alcohol (2 g/kg/day) for first 7 days and after that were treated by diazepam (0.4 mg/kg, ip, once daily), forced exercise (as below protocol) and diazepam (0.4 mg/kg, ip, once daily) in combination with forced exercise (as below

protocol) for 2 weeks. All doses mentioned about alcohol and diazepam were chosen based on previous studies.[18,19]

Evaluation of Alcohol withdrawal syndrome: In day 22, the rats were observed for 4 min at the 24 h of the ethanol-withdrawal period. All of the subjects groups were observed, and their 5 behaviors (Stereotyped behaviors, Agitation, Tail stiffness, abnormal posture, and abnormal gait) were recorded by camera. After computation of recorded data, behaviors were counted and analyzed, and a digit allocated to each one [Table 1]. The summation of these digits gives Alcohol Total Withdrawal Score (ATWS).[20-22]

Treadmill forced exercise protocol

Rats were allowed to run on a motor-driven leveled treadmill (ModelT408E, Diagnostic and Research Instruments Co., Taoyuan, Tai). The animal of groups of 4 and 5 was trained by treadmill for 45 min/day, for 5 days/week. The training speed was 12 miles/min (for 1st week) and reached14 miles/min (in the second week) by the end of the experiments. The slope and Intensity of exercise were settled as 0° at the first 10 min, 5° for second 10 min and 15° for last 25 min.[23-25]

Table 1: Rating scale for some behaviors signs induced by ethanol withdrawal in rats

Signs	Scoring
Stereotyped behaviors*	1: Rats showing only one stereotyped behavior
	2: Two stereotyped behaviors
	3: Three stereotyped behaviors
	4: Four stereotyped behaviors
	5: All of the stereotyped behaviors
Agitation	1: Rats showing mild or moderate agitation
	2: Very irritable
	3: Handling vocalization and moderately aggressive
	4: Handling vocalization and very aggressive
	5: Spontaneous vocalization and very aggressive
Tail stiffness	1: Mild tail rigidity
	2: Moderate tail rigidity
	3: Tail rigidity but mildly flexible during ambulation
	4: Tail rigid and not flexible during ambulation
	5: Tail very rigid and not flexible during ambulation
Abnormal posture	1: Mild head-down, back-hunched
	2: Moderate head-down, back-hunched
	3: Prominent head-down, back-hunched
	4: In addition hind legs wide apart
	5: In addition for limbs apart
Abnormal gait	1-2: Mild difficulty ambulating and rearing is normal
	3-4: Moderate difficulty ambulating and rearing
	5: Prominent difficulty ambulating and no rearing

*Grooming, sniffing, head weaving, gnawing and chewing

Measuring the blood cortisol

On the 22nd day after, the behavioral signs been recording whole blood of animals were collected, and their serum was separated and the fasting serum level of cortisol was measured based on µg/dL and by ELISA method.

Experimental design for nociception protocols

32animals were divided randomly into 4 groups:

- Group I, as negative control received normal saline (0.2 ml/rat *ip,* once daily) for 21 days
- Group II, as positive control received alcohol (2 g/kg/day by gavage, once daily) for 21 days and single dose of indometacin (5 mg/kg, ip) in test day
- Group III, dependent group received alcohol (2 g/kg/day by gavage, once daily) for 21 days
- Groups IV were treated by alcohol (2 g/kg/day by gavage, once daily) and forced exercise (as mentioned protocol) for 21 days. All doses mentioned about alcohol and indometacin were chosen based on previous studies.[18,26]

This treatment in 1st day two types of nociception test tail-flick and hot plate test was assessed and after this treatment in 22 days of treatment, three types of nociception method were applied for evaluation of pain.

