The effects of high-intensity interval training and iranian propolis extract on serum levels of TRPV4 and CYP2E1 proteins in patients with nonalcoholic fatty liver

Les effets de l'entraînement par intervalles de haute intensité et de l'extrait de propolis iranienne sur les niveaux sériques des protéines TRPV4 et CYP2E1 chez les patients atteints de stéatose hépatique non alcoolique

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Abstract

Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver failure, fibrosis, cirrhosis, and liver cancer, which can eventually lead to death.

Aim: To investigate the effects of high-intensity interval training (HIIT) and iranian propolis extract on serum levels of transient receptor potential cation channel subfamily V member 4 (TRPV4) and cytochrome P450 2E1 (CYP2E1) proteins in patients with NAFLD.

Methods: Thirty-two patients with NAFLD (mean±standard deviation of age: 45.1±3.6 years; body mass index: 30.0±3.6 kg/m^2) were assigned in a randomized control trial to one of the following groups: HIIT (n=8), propolis supplement (n=8), propolis + HIIT (n=8), and controls (n=8). The subjects participated in eight weeks of HIIT (one bout of 1-min intervals at 80-95% of the maximal heart-rate, interspersed by two min at 50-55% of the reserve heart-rate). The Propolis supplement was taken three times a day by the patients in the form of 50 mg tablet after the main meals. Body composition, liver injury test (eg; Alanine- and Aspartate- aminotransferase levels), liver ultrasound and serum levels of TRPV4 and CYP2E1 were measured before and after intervention. One-way analysis of variance was used to compare post-tests among the groups.

Results: HIIT significantly reduced serum levels of TRPV4 protein (p=0.001). The reduction in CYP2E1 was not significant in HIIT group (p=0.075). Propolis consumption had no significant effect on serum levels of CYP2E1 protein (p=0.059), and TRPV4 (p=0.072). There was a significant decrease in TRPV4 and CYP2E1 in the HIIT (p=0.001) and propolis supplement (p=0.032) groups.

Conclusion: HIIT and propolis supplementation can be used to reduce TRPV4 and CYP2E1, which in turn reduces oxidative stress and inflammation in patients with NAFLD.

Key words: ALT, AST, Liver enzymes, Oxidative stress, Physical activity, Sports, Visceral fat

Résumé

Introduction: La stéatose hépatique non alcoolique (SHNA) est la principale cause d'insuffisance hépatique, de fibrose, de cirrhose et de cancer du foie, pouvant éventuellement entraîner la mort.

Objectif: Étudier les effets de l'entraînement par intervalles de haute intensité (EIHI) et de l'extrait de propolis iranienne sur les niveaux sériques des protéines du canal cationique de la sous-famille V du récepteur potentiel transitoire 4 (TRPV4) et du cytochrome P450 2E1 (CYP2E1) chez les patients atteints de SHNA.

Méthodes: Trente-deux patients atteints de SHNA (Moyenne ± écart-type de l'âge : 45,1 ± 3,6 ans ; indice de masse corporelle : 30,0 ± 3,6 kg/m^2) étaient répartis de manière aléatoire dans le cadre d'un essai contrôlé randomisé dans l'un des groupes suivants: EIHI (n = 8), supplément de propolis (n = 8), propolis + EIHI (n = 8) et témoins (n = 8). Les patients avaient participé à huit semaines d'EIHI (une séance d'intervalle d'une minute à 80-95 % de la fréquence cardiaque maximale, entrecoupée de 2 minutes à 50-55 % de la fréquence cardiaque de réserve). Le supplément de propolis était pris trois fois par jour par les sujets sous forme de comprimé de 50 mg après les repas principaux. La composition corporelle, les tests de cytolyse hépatique (c'est-à-dire alanine et aspartate aminotransférases), l'échographie hépatique et les niveaux sériques de TRPV4 et de CYP2E1 étaient mesurés avant et après l'intervention. Une analyse de covariance à un facteur était utilisée pour comparer les tests post-intervention entre les groupes.

Résultats: L'EIHI a réduit de manière significative les niveaux sériques de la protéine TRPV4 (p = 0,001). La réduction de la CYP2E1 n'était pas significative dans le groupe d'EIHI (p = 0,075). La consommation de propolis n'avait aucun effet significatif sur les niveaux sériques de la protéine CYP2E1 (p = 0,059) et de TRPV4 (p = 0,072). Il y avait une diminution significative de TRPV4 et de CYP2E1 dans les groupes HIIT (p = 0,001) et supplément de propolis (p = 0,032).

