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Original Research

Interactive Effects of Endurance Swimming and Curcumin Supplementation on Serum Levels of Liver Alkaline Phosphatase in Male Rats Following Ethanol Abuse

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ABSTRACT

In this study, 48 male wistar rats approximately 12 weeks old with 240 ±10g weight are selected.(1st) 8 rats were randomly separated as control group and 40 rats in 5 equal group were gavaged with ethanol (5-7 g/kg weight of body) every 8 hours for 4 days. Six days after the final binge, (2nd) the rats were randomly divided into groups of alcohol with any intervention, exercise, curcumin, sham and exercise + curcumin. Endurance swimming training program was conducted for two weeks, daily and 20-60 min, equally in groups with exercise. There was daily intra-peritoneal injection of curcumin (50mg/kg weight of body), equally in groups with curcumin. Rats in sham group were injected with curcumin's solvent (dimethyl sulfoxide). The data were analyzed by ANOVA and Tukey's post hoc test at the significant level of $p< 0.05$. Plasma level of ALP enzyme ($p=0.0001$) significantly increased in the alcohol group compared to the control group. Also the result show that the was significantly decreased in training + curcumin group in comparison with curcuminand training groups. According the finding,alcohol abuse significantly increase Plasma level of ALP enzyme. It seems that Short-term exercise with curcumin, caninhibit enzymthat enhanced by alcohol abuse.

Key words: Endurance swimming, Curcumin supplementation, Alkaline phosphatase, Ethanol abuse.

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Introduction

Drinking patterns are changing and alcohol consumption is on the rise in many countries. Excessive use of alcohol and its complications contribute about 2.5 million deaths each year globally (1). According to statistics and evidence alcohol abuse is undeniable in Iran (2). It leads to social and family crisis and has also severe side effects and pathogenicity in the human body. Alcohol can cause at least 60 diseases in various organs of the body, with the highest mortality rate from liver diseases (3).Because approximately90% of ingested alcohol is metabolized in the liver (4). On the other hand, alcohol levels in the blood stream of the hepatic portal vein are much higher than those in the systemic bloodstream, which lead to intoxication, fibrosis, liver cancer and early death if left untreated.Increase of high-risk behaviors such as ethanol drink in the society have been recognized as one of risk factors of many diseases, including obesity, cardiovascular diseases (CVD) and metabolic syndrome (5, 6). Excess use of ethanol

has indirectly been described as one of the major factors of liver injuries (7-9). Ethanol exposure results in alcoholic fatty liver, inflammation, necrosis, fibrosis and cirrhosis as major reasons of mortality in developing countries (10). Role of cytokines in development of hepatic fibrosis has widely been studied. It has been reported that the most important injuries of ethanol are resulted from increases in oxidative stress and mitochondrial injury (11). One of the major liver enzymes is alkaline phosphatase (ALP), which is an important indicator of physiological health of the liver (12). In 1920, it was found that this enzyme increased in hepatic diseases (13). Alkaline phosphatase (EC3.1.3.1) mediates the hydrolysis of organic phosphates such as proteins, nucleotides and alkaloids in an alkaline medium. The enzyme is found in various forms in blood stream. It is also abundant in the liver and bones (14). It is worth noting that to treat hepatic damage caused by alcohol abuse, chemical medicines are commonly used with antioxidant and anti-inflammatory effects such as vitamin E and corticosteroids that prolonged use at inappropriate doses can cause problems. For example, high intake of vitamin E increases prostate cancer risk in men (15). To this purpose, researchers have recently proposed the use of non-pharmacological methods such as physical activity to reduce liver injury (16). The supportive role of chronic aerobic training in liver injury, inflammation reduction and hepatic fibrosis has been confirmed in previous studies (17). Regular physical activity is known as a protective factor against the development and progression of certain diseases. It may also have positive effects on the liver of obese diabetics, children with liver disease and the cardiac function of patients with fatty liver (18). However, these represent only part of the description of the effect of regular physical activity on various types of liver diseases. One of the hottest topics in today's world of training is the use of short-term training to achieve health goals. According to the results of previous studies, there is still a question whether it is possible to achieve more therapeutic efficacy with a shorter training period in those who are quitting alcohol. Previous research has shown that the effect of short-term training on potential hepatic damage due to alcohol abuse have not been studied yet. In recent years, in addition to physical activities, the use of herbal antioxidants for the treatment of various diseases has attracted researchers. Curcumin is a polyphenolic and active ingredient derived from turmeric rhizomes (Crucoma Longa) that has been approved for its antioxidant, anti-inflammatory and antimicrobial activity. Previous studies have confirmed its therapeutic effects on chronic and inflammatory diseases such as diabetes, obesity, rheumatic disorders and allergy (19). However, little research has been conducted on the effect of this substance on hepatic damage caused by excessive alcohol consumption (20). There was also no study investigating the concomitant effect of these two methods on hepatic ALP activity. Therefore, researchers are seeking for an effective, ideal and readily available treatment which is well tolerated by patients has a high specific effect on the liver in the short term with few side effects. The aim of this study was to investigate the interactive effect of endurance swimming and curcumin supplementation on serum levels of hepatic alkaline phosphatase in male rats that took alcohol.