2-1: Writhing test

In 22 days of treatment, all mentioned animal acetic acid (0.8%) was administrated in a volume of 10 ml/kg in rat. Nociceptive behavior is characterized by abdominal contraction known as writhing, described as an exaggerated extension of the abdomen combined with the out stretching of hind limbs. Total number of writhing following i.p. administration of acetic acid was recorded in 30 min after acetic acid injection. Percentage of inhibition of abdominal constrictions in each group was computed by following ratio: Treated mean-control mean × 100/control mean. This meted of nociception assessment was done as base

of previous studies. In additions, the onset of the first writhing was recorded as latency time.[27]

2-2: Tail-flick test

Before the start of treatment, this test was done in all mentioned animal. In this test, radiant heat (Tail-Flick Apparatus Model P-162, Pouyaye Armaghan Co., Iran) was applied for measurement of acute nociception responses in rat. Intensity of the thermal stimulus was adjusted to produce 5-6 s latency in tail-flick response. Five millimeters of the tail was submitted to noxious heating. To avoid damage to the tail, if the response did not occur, trial was automatically terminated at 12 s (cutoff time). The tail-flick test was measured in 1st day and last day of mentioned treatment as exercise (for 21 days), single dose of indometacin (5 mg/kg, ip) and saline (0.2 ml/rat). The percentage of nociception for each animal was calculated, using the following ratio: ([Posttreatment–pretreatment]/[pretreatment]) ×100. This method of nociception assessment was done as base of previous studies.[28]

2-3: Hot plate test

In this test, analgesic activity was measured with a thermostatically heated surface maintained at 55 ± 2C. Time of reaction was revealed as the time period from the instant animal was put on the hot plate until the moment the animal licked its feet or jumped out. The hot plate test was done twice in 1st day and last day of mentioned treatment as exercise (for 21 days), single dose of indometacin (5 mg/kg, ip) saline (0.2 ml/rat), the reaction time was again evaluated, but only once, this value represented the reaction time after treatment. The percentage of nociception was measured by following ratio: (Reaction time after treatment - reaction time before treatment) ×100/reaction time after treatment.[29]

Statistical analysis

Normality of continuous variables (ATWS, blood cortisol and pain perception) was assessed using Kolmogorov–Smirnov test. Based on this test, all variables were normally distributed. We also used Leven's test and Bartlett's test to assess homogeneity of variances between two and more than two groups, respectively. The results showed that variances are homogenous between tested groups. As parametric assumptions were met, we described continuous data as means ± standard error of the mean, compared the differences between positive and negative control groups by unpaired Student's *t*-test, and the differences between treatment groups by one-way ANOVA. Bonferroni's test was then used for group-by-group comparisons. Results were considered to be significant at 0.05 levels.

RESULTS

3-1: Alcohol total withdrawal score results in control and treatment groups

Our data indicate that ATWS in negative control group that received saline during protocol process was 14 ± 2 while ATWS in positive control group (dependent group) increased significantly by 53% ($P < 0.05$) in comparison with negative control and was 30 ± 1.6 [Figure 1].

In different protocol and administration of diazepam caused, 33% of significantly decrease in ATWS 20 ± 2 ($P < 0.05$) in comparison with positive control group [Figure 2]. Also treatment of animal by treadmill forced exercise caused 26% decrease ATWS 22 ± 1.3 in comparison with positive control group and in the last treatment group, combination therapy with diazepam and treadmill forced exercise caused 46% decreasing in ATWS and reached 16 ± 2 ($P < 0.05$) [Figure 2].

3-2: Blood cortisol level in control and treatment groups

Blood cortisol level in the negative control group was 7.2 ± 1.3 μg/dL after withdrawal syndrome period, while in the positive control group was significantly higher, and was reached 16 ± 2.1 μg/dL ($P < 0.05$) [Figure 3].

Administration of diazepam significantly decreased cortisol level (from 16 ± 2.1 to 6.8 ± 1.3 μg/dL ($P < 0.05$), that is, 57%). Treatment of animals by forced exercise caused significantly attenuation of cortisol level (i.e. 50%) in comparison with positive control group and reached to 7.9 ± 1.2 μg/dL. Combination therapy of dependent animal by of diazepam and forced exercise caused 63% decrease in cortisol level and reached to 5.8 ± 1.1 μg/dL ($P < 0.05$) [Figure 4].