Conclusion: L'EIHI et la supplémentation en propolis peuvent être utilisés pour réduire les niveaux de TRPV4 et de CYP2E1, ce qui réduit par conséquent le stress oxydatif et l'inflammation chez les patients atteints de NAFLD.

Mots clés: Activité physique, ALT, AST, Enzymes hépatiques, Graisse viscérale, Sports, Stress oxydatif

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease, with a worldwide prevalence of 25% (1, 2). NAFLD is caused by the deposition and pathological accumulation of large fat particles (mainly triglycerides) in the cytoplasm of liver cells in the absence of alcohol consumption (3). The increasing prevalence of fatty liver and steatohepatitis, along with obesity and metabolic syndrome, are the main causes of diseases such as cirrhosis and liver cancer (4). The prevalence of NAFLD has been reported to be 21.5% to 43.8% in Iran (5, 6). The two-hit theory is one of the most valid pathological hypotheses of this complication, which is related to insulin resistance and oxidative stress (7). The first hit is primarily a result of insulin resistance, increased dietary intake, and enhanced hepatic lipogenesis (7). The second hit is a mix of oxidative stress, lipid peroxidation, mitochondrial dysfunction, and the release of inflammatory mediators, all of which contribute to increasing liver injury, steatohepatitis, and fibrosis (8). Insulin resistance leads to hyperglycemia, which causes the pancreas to secrete more insulin for glucose homeostasis (9). Oxidative stress is a redox imbalance that results from overexpression of reactive oxidative stress (ROS) species or free radicals or reduced antioxidant defenses (10). Elevated levels of free radicals, increased DNA damage and lipid peroxidation along with decreased antioxidants have been observed in NAFLD patients (11). In addition to direct effect on hepatotoxicity, excess free fatty acid (FFA) induces ROS through mitochondrial-dependent oxidation or microsomal enzymes (12). Excessive ROS formation has a negative effect on DNA, protein and cell membranes and causes lipid peroxidation, which impairs mitochondrial function and ultimately damages liver cells (13). ROS also causes inflammatory cytokines (eg; tumor necrosis-α (TNF-α) and interleukin(IL)-6) and cell death (14). Research has shown that in the advanced stage of fatty liver, there is an increase in inflammation and lobular necrosis due to increased cytochrome P450 2E1 (CYP2E1) activity, which causes a rapid change in the expression of transient receptor potential cation channel subfamily V member 4 (TRPV4) in Kupffer cells (15). The results identified that in progressive NAFLD, the TRPV4 gene is methylated in its promoter regions and possibly suppresses the expression of the TRPV4 gene to reduce the level of this protein as NAFLD progresses. It was also reported that activation of the CYP2E1 protein in the liver of NAFLD mice was associated with DNA methylation in the TRPV4 promoter region, which significantly reduced the expression of this protein (16). A study looked at the role of TRPV4 in hematopoietic stem cells (HSC)-induced proliferation of transforming growth factor (TGF, a growth factor that is an important marker of tissue regeneration and an advanced pathophysiological event in NAFLD) and identified that TRPV4 plays an important role in 1TGF-induced hepatocyte proliferation (17). There was also a significant increase in TRPV4 expression in hepatic fibrotic tissue (18). The main mechanism that leads to NAFLD is not yet fully understood; but factors such as obesity, metabolic syndrome, insulin resistance, dyslipidemia, sedentary lifestyle and poor nutrition can contribute to this disease (19). Since there is no definitive cure for NAFLD; the issue of prevention is more important than treatment. Therefore, changing lifestyle with increasing physical activity and proper nutrition are useful in improving this disease (20). Natural antioxidants found in edible or medicinal plants often have the ability to inhibit free radicals as well as anti-inflammatory effects, which is a good therapeutic approach to prevent and treat liver disease due to the role of oxidative stress and inflammation in the development and progression of liver damage (21, 22). Propolis is one of these antioxidants reported to have great therapeutic effects (22). Propolis is a naturally sticky substance produced by bees from gum or resin made on various plants (23). The chemical composition of propolis includes more than 200 compounds, including flavonoids, many polyphenols such as phenolic acids, esters, phenolic aldehydes and ketones (22). It has been suggested that the antioxidant activity of propolis extract in radical oxygen uptake is four times higher than that of vitamin E (22). It seems that the antioxidant properties of propolis are mainly due to its flavonoids and phenolic compounds, which may protect the human body against harmful oxidative processes (24). Due to the strong antioxidant and anti-inflammatory effects of propolis, studies have been conducted on its protective effects on the liver (22, 24). Research has identified that propolis can reduce oxidative stress in liver tissue and plasma and reduce Alanine- and Aspartate-aminotransferase (ALT and AST, respectively) enzymes (23, 25). Regarding the beneficial role of exercise on prevention and treatment of metabolic syndrome disease, many studied have been conducted (26-29). So far, many studies have been performed on the effects of exercise on fatty liver and its associated risk factors, most of which reported reduction of liver fat, visceral fat, insulin resistance, IL6, TNF-α and liver enzymes such as AST and ALT (21, 30). Some studies reported aerobic and resistance exercises to be effective in improving fatty liver (21, 31).