Material and Methods

Animals

This was an experimental study and the population was male Wistar rats at Pharmacology Department of Tehran University of Medical Sciences. A total of forty-eight 3-month-old rats weighing 240±10 g were selected as the samples. Samples were housed in a rodent animal PVC cage with a metal mesh lid and wood chip floor with a 21±2 °C temperature, 40-60% humidity, a 12-hour light cycle (starting at 7 am) and 12 hours of darkness. Initially, eight rats were separated as control samples without any training or treatment. Animals had free access to food and water except during ethanol consumption. They consumed the municipal purified water in a PVC drink container available to them every morning. Compact rat food was provided to them. There was no intervention to make them adapt to the environment and the conditions at least one week after they were purchased. All interventions were performed in the light phase between 9 and 2 pm. The experimental protocol used in this study was based on the guidelines of the Research and Ethics Committee of the Faculty of Modern Technologies, Tehran University of Medical Sciences according to the National Institute for the Care and Use of Laboratory Animals

guidelines. It was designed and implemented with an emphasis on using the minimum required animal and minimizing the pain and suffering at various stages, with the code IR.IAU.B.REC.1398.011.

Ethanol abuse and how to stop it

Animals were not fed during ethanol consumption because each gram of ethanol produced about 7 kcal of energy. However, water was always available to them. Ethanol was gavaged into their stomach. Forty male rats were gavaged with 25% ethanol in vanilla supplement from Ensure Company every eight hours for four days (7). This initial dose was five grams per kg body weight of animal. Subsequent doses of ethanol were prescribed based on a six-point scale of dependency behavior (0=normal, 1=low activity, 2=ataxia or imbalance or motor imbalance, 3=ataxia + moving on abdomen or delay in standing reflex, 4=no standing reflex, and 5=no eye-blink reflex); the mice with higher scores received less ethanol. The maximum dose used was 7 g/kg/day (21). After a four-day period of ethanol consumption and weighing, food was returned to the animal cage and they were kept in their cages for six days without any intervention. From the day seven, after weighing, they were randomly divided into five groups of eight, including alcohol, training, curcumin, sham (curcumin solvent) and training + curcumin groups. Rats in the alcohol group received only water and food for two weeks.

Curcumin Administration

Curcumin was obtained from Merck Company, Germany. This substance was insoluble in water or normal saline, instead of that dimethyl sulfoxide (DMSO)10% was used. Dissolved curcumin was intraperitoneallyinjectedto the curcumin group on a daily basis. Calibrated insulin syringes were used for injection. Curcumin dosage was 50 mg/kg body weight (22). This dose of curcumin was used after quitting alcohol and continued for two weeks. To investigate the effect of injection-induced stress and the possible effect of dimethyl sulfoxide as a curcumin solvent, the sham group was treated with this substance.

Exercise Protocol

Six days after the last dose of ethanol (from day seven), the animals in the training group performed daily swimming exercises at 11 AM for two weeks. The endurance swimming program was initially twenty minutes. 2nd session, forty minutes and finally one hour after three sessions, One-hour practice time was considered in subsequent sessions (23). After each swimming session, the animals were towel-dried and returned to their cages. Training program was performed in a pool with20 - 22 °C water temperature (24). For adaptation to new situation, the animals were immersed in shallow water, prior to the study period.

Sampling

At the end of the course, 24 hours after the last training session, the rats were first anesthetized with ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg) following a 12-hour fasting period. Then, by cutting the skin in the abdomen and chest area, about 10 ml blood was drawn directly from their heart by opening their abdominal cavity. Blood samples were discharged into containers with anti-coagulative agent (EDTA) and centrifuged for 10 min at 3000 rpm. All samples were immediately frozen in nitrogen and stored at -80 °C. ALP enzyme activity was measured using standard kits and ELISA (ZellBio, Germany).

