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Research Article

Effect of Moderate Aerobic Exercise Combined with Cannabinoid Supplementation on P53 Gene Expression in Rats with High-Fat Diet-Induced Non-alcoholic fatty liver disease (NAFLD)

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Abstract

Introduction: In the present study, changes in P53 gene expression were investigated in rats fed a high-fat diet (HFD) after 6 weeks of moderate aerobic exercise and cannabinoid supplementation.

Methods: In this experimental study, 40 male Wistar rats were divided into 5 groups (n=8 per group): healthy control, high-fat diet (HFD), HFD + aerobic exercise (Tr), HFD + cannabinoid supplement (Sup), and HFD + Tr + Sup. Rats in the HFD group received a special high-fat diet for 2 months before the main protocol. Rats in the exercise groups ran on a rodent treadmill for 6 weeks, 5 days per week. Cannabinoids supplements were administered via gavage at a dose of 100 ng/kg after each exercise session to rats in the supplement group. To confirm Non-alcoholic fatty liver disease (NAFLD) induction based on HFD, the rats' livers were examined using a Zonecare-Q9 ultrasound device before the start of the exercise and supplementation protocol. Finally, after the exercise and supplementation protocol, the rats were sacrificed, and the P53 variable was measured by Real-Time PCR. For data analysis, an independent t-test, Bonferroni test, and two-way ANOVA were used.

Results: Ultrasound results showed that the high-fat diet in the HFD group induced grade 2 Non-alcoholic fatty liver disease (NAFLD) compare to the healthy control group. In the HFD group, the P53 gene expression in liver tissue was significantly increased in comparison to the healthy control group ($p<0.05$). Compared to the HFD group, the Sup group showed a significant decrease in P53 ($p<0.05$). The HFD+Tr and HFD+Tr+Sup groups did not demonstrate a significant effect on P53 in comparison to the HFD group ($p<0.05$).

Conclusion: The high-fat diet can lead to grade 2 Non-alcoholic fatty liver disease (NAFLD), while cannabinoid supplements, by reducing cellular stress and modulating P53 gene expression, may improve cellular signaling processes and provide a basis for developing new therapeutic strategies for liver cancer patients.

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1. Introduction

Overweight and obesity prevalence has increased dramatically worldwide in recent decades, with global obesity rates doubling since 1980 and approximately one-third of the world's population now classified as overweight or obese (1–3). Obesity, characterized by excessive fat accumulation, is typically caused by an energy imbalance, and high-fat diets (HFD) play a major role in its development. One of the major consequences of obesity associated with high-fat diets is non-alcoholic Non-alcoholic fatty liver disease (NAFLD), which has become one of the primary causes of liver disease worldwide, especially in Western countries. Although NAFLD is highly prevalent, only a small percentage of patients progress to more advanced stages such as inflammation, fibrosis, or chronic liver disease, with most experiencing only the early stages of the disease (4). Obesity and hyperglycemia increase p53 gene expression. This upregulation leads to the release of pro-inflammatory cytokines and exacerbates inflammation, which can result in metabolic disturbances that ultimately contribute to the development and progression of hepatocellular carcinoma (HCC) (5). For example, under hyperglycemic conditions or with excessive caloric intake, p53 activation can induce systemic insulin resistance (6). Furthermore, increased p53 expression can disrupt adipose tissue homeostasis, as p53 plays a crucial role in regulating the formation of white and brown adipose tissue and preventing adipocyte differentiation (7). The dual role of P53 in NAFLD progression is also notable. In high-fat diets, excessive p53 activity can lead to inflammation and cellular damage in the liver, whereas in the early stages of the disease, this protein may play a protective role and prevent disease progression (8). In animal models of NAFLD fed high-fat diets, increased p53 expression is associated with steatosis and inflammation, and inhibition of p53 can prevent these harmful effects (9). In this context, the use of natural supplements with antioxidant and anti-inflammatory properties, such as cannabis components, has attracted particular attention to managing high-fat diet complications. Cannabis contains bioactive compounds, including cannabinoids, which reduce oxidative stress and inflammation through various signaling pathways (10). Specifically, endocannabinoids (eCBs) influence p53 activity in the liver via cannabinoid receptor type 1 (CB1R). CB1R inhibition induces changes in p53 expression and activity, leading to the increased expression of miR-22. MiR-22 specifically regulates SIRT1 and PPARα, and these pathways can improve liver status in animal models of hepatic steatosis (11). Exercise is also an effective non-pharmacological intervention in reducing Non-alcoholic fatty liver disease (NAFLD) disease. Evidence indicates that exercise prevents the development of Non-alcoholic fatty liver disease (NAFLD)s in advanced stages and improves metabolic capacity (12). Furthermore, exercise modulates P53-related pathways and protect against liver injury (13). Given the existing evidence regarding the anti-inflammatory and antioxidant effects of natural supplements such as cannabis and exercise, this study aims to investigate the combined effects of exercise and cannabis supplementation on p53 gene expression in animal models with high-fat diet-induced Non-alcoholic fatty liver disease (NAFLD) disease.