To the best of the authors' knowledge, the role of high-intensity interval training (HIIT) exercise on CYP2E1 and TRPV4 levels has not been studied. Therefore, the aim of the present study was to investigate the effect of HIIT and iranian propolis extract on serum levels of TRPV4 and CYP2E1 proteins in patients with NAFLD.

Methods

Study design and participants

This study was a four-group randomized clinical trial with a pretest-posttest design. Thirty-two patients with NAFLD were randomly assigned to one of the four groups of HIIT (n=8), propolis supplement (n=8), propolis + HIIT (n=8), and controls (n=8) in a randomized control trial. Subjects were overweight or obese type 1, and with NAFLD (grades 2 and 3) who were diagnosed by liver ultrasound and increased ALT and AST. All ultrasounds and tests were

performed in one center (ie; private clinic and laboratory under the supervision of a radiologist). Before conducting the research, all test conditions were explained in detail in a briefing session and the consent form, personal information questionnaire, general health questionnaire, physical activity log, were given to them. The research was performed in accordance with the ethical standards of the Helsinki Declaration. The study was approved by the ethics committee board of Qazvin University of Medical Sciences (Approval ID: IR.QUMS.REC.1397.260). Informed consent was signed by subjects prior to the beginning of study.

Exercise protocol

The subjects participated in eight weeks of HIIT (one bout of 1-min intervals at 80–95% of the maximal heart-rate), interspersed by 2 min at 50-55% of the reserve heart-rate). The exercise protocol is detailed in table 1.

Table 1. Exercise protocol.

Phase	Warm-up	Main program	Cool down	Duration (min)
Week 1	Jogging and stretching (10 min)	Adaptation exercises with 50% intensity of heart-rate (HRR)	Jogging and stretching (10 min)	40
Week 2-5	Jogging and stretching (10 min)	6-7 intervals × (1 min with intensity of 80-95% + 2 min with intensity of 55-50% HRR)	Jogging and stretching (10 min)	40-45
Week 6-8	Jogging and stretching (10 min)	8-9 intervals × (1 min with intensity of 95-80% + 2 min with intensity of 55-50% HRR)	Jogging and stretching (10 min)	45-50

The above protocol was adapted from the protocol of Hood et al. (32), who performed one minute of vigorous activity and one minute of active rest on sedentary people. To determine the intensity of exercise, subjects were asked to record their resting heart-rate for three consecutive days before getting out of bed. Then, the average recorded numbers were considered as the resting heart-rate of the individual and were used according to the Karvonen equation to determine the intensity of exercise, which was adjusted according to the principle of overload by increasing the fitness and aerobic capacity of the subjects (33).

Supplementation

The Propolis supplement was taken three times a day by the subjects in the form of 50 mg tablet after the main meals. Propolis supplement was made by Soren Tak Toos Company (approved by the Food and Drug Administration) and was taken three tablets of 50 mg daily for eight weeks after the main meals.

Measures

Pre- and post-intervention tests included fasting blood lipid levels (eg; total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol), fasting blood sugar, ALT, AST, and ultrasound of the liver and bile ducts. Body composition was also measured with a Zeus composite pneumatic device. Before starting the training and supplementation protocol, subjects were fasting for 12 hours. To record the dependent variables (ie; TRPV4 and CYP2E1), blood samples were taken at five cc in sitting position. The amounts of these proteins were measured by enzyme-linked immunosorbent assay method with specialized kits made by ZellBio Company. The sensitivities of these kits were 0.2 ng/ml for CYP2E1 and 2 ng/l for TRPV4. Serum samples were separated by centrifugation (3000 rpm for 15 minutes at 4° C) and stored at -20° C until biochemical analysis. All tests and measurements were performed in the exercise physiology laboratory, the gymnasium of Imam Khomeini international university and the physiology laboratory of Qazvin medical sciences.