Statistical analysis

Shapiro-Wilk test was used to check the normality of data. One-way analysis of variance (ANOVA) was used to determine the difference between indices among different groups and if meaningful, Tukey's post-hoc test was employed to find the difference. Data were analyzed in SPSS 21. ($P< 0.05$ considered as significant).

Results

At the end of the study, mean and standard deviation of serum level of hepatic ALP in different groups were presented in (Table 1) Results of one-way ANOVA showed a significant difference in serum levels of ALP in groups (F=10.1, p=0.0001). Serum levels of ALP increased significantly in the alcohol group compared to the control group (p=0.0001). In addition, according to Tukey's post-hoc test, the mean serum levels of the enzyme in training + curcumin ,not decreased compared to sham , alcohol , curcumin and training groups .The mean serum levels of the enzyme in curcumin group not decreased compared to sham and alcohol groups. Although the mean serum levels of the enzyme in training groups decreased compared to sham and alcohol groups, but compared to curcumin group, enzyme level was not impressive decrease (Table 2).

Table 1: Mean, standard deviation and one-way ANOVA (u/l) in different group

Alkaline phosphatase	Mean±standard deviation	Results of one-way ANOVA	
Groups		P	F
Control Curcumin Training Training + Curcumin Sham Alcohol	2.62±1.06 5.50±1.9 5.75±1.28 3.62±1.06 6.0±0.75 6.1±0.99	10.1	*0.0001

Table 2: Results of Tukey's post-hoc test for serum levels of ALP (u/l) among different groups

Comparison of groups	Sham	Alcohol	Training	Curcumin	Training+ Curcumin
Control	*0.0001	*0.0001	*0.0001	*0.0001	0.59
Training + Curcumin	*0.004	*0.003	*0.01	*0.04	
Curcumin	0.92	0.91	0.99		
Training	0.9	0.9			
Alcohol	1.00				

* Significant at $p<0.05$.

Discussion

Results of the study showed that alcohol abuse was associated with a significant increase in serum level of ALP in the alcohol group compared to the control group, which may indicate the toxic effects of high alcohol consumption on hepatic cells. Previous studies have found concordant findings regarding the effect of alcohol abuse on this enzyme (12, 14, 25). Alcohol abuse has been shown to contribute to liver cell necrosis, hepatocyte membrane damage, lack of cell membrane function, and cell leakage. Following alteration in hepatic parenchymal structure due to alcohol abuse, serum levels of ALP ascend in the blood. Changes in serum levels of ALP are one of the important biomarkers used in the evaluation and diagnosis of liver lesions caused by excessive alcohol consumption (3). The obtained results are consistent with the results of previous human studies (26, 27). Accordingly, serum levels of ALP increase in alcoholics by up

to one and a half times (12). To sum up, it seems that the elevated serum levels of ALP in alcoholic liver disease are due to the of plasma membrane destruction in hepatic cells.

The most important finding of the present study was a decrease in serum levels of ALP after two weeks in curcumin, training + curcumin and training groups compared to sham and alcohol groups, which was significant in the training + curcumin group compared to the curcumin and training groups and it significantly inhibited the mentioned index. Various studies have found concordant findings regarding the effect of alcohol abuse on this enzyme (12, 14, 25). According to research by Barani et al. who compared the effect of resistance training and combo training on plasma levels of ALP in women with fatty liver. The decrease in plasma levels of ALP in the resistance training group was significant compared to the combo training group (23). In contrast, according to the study by Siadat, there was no significant difference between resistance training and combo training in ALP levels in men with fatty liver (28). In this regard, Aliyeh stated that there is no significant difference between the effects of eight weeks of resistance training with varying intensities on this enzyme in obese men. On the other hand, six to twelve weeks of continuous training along with interval training significantly increased the enzyme levels (29). Based on Barzgarzadeh's research, twelve weeks of interval training significantly reduced this enzyme compared to the continuous training group (30). In a research by Tartibian et al., the effect of nine weeks of aerobic training on ALP was evaluated. This study showed that plasma levels of ALP decreased significantly compared to the control group (31). In contrast, in a study on obese women, Skrypnik et al. showed that endurance and non-endurance training had little effect on plasma levels of alkaline phosphatase in these individuals (32). Akan also showed in his research on adolescents that serum levels of alkaline phosphatase increases in this group after playing football (33). The mentioned results indicate that training can lower serum levels of ALP and physical activities can improve liver injury, but the possible reason for the inconsistency of findings may be related to the severity and duration of training as they can affect the activity of enzymes.

