**Original Article**

Evaluation of the Effect of Aerobic Exercise and Curcumin Consumption on HPG Axis (Hypothalamus-Pituitary-Gonadotropic) in Alcohol Binge Drinking Rats

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ABSTRACT

Background and Objectives: Alcohol consumption has many health side effects. It is well known male gender is a serious risk factor to be an alcohol consumption and finding a way to reduce these complications in the short term is essential. The aim of this study was to investigate the interaction effects of aerobic exercise and curcumin on LH, FSH, Ts and GnRH following alcohol-induced rats.

Materials and Methods: 30 adult male Sprague Dawley rats with weighting 220-250 g and 8 weeks of age were obtained for this study, and randomly assigned into five groups (n=6) including: Dextrose-Saline control (Dext-Con), ethanol-control (Eth-Con), ethanol-curcumin (Eth-Cur), ethanol-swimming training (Eth-SWT) and ethanol-SWT+curcumin (Eth-SWT+Cur). The project duration consisted of 4 days of addiction, 6 days of quitting, 14 days of swimming training (60 min/day) and curcumin (50 mg/kg) interventions, and finally animal sacrificed. Blood sample was collected and LH, FSH, Ts, and GnRH level were measured by using ELISA (enzyme-linked immunosorbent assay) kit. Analysis of variance (ANOVA) was performed using SPSS version 21.

Results: In the Eth-Con group, alcohol reduced the level of LH, FSH, Ts, and GnRH compared to other specially the Dext-Con group ($p=0.001$). In the Eth-SWT+Cur group, significantly increased of these hormones was observed ($p=0.001$). Exercise alone had no significantly effect on FSH and GnRH level.

Conclusions: Likely curcumin along exercise could improve HPG axis biomarker after a decline due to excessive alcohol consumption in rat. Lack of exercise effect alone can be due to Exercise-induced oxidative stress.

Keywords: Alcohol drinking, HPG axis, LH, FSH, GnRH, Testosterone, Aerobic exercise, Curcumin

Introduction

Alcohol addiction is described by a degree of clinical heterogeneity, that based on a greater diversity of the involved neurocircuitries and neuronal systems (1). Ethanol is one of the most widespread used recreational drinks (2, 3). Therefore, sexual dimorphisms in alcohol degradation are important when attempting to explain the differences of gender in alcohol addiction (4). Alcohol and its metabolite acetaldehyde have been classified as a group one carcinogens by several studies on different disorders (2, 3). Acute and chronic alcohol misuse and associated harmful effects have been investigated widely in causing damage to different organs and functions especially in reproductive function

disorders in humans and experimental animals (4, 5). A direct association of alcohol addiction with metabolic functions and other hormones involved in reproduction at the gonadal level of the hypothalamus-pituitary-gonadotropic (HPG) axis is well established (4).

The HPG axis is a critical part in the reproductive and immune systems development and body's systems regulation that any alteration in this axis may cause hormones produced fluctuation or various systemic effects on the body. The axis controls reproduction through Gonadotropin-releasing hormone (GnRH) to make luteinizing hormone (LH), follicle-stimulating hormone (FSH), oestrogen and testosterone. This

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HPG axis is a multiple level hormonal system which involving with brain or pituitary with feed forward and feedback elements. Fundamentally, one of the mechanisms that medications or drugs may damage male fertility is the altering the HPG axis. High alcohol drinking or alcoholism cause alterations in the pituitary-gonadotropic (PG) axis, resulted in testicular dysfunction (4). Moreover, alcohol oxidation competes with the production of testicular and testosterone. These mechanisms leading to reduce in sperm density and the volume of semen (6). There are still no unified results in this area. Some researchers believe that alcohol act via the HPG axis to vary the pituitary gonadotropic hormone secretions (5, 7), while other authors stated that it acts directly on testicular tissue (8).

Nowadays, numerous substances change the delicately balanced HPG axis, such as dietary phytochemicals or natural plants could causing an alteration in intratesticular testosterone concentrations or a reduction in pituitary-secreted gonadotropins (9). The main constituent of the spice turmeric is Curcumin, which is a polyphenolic phytochemical with antioxidant, anti-inflammatory, anticancer, antiseptic, and hypocholesterolemic properties, which is effective in reducing the complications of diseases especially testicular dysfunction (10-15). In addition, Curcumin consumption improved alcohol-mediated fetal cardiac apoptosis, suggesting that curcumin play a protective role against alcohol abuse caused cardiac damage during pregnancy (15-18). Moreover, curcumin was also known to hinders the conversion of alcohol to acetaldehyde in testicular microsomal fractions (19).