Effect of exercise and alcohol dependency in writhing test

Administration of alcohol in dependent group induced significant alter in pain response when

compared to negative control group ($P < 0.05$). Forced exercise inhibited the increase of pain perception which induced by alcohol dependency ($P < 0.05$). As well as indometacine significantly decrease the number of writhing as a reference drug ($P < 0.05$) [Table 2].

Effect of exercise and alcohol dependency tail-flick test

Table 3 indicates the effect of alcohol dependency on tail-flick test response in rat. Alcohol dependency significantly decreased the tail-flick test time compared to the control ($P < 0.05$). Forced exercise by treadmill significantly increased the tail-flick test time in alcohol dependent rat. Furthermore, indometacin (10 mg/kg) significantly increased the tail-flick test time ($P < 0.05$) [Table 3].

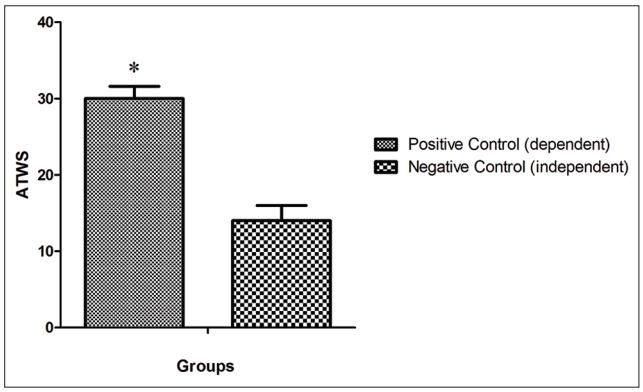

Figure 1: Comparison of severity of the alcohol withdrawal syndrome between the animal of the negative control group and positive control group. *Shows the significant difference (P<0.05) in comparison with the negative control. ATWS: Alcohol total withdrawal score

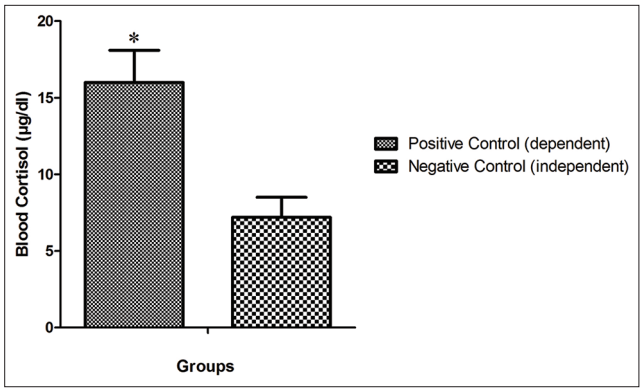

Figure 3: Comparison of blood cortisol levels between the animal of the negative control group and positive control group. *Shows the significant difference (P<0.05) in comparison with the negative control

Effect of exercise and alcohol dependency on hot plate test

Table 3 indicates the effect of alcohol dependency on hot plate test response in rats. Alcohol dependency significantly decreased the hot plate test compared to the control ($P < 0.05$). Forced exercise by treadmill significantly increased the hot plate test time in alcohol dependent rat. Furthermore, Indometacin (10 mg/kg) significantly increased the hot plate test ($P < 0.05$) [Table 4].