Research has also been conducted on the effect of curcumin supplementation on serum levels of alkaline phosphatase. Rukkumani et al. showed that alcohol increases serum levels of alkaline phosphatase, but curcumin inhibits the impact of alcohol on ALP levels (34). Farzanegi studied the effect of eight weeks of endurance training and curcumin supplementation on the ALP enzyme in rats intoxicated by lead, which showed that the ALP enzyme decreased in the curcumin and training + curcumin groups compared to the control group. In the training + curcumin supplement group, plasma levels of ALP decreased significantly, but in the curcumin group, the decrease in the enzyme was not significant compared to the control (35). In contrast, findings of Feng et al. showed that curcumin supplement had a significant effect on the levels of ALP enzyme in the mice intoxicated with carbon tetrachloride (36). They showed that serum levels of alkaline phosphatase were significantly lower in the training + curcumin group compared to other groups, which is consistent with the results of the present study. Since tumor necrosis factor-alpha and interleukins are involved in the development of hepatic necrosis, curcumin inhibits the secretion of these factors from macrophages and ultimately reduces the toxic effects of alcohol on liver tissue, resulting in a decrease in serum levels of ALP. Thus, by summarizing the above findings, results of this study suggest that the two interventions can enhance each other's protective effect in the short run. Therefore, the present study showed for the first time that endurance swimming training with curcumin supplementation ameliorates the pathological damage caused by excessive alcohol consumption such as changes in serum levels of ALP in the liver.

Conclusion

This finding indicates the impact of lifestyle changes such as regular physical activity and nutrition on suppressing the negative impact of alcohol abuse. According to previous studies and the results of the present study, it is concluded that high alcohol consumption leads to elevated levels of ALP in the liver. Short-term endurance swimming training with curcumin supplementation seems to inhibit the enzyme levels and reduce the hepatic impact of excessive alcohol intake in the short term. It is however suggested

to examine two interventions on different hepatic variables by changing the dosage and the type of practice protocol.

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چکیده فارسی

تأثیرات تعاملی شنای استقامتی و مکمل کورکومین بر سطح سرمی آلکالین فسفاتاز کبد در رتهای نر ویستار مبتلا به سوء مصرف الکل

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آنزیم آلکالین فسفاتاز (ALP) شاخصی جهت آسیب های کبدی می‌باشد.هدف پژوهش حاضر تاثیر شنا و مصرف کورکومین درکوتاه مدت بر سطح سرمی آلکالین فسفاتاز در اثر سوءمصرف الکل می‌باشد. در این مطالعه تجربی، ۴۸ سر موش صحرایی نر، سن ۱۲ هفته و وزن ۱۰ ± ۲۴۰ گرم انتخاب شدند. هشت موش به عنوان گروه کنترل جدا شد. به چهل موش طی چهار روز هر هشت ساعت یک‌بار، ۷-۵ گرم به ازای هر کیلوگرم وزن بدن اتانول تزریق شد. شش روز پس از آخرین دوز مصرف اتانول، موش‌ها به روش تصادفی به گروه‌های الکل، تمرین، کورکومین، شم و تمرین + کورکومین تقسیم شدند. حیوانات گروه تمرین، طی دو هفته، هر روز به مدت ۲۰-۶۰ دقیقه شنا کردند. به حیوانات گروه‌های کورکومین و تمرین + کورکومین ۵۰ میلی‌گرم به ازای هر کیلوگرم وزن بدن، کورکومین تزریق شد. به گروه شم، دی‌متیل سولفوکساید (حلّال کورکومین) تزریق شد. برای تجزیه و تحلیل داده‌ها از نرم افزار SPSS V ۲۱ و آزمون آنالیز واریانس یک طرفه و تعقیبی توکی استفاده و سطح معنی‌داری p<۰/۰۵ در نظر گرفته شد.

سطح سرمی آلکالین فسفاتاز در گروه الکل در مقایسه با کنترل افزایش معنی‌داری داشت (p = ۰/۰۰۰۱). سطح سرمی این آنزیم در گروه تمرین نسبت به گروه‌های کورکومین و تمرین همراه با کورکومین و شم کاهش معناداری داشت (p = ۰/۰۰۰۱). مصرف بیش از حد الکل منجر به افزایش معنی‌دار سطح سرمی آنزیم ALP کبد می‌گردد. تمرین ورزشی شنا در کوتاه‌مدت، می تواند میزان پلاسمایی افزایش یافته آنزیم مذکور در اثر سوءمصرف الکل را مهار کند.

واژه‌های کلیدی: تمرین شنا، کورکومین، آلکالین فسفاتاز، سوءمصرف الکل