Data Analysis

Shapiro-Wilk test was used to determine data normality. Quantitative and qualitative data were expressed as mean ± standard deviation and number (%), respectively. Two-way analysis of covariance and Bonferroni post hoc test were also used. Paired t-test was used for the effect of intragroup variable. Statistical analyzes were performed at the significance level of 0.05 using SPSS statistical software (version 23).

Results

The mean±SD of age and body mass index (BMI) of the total sample were 45.1±3.6 years; and 30.0±3.6 kg/m^2, and 54% of the subjects were female. General characteristic of subjects of each group are detailed in table 2. As shown, the weight in HIIT + Propolis changed significantly in the post-test, while the weight in the other groups did not change significantly. Regarding BMI, it was observed that the group that exercised or supplemented experienced a significant change in BMI, while in the supplement group, there was no significant changes. As shown in table 2, the results suggested that the amount of CYP2E1 in the supplement group and the amount of TRPV4 in the HIIT + Propolis group in the post-test were significantly lower than the pre-test. No significant change was observed in other groups (Table 2).

After eight weeks of intervention, there was a significant difference between the mean post-test scores of both data (ie; CYP2E1 andTRPV4) (Table 3). The amount of CYP2E1 in the "HIIT + Propolis" group decreased significantly compared to other groups ($p = 0.032$). The exercise and the supplement-exercise groups had a significant decrease in TRPV4 ($p = 0.001$) (Figure 1).

Table 2. General characteristics of the subjects in pre-test and post-test (n=32).

	HIIT + Propolis (n=8)		Propolis (n=8)		HIIT (n=8)		Control (n=8)	
Data	**Pre-test**	**Post-test**	**Pre-test**	**Post-test**	**Pre-test**	**Post-test**	**Pre-test**	**Post-test**
Age (year)	45.80±4.65		45.80±3.49		42.40±2.60		46.40±3.28	
Height (cm)	171.20±4.50		166.20±4.35		174.40±5.39		163.20±4.29	
Weight (kg)	87.16±7.96	85.06±8.11*	85.14±6.72	84.94±6.18	83.40±8.3	82.08±8.13	86.04±4.15	85.62±4.14
BMI (kg/m²)	29.33±3.32	28.34±3.62*	30.63±3.02	30.66±3.10	27.28±4.19	26.56±3.83*	32.94±1.82	31.57±2.10
CYP2E1	6.98±1.59	5.37±0.94*	7.76±1.29	5.78±1.33*	6.72±1.67	5.41±0.77	6.27 ±1.15	6.34±1.18
TRPV4	72.13±8.87	67.12±10.76*	64.95±8.14	63.23±8.03	67.54 ±9.36	61.58±8.92*	72.84±8.05	75.16±6.58

BMI: Body mass index. CYP2E1: Ehanol-inducible cytochrome P450-2E1. HIIIT: Hight interval intermittent training. TRPV4: Transient receptor potential cation channel subfamily V. Data were mean ± standard deviation. .*p≤0.05 (pre-test vs. post-test) for the same group.

Table 3. Comparison of physical and physiological variables in groups (ANCOVA test) (n=32).

Data	Sum of squares	Degree of freedom	Mean squares	Fisher's F Ratio	Significance level	Eta-square
CYP2E1	8.641	3	2.88	3.99	0.018	0.30
TRPV4	329.164	3	1.9.87	11.07	0.001	0.55

ANCOVA: Analysis of covariance. CYP2E1: Ethanol-inducible cytochrome P450-2E1. TRPV4: Transient receptor potential cation channel subfamily V.

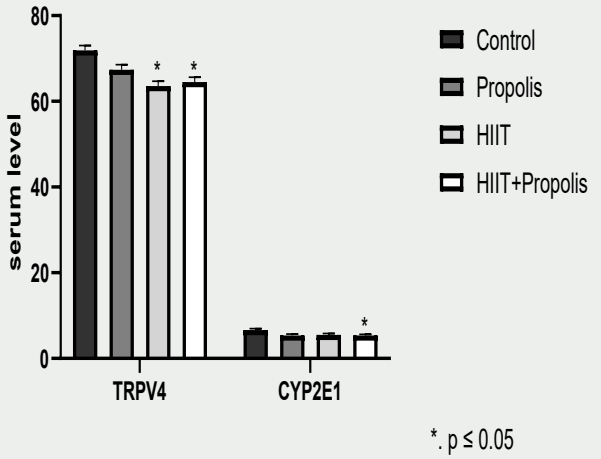

Figure 1. Bonferroni post-hoc test for means of test groups.