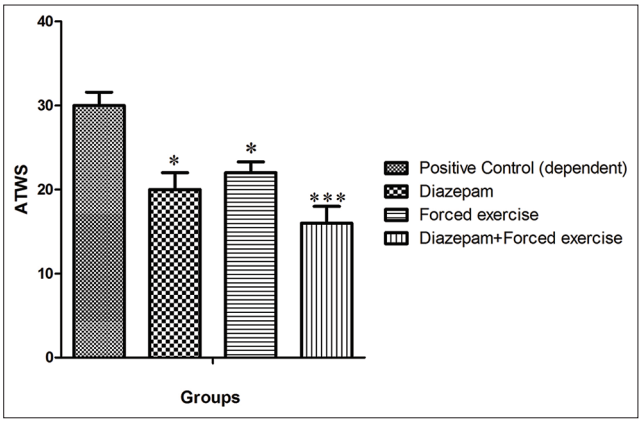

Figure 2: The severity of the alcohol withdrawal syndrome in the dependent animal of groups of under treatment by Diazepam, under treatment by forced exercise, and under treatment by combination of Diazepam and forced exercise, in comparison with the positive control group. ***Shows the significant difference (P<0.001) and *shows the significant difference (P<0.05) in comparison with group under treatment by Diazepam, forced exercise, and their combinations. ATWS: Alcohol total withdrawal score

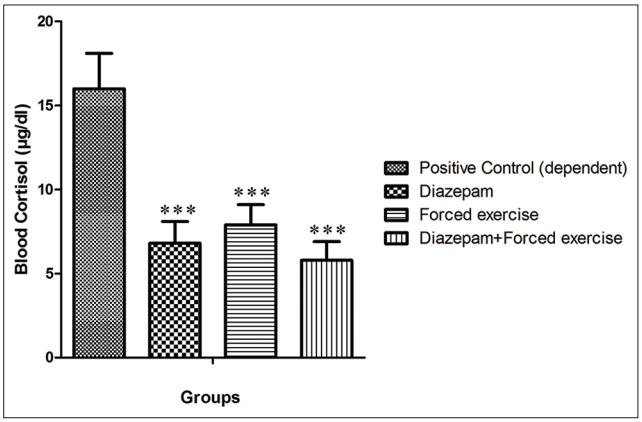

Figure 4: Comparison of blood cortisol levels in dependent animal of groups of under treatment by Diazepam, under treatment by forced exercise, and under treatment by combination of Diazepam and forced exercise, in comparison with the positive control group. ***shows the significant difference (P<0.001)

Table 2: Effect of forced exercise in acetic acid-induced writhing test in alcohol dependent rat

Treatment	Latency time (s)	Writhing test (mean±SEM)	Inhibition %	*P*
Control (0.2 ml/rat)	336±41	62±5.3	-	-
Alcohol	390±51	49±1.8	20	0.0108 vs. control
Alcohol+exercise	612±20	39±0.3	37	<0.001 vs. control <0.001 vs. alcohol treated
Alcohol+indomethacin (5 mg/kg)	785±67	16.5±3.2	74	<0.001 vs. control <0.001 vs. alcohol treated

Value with $P<0.05$ was taken as statistically significant, $n=8$ for each group. SEM=Standard error of the mean

Table 3: Effect of forced exercise in tail flick test in alcohol dependent rat

Group	Pretreatment (s)	Posttreatment (s)	Percentage of inhibition
Control (0.2 ml/rat)	5.4±0.2	5.5±0.2	4.5
Alcohol	5.5±0.2	4.2±0.3	−23*
Alcohol+ exercise	5.4±0.4	8.5±0.3	57*#
Alcohol+ indomethacin (5 mg/kg)	5.3±0.8	10.1±0.1	90*#

Values are mean±SEM. *$P<0.05$, vs. control, #$P<0.05$, versus alcohol dependent animal ($n=8$). SEM=Standard error of the mean

Table 4: Effect of forced exercise in hot plate test in alcohol dependent rat

Group	Pretreatment (s)	Posttreatment (s)	Percentage of inhibition
Control (0.2 ml/rat)	4.6±2.1	4.7±0.4	2
Alcohol	5.3±1.8	4.7±0.7	−11*
Alcohol+ exercise	5.2±1.2	7.2±0.3	38*#
Alcohol+ indomethacin (5 mg/kg)	5.1±0.5	8.8±0.6	72*#