Briefly, alcohol can damage the performance of the HPG axis (4, 19). In contrast, regular physical activity and consumption of curcumin alone have reduced the complications or decreased dysfunction of the HPG axis (15, 17, 18, 20-22). However, few studies have examined the simultaneous effect of these two variables (22, 23). The purpose of this study was to reduce the effects of excessive alcohol consumption in a short period. It is imperative to investigate that at least time and low doses of interventions reduce alcohol-related negative complications. Moreover, regular exercise leads to the adaptation in antioxidant capacity, protecting cells against harmful effects of oxidative stress (24). The relationship between antioxidant effects and HPG function improvement

has been observed in male rats and female mice (15, 18). The synergistic effect of exercise and natural medications are widely proven to cure various illnesses (25). Therefore the aim of this study was to investigate the simultaneous effects of short-term aerobic and turmeric training on HPG axis biomarkers including of the level of LH, FSH, Ts, and GnRH (As the most important activity-based biomarkers of HPG axis) in rats.

Materials and Methods

Experimental animals and ethical aspects

In present study, 30 adult male Sprague-Dawley rats (220-250 g) in weight, and 8 weeks of age were obtained from the animal centre house of Islamic Azad University, Central Tehran Branch. The animals transferred to the laboratory polycarbonate cages as separate groups. They were reared at a temperature of 22 ± 2 °C and $55 \pm 5\%$ moisture under 12/12 h light/dark cycle. The day cycle began on 7.00 a.m. The capable rats of swimming exercise were randomly assigned into five groups (n=6): Dextrose-Saline control (Dext-Con, received a mixed of dextrose and Saline), ethanol-control (Eth-Con), ethanol-curcumin (Eth-Cur), ethanol-swimming training (Eth-SWT) and ethanol-SWT+curcumin (Eth-SWT+Cur). All procedures involving animal experiments were approved and carried out in strict accordance with the research guidelines for the care and use of laboratory animals by the Animal Care and Use Committee (ACUC).

Binge ethanol induction

The entire project duration consisted of 4 days of alcohol gavage (25% ethanol w/v in vanilla Ensure TM; Abbot Laboratories, Columbus, OH)(22, 23, 26), 6 days of quitting, 14 days of exercise and curcumin interventions, and finally animal sacrificed 48 hours after last exercise session. Access to food was restricted in all groups to control isocaloric diet during alcohol gavage. However, the calorie equivalent of alcohol was used in the healthy glucose group. The experiential groups were received Ethanol according to previous methods (22, 23, 26). The initial dose of alcohol was administered 5 g/kg of body weight, and then subsequent doses were administered depending on the behaviour of ethanol up to a maximum of 7 g/kg of the body weight per day. After ethanol induction, the diet converted to the

standard of animals consumed pellet food (Behparvar Co., Karaj, Iran).

Curcumin supplement preparation

Curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) was purchased from Merck Company (Merck, 820354) and mixed with 10 mg/ml into curcumin solvent (Dimethyl sulfoxide: DMSO, Merck, 109678). The solution was intraperitoneally injected for 2 weeks (five times per week, the same as swimming sessions) with the dose of 50 mg/kg body weight.

Swimming training protocol

Swimming training was considered in this study. The training protocol was performed by using a swimming tank (Razi Rad Co. 0.5 m_0.45 m_0.45 m) with temperature of 20-22°C. The experimental groups were conducted swimming training with each session of 60 min/day for the period of 2 weeks.

Blood sampling and determination of biochemical variables

Animals from all groups were euthanized with ketamine/xylazine (80/8 mg/kg i.p.) and sacrificed by cervical dislocation at the end of procedure. Blood samples were collected through a heart puncture to evaluate the biochemical variables. In this study, LH, FSH, Ts, and GnRH level were measured by using ELISA (enzyme-linked immunosorbent assay) kit.

Statistical Analysis

All data are presented as mean \pm standard deviation. Shapiro-Wilk test was conducted to determine the data are normally distribution. Analysis of variance (ANOVA) was performed using SPSS version 21. Two-way ANOVA was used for comparing the effect of exercise and Curcumin and the combination of them on biochemical parameters. A Tukey post-hoc analysis was used to heck for significant differences among the main effects of each dependent variable. Statistical significance was considered when p value was less than 0.05.