CYP2E1: Ethanol-inducible cytochrome P450-2E1. HIIIT: High interval intermittent training. TRPV4: Transient receptor potential cation channel subfamily V.

Discussion

The current study's major findings were that, although eight weeks of HIIT lowered serum CYP2E1 levels, the reduction was not statistically significant. As a result, it indicates that such activity would be ineffective in lowering oxidative stress caused by CYP2E1 during the evolution of fatty liver. HIIT with propolis supplementation, on the other hand, significantly lowered CYP2E1.

As noted in the research literature (34), increased activity of the CYP2E1 protein due to fatty liver and nonalcoholic steatohepatitis has been reported in humans and rodents (34). Increased CYP2E1 and increased insulin resistance appear to cause each other to form a positive feedback loop. In a study that aligns with our research, the effects of 16 weeks of high-fat diet and exercise on ethanol-inducible cytochrome P450 (CYP) gene expression were investigated (34). It was reported that a high-fat diet reduced CYP2A4, CYP2B10, CYP2E1 and CYP3A11 mRNA expression, while increasing CYP1A1 and CYP4A10 mRNAs (34). The authors also identified that CYP2E1 gene expression did not change under the influence of exercise (34).

A similar related study reported that endurance training reduced ROS production due to acute consumption of alcohol, triglycerides, and serum ALT and AST; but the level of mRNA and CYP2E1 protein remained unchanged (15). This decrease was also seen in the "exercise + alcohol" group, but did not completely prevent the progression of liver damage (15). This may be related to the increased expression of alcohol-induced CYP2E1 (15). In that study, researchers identied that endurance training could reduce the accumulation of damaged mitochondria caused by alcohol and mitophagy due to acute alcohol consumption, but could not affect the acute expression of alcohol-induced CYP2E1 (15). Numerous studies have individually examined the effects of one type of aerobic exercise on the condition of patients with metabolic syndrome disease, and the results of this study have shown a reduction in liver enzyme levels in line with our research (35-38). A study identified that endurance exercise protocol decreased serum lipid, ALT and AST levels in patients with NAFLD (39).

Propolis is one of the powerful antioxidants that has strong therapeutic effects (40). In this study, we identified that although this supplement had no significant effect, in interaction with HIIT protocol, Propolis can have a significant effect on reducing serum levels of CYP2E1 protein, thereby reducing oxidative stress and inhibiting the progression of fatty liver. Consistent with our study, some authors highlighted that propolis is a potent inhibitor of the CYP2E1 protein due to its flavonoid content and antioxidant effects, which may be responsible for the protective effect of propolis on the liver (22). Another cohort study reported that quercetin (a propolis flavonoid) inhibited CYP2E1 in human liver cell microsomes and reduced oxidative stress (41). Other studies have examined the effect of propolis on other factors associated with fatty liver and have shown that propolis reduces the enzymes ALT and AST, triglycerides and the fat content of the liver and its flavonoids are able to inhibit free radicals and thus protect the membrane (35). In our study, the results showed that HIIT alone and in interaction with propolis supplementation can significantly reduce serum levels of TRPV4 protein. Previous studies have reported that increased CYP2E1 increases TRPV4 activity, which ultimately leads to decreased CYP2E1 activity(15, 42) . Increasing CYP2E1 rises

TRPV4 activity, which ultimately leads to decreased CYP2E1 activity (15, 42). In our study, although HIIT did not significantly alter serum CYP2E1 levels, it did significantly reduce TRPV4. Therefore, we can conclude that no relationship was observed between CYP2E1 and TRPV4 and the decrease in TRPV4 was independent of changes in CYP2E1 and under the influence of other mechanisms.

In line with our results, a study identified that exercise has a significant effect on the expression of TRPV4 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (43). Running on a treadmill decreased TRPV4 and consequently increased PGC-1α in adipose tissue of mice (43). Another study reported that the TRPV4 protein content in the adipose tissue of trained mice was significantly reduced, which was associated with increased PGC-1α expression (44). These findings suggested that a decrease in exercise-induced TRPV4 protein levels may have protective effects against acute adipose tissue inflammation (44). Contrary to these results, a study reported an increase in TRPV4 in the anterior cortex of mice following acute hypoxic exercise, which was attributed to premature fatigue in hypoxic exercise (45).