Values are mean±SEM. *$P<0.05$, vs. control, #$P<0.05$, versus alcohol dependent animal ($n=8$). SEM=Standard error of mean

DISCUSSION

Alcohol use disorders are a major public health concern. Despite the demonstrated efficacy of a number of different treatments for alcohol dependency, its withdrawal syndrome remains a major problem.[1] Healthy lifestyle changes and nonpharmacotherapy may contribute to long-term maintenance of recovery, and interventions targeting physical activity, in particular, may be especially valuable as an adjunct to alcohol treatment.[14] The role of exercise as an adjunct to alcohol treatment has been explored on participants receiving inpatient alcohol rehabilitation treatment.[14,30,31] Many previous studies were done in the field of treatment of alcohol dependency and management of withdrawal syndrome, all of this experimental study had been conducted by groups of sedative and hypnotic medications such as diazepam, chlordiazepoxide, and topiramate.[31,32] This study showed that forced exercise with treadmill and its combination with diazepam, as standard treatment of alcohol dependency, can be effective in alcohol withdrawal syndrome management. Our date showed that dependent groups under treatment by forced exercise with treadmill or dependent group under treatment by diazepam (0.5 mg/kg) alone showed significant decrease in alcohol abandonment sign and its severity ($P < 0.05$). Also dependent group under treatment by exercise in combination with diazepam significantly attenuate the withdrawal signs in comparison with positive control (dependent without treatment) ($P < 0.001$). Our results show that physical activity by treadmill can ameliorate severity of withdrawal symptom. Recent studies showed that exercise can abolish these symptoms by attenuating of pain perception, depression, and reducing anxiety probably by increasing release of the opioid like peptides such as endorphin.[33-37] These results can arguable with this concept that exercise plays an important role in the prevention and treatment of alcoholic addictive disorders. Furthermore, we can argue these findings by describing the possible 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 some neurotransmitter such as dopamine, serotonin, and GABA.[37,38] On the other hand, exercise has been shown to result in acute improvements in positive-activated affect and alleviate mood disturbance and withdrawal symptoms in patient attempting to quit Alcohol.[30] These positive reinforcing properties may be mediated in part by exercise effects on the endogenous opioid system and potentiating of dopaminergic systems linked importantly to the experience of enhanced mood and experienced pleasure.[39,40] Furthermore, our previous study demonstrated that exercise can be effective in alleviation of morphine dependency and cortisol level of withdrawal syndrome periods.

The present study indicates that the chronic abuse of alcohol and its abstinence syndrome can increase the activity of the CRF-secreting cells and activates adrenal cortex.[7,9] Alcohol dependency increases the stress parameter and hypothalamic-pituitary-adrenal axis activity, by changes in gene expression of CRF in selective neurons of the para-ventricular nucleus.[9,41,42] The result of our experimental study indicate that alcohol 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 a cessation period ($P < 0.05$). We can argue this result with the basic concept that the alcohol abandonment cause increase in the level of stress in rats and consequently raising the cortisol secretion in the withdrawal period in rats.

On the other hand, by applying the treatment protocols with diazepam, exercise, and exercise in combination with diazepam, a significant reduction in the blood cortisol level was reached; in comparison with the dependent positive control group was statistically significant ($P < 0.05$). Generally our study results showed that treatment protocols decreased stress level in animal in the withdrawal syndrome period and consequently cortisol level. We conclude our results that there is significant difference in withdrawal syndrome, cortisol, levels between positive control group and the group treated by diazepam, exercise and diazepam in combination with exercise.

The consequences of the current study indicated that indometacin as typical antiinflammatory and pain killer drug reduce the acetic acid induced writing test) abdominal pain constriction (and increase the latency time of abdominal pain expression in comparison with control group. This study also confirmed that alcohol dependency alter the acetic acid induced writing test) abdominal pain constriction (and increase the latency time of abdominal pain expression in comparison with control group. We can discuss our study results with basic concept that neuroanatomical regions involved in pain transmission have been shown to play an important role in the development of alcohol dependence and addiction.[12] Our study showed that forced exercise ameliorated the acetic acid induced abdominal constriction in alcohol dependent group and increase the latency time of abdominal pain expression in comparison with control and alcohol dependent group.