Results

Alcohol consumption reduced the level of LH compared to the other groups especially Dex-Con group. The exercise and curcumin consumption significantly increased the concentration of LH compared to the ethanol groups ($F=472.21$, $P=0.001$, $\eta=0.938$), ($F=511.27$, $P=0.001$, $\eta=0.905$) respectively. Moreover, the elevation of LH was more pronounced

in the group of interaction of exercise and curcumin compared to the other ethanol groups ($F=379.64$, $P=0.001$, $\eta=0.954$) (Figure-1).

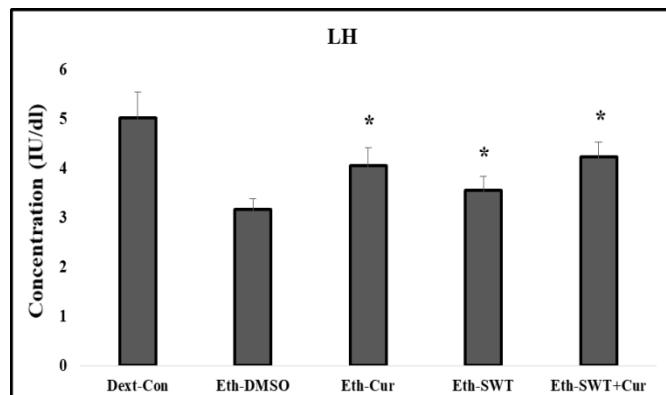


Figure 1. The concentration of LH among studied groups. Dext-Con; Dextrose-Saline control, Eth-DMSO; ethanol-DMSO, Eth-Cur; ethanol-curcumin, Eth-SWT; ethanol + swimming training, Eth-SWT + Cur ethanol + swimming training + curcumin. * $p<0.05$ as compared to Dext-Con.

The level of FSH is reduced through alcohol consumption compared to the other groups especially Dex-Con group. Curcumin consumption significantly increased the concentration of FSH compared to the Eth-Con group ($F=598.46$, $P=0.001$, $\eta=0.875$). The exercise alone had no significantly effect on FSH level ($F=638.41$, $P=0.036$, $\eta=0.095$). Furthermore, the level of FSH also significantly increased in the group of interaction of exercise and curcumin consumption compared to the other ethanol groups ($F=379.36$, $P=0.001$, $\eta=0.954$) (Figure-2).

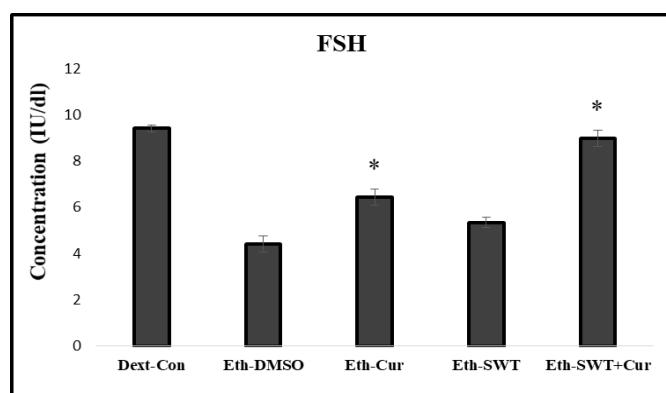


Figure 2. The concentration of FSH among studied groups. Dext-Con; Dextrose-Saline control, Eth-DMSO; ethanol-DMSO, Eth-Cur; ethanol-curcumin, Eth-SWT; ethanol + swimming training, Eth-SWT + Cur ethanol + swimming training + curcumin. * $p<0.05$ as compared to Dext-Con.

Alcohol consumption reduced the level of testosterone compared to the other groups especially Dex-Con group. The exercise ($F=448.15$, $P=0.001$, $\eta=0.973$), and Curcumin ($F=398.26$, $P=0.001$,

$\eta=0.972$) increased the concentration of testosterone compared to the other ethanol groups. Additionally, the interaction of exercise and curcumin was more pronounced on testosterone level improved compared to the other ethanol groups ($F=387.34$, $P=0.001$, $\eta=0.954$)(Figure-3).