In our study, consumption of propolis alone did not change the serum levels of TRPV4 protein, but when combined with HIIT, it decreased significantly this protein. Although studies have examined the protective effects of propolis supplementation on the liver, no study on the effects of propolis on TRPV4 have been found in databases. One study looked at the effect of apigenin on TRPV4 and its role in vasodilation. Apigenin is a plant-derived flavonoid from propolis flavonoids that may help prevent cardiovascular disease (46). It was found that epinephrine increases TRPV4 expression in Human embryonic kidney cells (ie; cells made by growing human kidney embryonic cells in culture medium) and causes vasodilation (46). One reason for the difference in results can be due to the discrepancy of the sample tissue and the difference in the effects of propolis flavonoids (46). Since our study was the first one who analysed the effects of HIIT and propolis supplementation on serum levels of CYP2E1 and TRPV4 in human subjects, no further comparison was possible. It is important to note that further exploration of the potential mechanisms underlying the observed effects of HIIT and propolis supplementation on CYP2E1 and TRPV4 would enhance the critical analysis of the research. Examining the interplay between these proteins and their potential contributions to the pathophysiology of NAFLD, along with considering the potential limitations of animal studies in translating findings to human subjects in future research, would add depth to the critical analysis. For example, one Iranian study have explored the potential of HIIT and continuous exercise training (CET) to induce hepatic miR-122 expression in rats with NAFLD and type 2 diabetes mellitus (T2DM) (47). The authors included 40 Wistar rats, which were divided into two groups: non-T2DM (NDC) and T2DM (DC), which was induced using a high-fat high-fructose diet (HFHFD) (47). DC rats were further categorized into three groups: diabetic control (HFHFD + DC), CET (HFHFD + CET), and HIIT (HFHFD + HIIT) (47). After eight weeks of exercise on a rodent treadmill, the authors have measured miR-122 and the expression of its target genes in the liver (47). The authors reported that HIIT i) Demonstrated a significant decrease in the expression of fatty acid synthase, acetyl-coa carboxylase, and Sterol Regulatory Element-Binding Protein 1c compared to HFHFD + DC, and ii) Showed a partial increase in miR-122 expression compared to HFHFD + DC (47). The authors concluded that exercise training emerges as a non-pharmacological intervention for improving NAFLD in diabetic rats through the induction of miR-122. Notably, HIIT exhibited a more pronounced effect on NAFLD amelioration compared to CET (47).

Limitations

The current research exhibits certain limitations, with the primary concern being the modest sample size. Specifically, our study comprised 32 patients, potentially limiting the representativeness of the findings to the broader population affected by NAFLD. A more extensive sample size would have bolstered the study's findings, rendering them more robust and applicable to a wider context. The second limitation is associated with the absence of a long-term follow-up. Our study focused solely on evaluating the impacts of interventions over a brief period (ie; eight weeks). Given that NAFLD is a chronic condition, it would have been more advantageous to explore the enduring effects and sustainability of the observed changes in TRPV4 and CYP2E1 levels. This would contribute significantly to comprehending the lasting impact of interventions, a crucial aspect for informed clinical decision-making. The third limitation pertains to the non-evaluation of several critical potential confounding factors, including dietary habits, medication usage, and comorbidities. The absence of assessment for the aforementioned factors introduces a potential source of influence on the study outcomes, necessitating consideration or control in both the study design and analysis. Recognizing and accounting for variables such as dietary patterns, medication regimens, and concurrent health conditions is essential to enhance the study's robustness and the accuracy of its findings. Incorporating these aspects into the research framework would contribute to a more comprehensive understanding of the relationship between interventions and outcomes. The fourth limitation is related to the difficulty of examining different enzymes in the liver of the subjects. Additional research with expanded sample sizes, meticulously designed control groups, and thorough different outcome measures is imperative to substantiate the present findings and ascertain the clinical significance of HIIT and propolis supplementation in the management of NAFLD. Rigorous investigation with these enhancements is crucial for providing more robust evidence, fostering confidence in the observed effects, and facilitating a more nuanced understanding of the potential benefits of HIIT and propolis supplementation in the clinical context of NAFLD treatment.

Conclusion

Propolis and HIIT would be a good strategy for non-pharmacological treatment of NAFLD by reducing CYP2E1 and TRPV4, as reducing these two proteins can lead to reduction of stress-oxidative levels, improve liver enzymes, insulin resistance and liver fat contents. Since no similar research has been done in this regard, a definitive statement needs further investigation.

DECLARATION: In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT (48-50).

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