The inhibitory action of exercise on pain perception of the dorsal horn may be mediated by activation of opioid-like peptide and releasing of dopamine and serotonin.[43] Previous study demonstrated that the exercise can change the pain signaling and alter and attenuate the inflammation induced pain in digestive systems.[44]

Present study indicated that the indometacin as standard antiinflammatory and pain killer drug increase the percent of pain inhibition in comparison with the control group in a hot plate and tail-flick test. Furthermore, our study demonstrated that alcohol dependency increase the percent of pain perception in comparison with the control group in a hot plate and tail-flick test. The probability that alcohol addiction and pain conduction share overlapping neural substrates suggests that alcohol abuse would alter the pharmacology and neurochemistry of pain perception.[12] Our study indicated that forced exercise increase the percent of pain inhibition in comparison with the control group in a hot plate and tail-flick test. Previous study demonstrated that the forced exercise has a neuroprotective effect, and is used for treatment of some neurodegenerative and central nervous system disease such as depression, neuropathic pain and

neuroinflammation.[45] We can argue our data results with the basic concept that forced exercise can modulate opoidergic system and alter the pain perception level.

CONCLUSIONS

Our study showed that the forced exercise can be an effective adjunct therapy to reduce symptoms of alcohol withdrawal syndrome. Our study demonstrated that alcohol dependency can reduce pain perception threshold and forced exercise will assist this kind of pain perception alterations alcoholic.