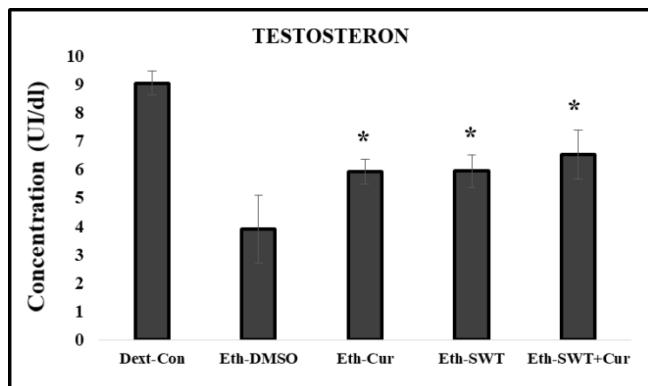


Figure 3. The concentration of Testosterone among studied groups. Dext-Con; Dextrose-Saline control, Eth-DMSO; ethanol-DMSO, Eth-Cur; ethanol-curcumin, Eth-SWT; ethanol + swimming training, Eth-SWT + Cur ethanol + swimming training + curcumin. * $p<0.05$ as compared to Dext-Con.

The level of GnRH is reduced through alcohol consumption compared to the other groups especially Dex-Con group. Curcumin consumption significantly increased the concentration of GnRH ($F=525.36$, $P=0.001$, $\eta=0.904$) compared to the other ethanol groups. The exercise alone had no significantly effect on GnRH level compared to the other ethanol groups ($F=652.39$, $P=0.059$, $\eta=0.165$). Furthermore, the level of GnRH also significantly increased in the group of interaction of exercise and Curcumin consumption compared to the other ethanol groups ($F=417.22$, $P=0.001$, $\eta=0.971$)(Figure-4).

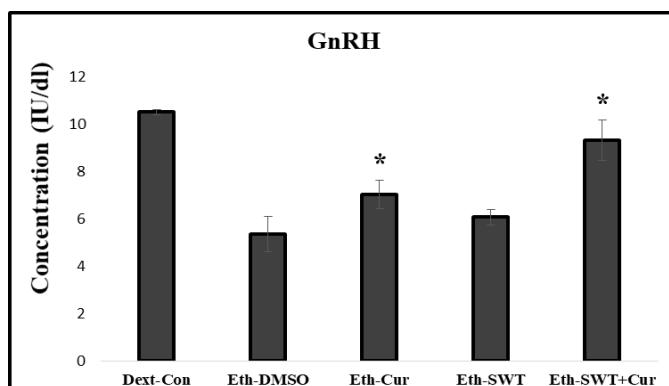


Figure 4. The concentration of GnRH among studied groups. Dext-Con; Dextrose-Saline control, Eth-DMSO; ethanol-DMSO, Eth-Cur; ethanol-curcumin, Eth-SWT; ethanol + swimming training, Eth-SWT + Cur ethanol + swimming training + curcumin. * $p<0.05$ as compared to Dext-Con.

Discussion

The findings of present study clearly demonstrate that LH, FSH, testosterone, and GnRH level decreased in rats who addiction with alcohol and a period of 2 weeks swimming training and Curcumin consumption had significantly effect on the improving of these factors. Based on the changes observed in the present study, it can be stated that excessive alcohol consumption affects the performance of the HPG axis. Exercise alone reduces these effects to some extent. Curcumin alone produces a better effect than exercise. The interactive effect of both independent variables led to further improvement. It can be noted that exercise has less effect for two reasons. First, high intensity exercise increases oxidative stress. Another reason was the shortness of training. It is likely that increasing the workout period can produce better effects through better adaptation. It also increases the intensity of exercise led to increase lactic acid, and lactic acid increases testosterone secretion from testis (27). This may decrease secretion of other biomarkers in the exercise group alone by negative feedback compared to the curcumin interaction group. However, the interaction of exercise and curcumin with the antioxidant properties of turmeric may counteract the exercise-induced oxidative stress.

It is well established that alcohol drinking inhibits testosterone production and probably causes testicular dysfunction (4). Because the HPG axis are steroid hormones, this change can affect other organs of the body. The early sex hormone activity model of alcohol addiction may prove to be a valuable tool in the development of preventive and therapeutic strategies (1), as the levels of corticosterone was increased in male rats which consumed alcohol (28). The mechanisms underlying the fertility damage by alcohol are not yet fully clarified. Furthermore, it is well established that curcumin protect damaged organs affected by alcohol consumption (18, 29-31). The results of present study may portend that alcohol acts on HPG axis biomarkers dysfunction through both pathways via disruption of the hypothalamus-pituitary-gonadal axis and directly on the testis activity biomarkers after two weeks of aerobic exercise and curcumin consumption. This study also shows that the administration of alcohol reduced the level of LH, however exercise and curcumin consumption significantly increased the concentration of LH, but the elevation of LH was more pronounced