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REFERENCES

1. Smith L, Watson M, Gates S, Ball D, Foxcroft D. Meta-analysis of the association of the Taq1A polymorphism with the risk of alcohol dependency: A HuGE gene-disease association review. Am J Epidemiol 2008;167:125-38.
2. Bouza C, Angeles M, Muñoz A, Amate JM. Efficacy and safety of naltrexone and acamprosate in the treatment of alcohol dependence: A systematic review. Addiction 2004;99:811-28.
3. Ferri M, Amato L, Davoli M. Alcoholics Anonymous and other 12-step programmes for alcohol dependence. Cochrane Database Syst Rev 2006;19:CD005032.
4. Krystal JH, Cramer JA, Krol WF, Kirk GF, Rosenheck RA, Veterans Affairs Naltrexone Cooperative Study 425 Group. Naltrexone in the treatment of alcohol dependence. N Engl J Med 2001;345:1734-9.
5. Addolorato G, Leggio L, Abenavoli L, Agabio R, Caputo F, Capristo E, *et al.* Baclofen in the treatment of alcohol withdrawal syndrome: A comparative study vs diazepam. Am J Med 2006;119:276.e13-8.
6. Muzyk AJ, Leung JG, Nelson S, Embury ER, Jones SR. The role of diazepam loading for the treatment of alcohol withdrawal syndrome in hospitalized patients. Am J Addict 2013;22:113-8.
7. Bruijnzeel AW, Small E, Pasek TM, Yamada H. Corticotropin-releasing factor mediates the dysphoria-like state associated with alcohol withdrawal in rats. Behav Brain Res 2010;210:288-91.
8. Breese GR, Sinha R, Heilig M. Chronic alcohol neuroadaptation and stress contribute to susceptibility for alcohol craving and relapse. Pharmacol Ther 2011;129:149-71.
9. Huang MM, Overstreet DH, Knapp DJ, Angel R, Wills TA, Navarro M, *et al.* Corticotropin-releasing factor (CRF) sensitization of ethanol withdrawal-induced anxiety-like behavior is brain site specific and mediated by CRF-1 receptors: Relation to stress-induced sensitization. J Pharmacol Exp Ther 2010;332:298-307.
10. Stalder T, Kirschbaum C, Heinze K, Steudte S, Foley P, Tietze A, *et al.* Use of hair cortisol analysis to detect hypercortisolism during active drinking phases in alcohol-dependent individuals. Biol Psychol 2010;85:357-60.
11. Bokhan N, Gavrilova V, Gusev S, Ivanova S. 1581– Steroid hormones levels in alcohol dependent patients under conditions of social isolation. Eur Psychiatry 2013;28:1.
12. Egli M, Koob GF, Edwards S. Alcohol dependence as a chronic pain disorder. Neurosci Biobehav Rev 2012;36:2179-92.
13. Fishbain DA, Rosomoff HL, Rosomoff RS. Drug abuse, dependence, and addiction in chronic pain patients. Clin J Pain 1992;8:77-85.
14. Brown RA, Abrantes AM, Read JP, Marcus BH, Jakicic J, Strong DR, *et al.* A pilot study of aerobic exercise as an adjunctive treatment for drug dependence. Ment Health Phys Act 2010;3:27-34.
15. Smith MA, Lynch WJ. Exercise as a potential treatment for drug abuse: Evidence from preclinical studies. Front Psychiatry 2011;2:82.
16. Williams DM, Dunsiger S, Whiteley JA, Ussher MH, Ciccolo JT, Jennings EG. Acute effects of moderate intensity aerobic exercise on affective withdrawal symptoms and cravings among women smokers. Addict Behav 2011;36:894-7.
17. Lynch WJ, Peterson AB, Sanchez V, Abel J, Smith MA. Exercise as a novel treatment for drug addiction: A neurobiological and stage-dependent hypothesis. Neurosci Biobehav Rev 2013;37:1622-44.
18. Nixon K, Crews FT. Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. J Neurochem 2002;83:1087-93.
19. Gatch MB. Effects of benzodiazepines on acute and chronic ethanol-induced nociception in rats. Alcohol Clin Exp Res 1999;23:1736-43.
20. Erden BF, Ozdemirci S, Yildiran G, Utkan T, Gacar N, Ulak G. Dextromethorphan attenuates ethanol withdrawal syndrome in rats. Pharmacol Biochem Behav 1999;62:537-41.
21. Lal H, Prather PL, Rezazadeh SM. Anxiogenic behavior

in rats during acute and protracted ethanol withdrawal: Reversal by buspirone. Alcohol 1991;8:467-71.
22. Kliethermes CL. Anxiety-like behaviors following chronic ethanol exposure. Neurosci Biobehav Rev 2005;28:837-50.
23. Albeck DS, Sano K, Prewitt GE, Dalton L. Mild forced treadmill exercise enhances spatial learning in the aged rat. Behav Brain Res 2006;168:345-8.
24. Saadipour K, Sarkaki A, Alaei H, Badavi M, Rahim F. Forced exercise improves passive avoidance memory in morphine-exposed rats. Pak J Biol Sci 2009;12:1206-11.
25. Mello PB, Benetti F, Cammarota M, Izquierdo I. Effects of acute and chronic physical exercise and stress on different types of memory in rats. An Acad Bras Cienc 2008;80:301-9.
26. Golshani S, Karamkhani F, Monsef-Esfehani HR, Abdollahi M. Antinociceptive effects of the essential oil of *Dracocephalum* kotschyi in the mouse writhing test. J Pharm Pharm Sci 2004;7:76-9.
27. Naddaf H, Najafzadeh H, Fallah H, Saremipoor P. Comparison the analgesic effect of electeroacupuncture and tramadol on writhing test in rat. Pharm Sci 2013;18:205-7.
28. Silva ML, Silva JR, Prado WA. Retrosplenial cortex is involved in analgesia induced by 2-but not 100-Hz electroacupuncture in the rat tail-flick test. J Acupunct Meridian Stud 2012;5:42-5.
29. Chavan M, Wakte P, Shinde D. Analgesic and anti-inflammatory activity of Caryophyllene oxide from Annona squamosa L. bark. Phytomedicine 2010;17:149-51.
30. Brown RA, Abrantes AM, Read JP, Marcus BH, Jakicic J, Strong DR, *et al.* Aerobic exercise for alcohol recovery: Rationale, program description, and preliminary findings. Behav Modif 2009;33:220-49.
31. Amato L, Minozzi S, Vecchi S, Davoli M. Benzodiazepines for alcohol withdrawal. Cochrane Database Syst Rev 2010;CD005063.
32. Martinotti G, Di Nicola M, De Vita O, Tedeschi D, Guerriero L, Guglielmo R, *et al*. PW01-237-Low-dosage topiramate in alcohol dependence: A randomized, double-blind, placebo-controlled trial. Eur Psychiatry 2010;25:1665.
33. Krogh J, Nordentoft M, Sterne JA, Lawlor DA. The effect of exercise in clinically depressed adults: Systematic review and meta-analysis of randomized controlled trials. J Clin Psychiatry 2011;72:529-38.
34. Tesarz J, Schuster AK, Hartmann M, Gerhardt A, Eich W. Pain perception in athletes compared to normally active controls: A systematic review with meta-analysis. Pain 2012;153:1253-62.
35. Ströhle A. Physical activity, exercise, depression and anxiety disorders. J Neural Transm 2009;116:777-84.
36. Fuss J, Ben Abdallah NM, Vogt MA, Touma C, Pacifici PG, Palme R, *et al*. Voluntary exercise induces anxiety-like behavior in adult C57BL/6J mice correlating with hippocampal neurogenesis. Hippocampus 2010;20:364-76.
37. Carek PJ, Laibstain SE, Carek SM. Exercise for the treatment of depression and anxiety. Int J Psychiatry Med 2011;41:15-28.
38. Greenwood BN, Foley TE, Le TV, Strong PV, Loughridge AB, Day HE, *et al.* Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. Behav Brain Res 2011;217:354-62.
39. Vuckovic MG, Li Q, Fisher B, Nacca A, Leahy RM, Walsh JP, *et al*. Exercise elevates dopamine D2 receptor in a mouse model of Parkinson's disease: *In vivo* imaging with [18F] fallypride. Mov Disord 2010;25:2777-84.
40. Boecker H, Tölle TR, Valet M, Sprenger T. Effects of Aerobic Exercise on Mood and Human Opioidergic Activation Measured by Positron Emission Tomography. Functional Neuroimaging in Exercise and Sport Sciences. New York: Springer Science/Business Media; 2012. p. 499-510.
41. Sinha R, Fox HC, Hong KI, Hansen J, Tuit K, Kreek MJ. Effects of adrenal sensitivity, stress-and cue-induced craving, and anxiety on subsequent alcohol relapse and treatment outcomes. Arch Gen Psychiatry 2011;68:942-52.
42. Roberto M, Cruz MT, Gilpin NW, Sabino V, Schweitzer P, Bajo M, *et al.* Corticotropin releasing factor-induced amygdala gamma-aminobutyric Acid release plays a key role in alcohol dependence. Biol Psychiatry 2010;67:831-9.
43. Hagen KB, Dagfinrud H, Moe RH, Østerås N, Kjeken I, Grotle M, *et al.* Exercise therapy for bone and muscle health: An overview of systematic reviews. BMC Med 2012;10:167.
44. Light AR, Bateman L, Jo D, Hughen RW, Vanhaitsma TA, White AT, *et al.* Gene expression alterations at baseline and following moderate exercise in patients with chronic fatigue syndrome and fibromyalgia syndrome. J Intern Med 2012;271:64-81.
45. Tajiri N, Yasuhara T, Shingo T, Kondo A, Yuan W, Kadota T, *et al.* Exercise exerts neuroprotective effects on Parkinson's disease model of rats. Brain Res 2010;1310:200-7.

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