in the group that performed exercise and received curcumin. These findings are in consonant with reports from Ren et al. (32) and Oremosu et al.(33). Some authors who report that the negative feedback of testosterone on the hypothalamus–pituitary–gonadal (HPG) axis promotes an increase in LH (8, 34, 35). Recent evidence suggests that due to the intensity of exercise by increasing lactic acid and its effect on HPG axis, testicular gland to increase testosterone secretion improve semen quality, hormone values, and male fertility (20, 21, 27, 36, 37). Curcumin shows to have efficacious protection against alcohol-induced tissue damage (17, 30). Our LH finding shows that the animals that were treated with alcohol had low LH levels, but after 2 weeks of exercise and consuming Curcumin, the LH level reached to acceptable level. It means that there is an interaction of two variables to have synergistic effect on the level of LH hormone.

The results of present study indicated that the level of FSH is reduced via alcohol consumption, however, Curcumin significantly increased the concentration of FSH, but exercise alone had no effect on FSH level. Furthermore, the level of FSH also significantly increased in the group of exercise and Curcumin. Exercise training alone had no effect on FSH level, that may associated to the intensity of exercise or needs to perform longer than two weeks to be effective. It has been shown that alcohol could decline the production of LH and FSH through influencing on the anterior pituitary gland (32). Contrary, it has been demonstrated that chronic alcoholics had a significant increased on FSH and LH levels (8). The results of alcohol on HPG axis pathway are controversy that may be related to research condition or other factors, which can effect on this axis. Furthermore, alcohol consumption not only able to reduce LH and FSH synthesis but can prevent the secretion of these hormones. Curcumin has antioxidant properties and oral administration of curcumin with alcohol showed a significant reduction in negative alcohol effects side in mice model (29). Aerobic exercise alongside Curcumin consumption seems to improve alcohol effects.

In the present study, a significantly decrease was observed in the level of testosterone within alcohol consumption, however, exercise and Curcumin increased the concentration of testosterone. In addition, the interaction of exercise and Curcumin

was more effective on testosterone level improved. This hormone is essential for spermatogenesis which reduced in alcohol binge drinking rats in agreement with previous reports (8). Numerous constituents vary the delicate balance of HPG axis, that causes either a reduction in pituitary-secrete gonadotropins or a modification of testosterone concentrations (9). Alcohol has a direct toxic effect on the testis that leads to decreased seminiferous tubular function (30). A study has proven that the significant reduction of sperm motility and sperm count in animals with alcohol treated resulted in inimical to male fertility (33). Curcumin suppress the conversion of alcohol to acetaldehyde in testicular microsomal fractions (19). The effectiveness of exercise and Curcumin consumption are in increasing testosterone levels explained may directly effect of HPG axis and GnRH release from the hypothalamus.

GnRH is the important key regulator of the reproductive axis. The level of GnRH in the present study is reduced through alcohol consumption, while curcumin significantly increased the concentration of GnRH, and curcumin alongside exercise treatment increased GnRH. It has been shown that testosterone levels modify HPG axis, and high level of this hormone suppresses the fluctuation of GnRH pulsatile release from the hypothalamus (38). Our data indicated that exercise alone had no markedly effect on inducing elevation GnRH level, which can be due to the intensity of exercise and exercise-induced oxidative stress or the short term of our selected protocol and low adaptation. In matching with our results, regular moderate exercise treatment had no effects on the GnRH level (39). In contrast, a study has proven that moderate exercise improve the performance of the pituitary-gonadal axis and fertility (40). Thus, the results confirm that Curcumin with exercise have a synergistic impact on the HPG axis biomarkers that will probably improve fertility performance. Note that these findings have been reported in male rats and should be generalized.

Conclusion

Reduction of the HPG axis activity under the influence of each factor in particular alcohol can decrease the hormone biomarkers of this axis especially LH and FSH and may leads to decrease spermatogenesis and fertility function consequently. To sum up, the findings of the present study indicated that likely consumption of the curcumin combined

with aerobic exercise is a therapeutic effective factor to reverse the HPG axis damage via alcohol effect in-vivo. Notice that in the present study, smaller groups and shorter periods were used to minimize injury to animals. Therefore, this may affect the results. Future studies should also document the findings of gene expression and histology.

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