2. Materials and Methods

Animals

In this experimental study, 40 male Wistar rats aged 10–12 weeks with an average weight of 240 grams were purchased from the Pasteur Institute of Iran (Tehran, Iran). They were housed under standard conditions in the animal biology laboratory of the Islamic Azad University, Science and Research Branch. The animals were acclimated to laboratory conditions for two weeks with a 12-hour light/dark cycle, an average temperature of 22±2°C, and a relative humidity of 55±5%. They were kept in ten rodent-standard cages measuring 30 × 15 × 15 cm, in accordance with the animal care checklist of Islamic Azad University, Science and Research Branch, Faculty of Medical Sciences and Technologies.During the first two weeks of acclimatization, the subjects were fed a normal diet consisting of pellets from the Javan-e Khorasan Company. These pellets had a composition of 19.6% protein, 3% fat, 6% fiber, 50.4% carbohydrates, 13% water, and other components. The present study was conducted under the supervision of the Animal Ethics Committee of the Islamic Azad University, Science and Research Branch, Tehran, Iran. It

High-fat diet

Except for the healthy control group, which was randomly selected, all other subjects were placed on a high-fat diet for two months by adding 40% vegetable shortening to their meals compared to the normal diet. The amount of added fat was calculated as 37% of the normal diet weight. The subjects' weights were measured twice a week before starting the diet and during the high-fat diet period using a Sunli BLK-500 digital scale with 0.1 g precision.

After two months, the 32 rats on the high-fat diet were randomly divided into four groups of eight: the high-fat diet group (HFD), the HFD plus exercise group (HFD+Tr), the HFD plus supplement group (HFD+Sup), and the HFD plus supplement and exercise group (HFD+Tr+Sup).

Before starting the exercise and supplementation protocol, four rats (one from the control group and three from the obese group) were randomly selected for liver ultrasound by a veterinarian. Except for one healthy control rat, all other tested subjects showed evidence of grade 2 Non-alcoholic fatty liver disease (NAFLD), which was confirmed.

Table 1. The data on weight, water consumption, and liver weight of the experimental animals.

Group	Body Weight(g) Mean ± SD	Water Intake (cm^3) Mean ± SD	Liver Weight (g)
Control	275.6± ۵٫۱۷	265±10.5	7.260
HFD	386.4±۷٫۱۹	370±13.5	13.388
HFD+Tr	357.9±۹٫۲۹	344±17.1	9.914
HFD+ Sup	۳۶۶±39.46	325±15.4	9.053
HFD+Tr+Sup	324.14±21.58	331±17.5	9.180

Laboratory measurements.

Liver P53 Gene Expression

To evaluate the P53 gene expression in each group, Real-Time PCR was performed. For primer preparation, the following were used: 10 µL of distilled water containing a lyophilized primer, 0.5 µL each of forward and reverse primers, 1 µL of cDNA, and 8 µL of DEPC (Diethyl pyrocarbonate) water. For gene expression analysis by qRT-PCR, total cellular RNA was extracted from a Qiazol solution, following the Sinagen Company protocol. The quality of the extracted RNAs was assessed by a spectrophotometer. To prepare single-stranded cDNA, an OligoDt primer and reverse transcriptase enzyme were applied according to the relevant protocol. Each PCR reaction was performed on an ABI Step One device according to the manufacturer's protocol. Real-Time PCR cycles for the P53 gene were performed at three temperatures: 94°C, 60°C, and 72°C. A melting curve was generated to verify the accuracy of the PCR reaction. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference gene for P53. Control and experimental genes were expressed simultaneously. Table 1 lists the primers used.

Table 2. Specifications of the primers used in the study

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCTCAGCATCTTATCCGAGTGG	22	60.29	54.55	3.00	1.00
Reverse primer	TGGATGGTGGTACAGTCAGAGC	22	61.74	54.55	4.00	2.00

Products on target templates

>NM_001127233.2 Mus musculus transformation related protein 53 (Trp53), transcript variant 2, mRNA

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product length = 128
Forward primer  1    CCTCAGCATCTTATCCGAGTGG  22
Template        675  ..C.............G....  696

Reverse primer  1    TGGATGGTGGTACAGTCAGAGC  22
Template        802  ............T.C.......  781
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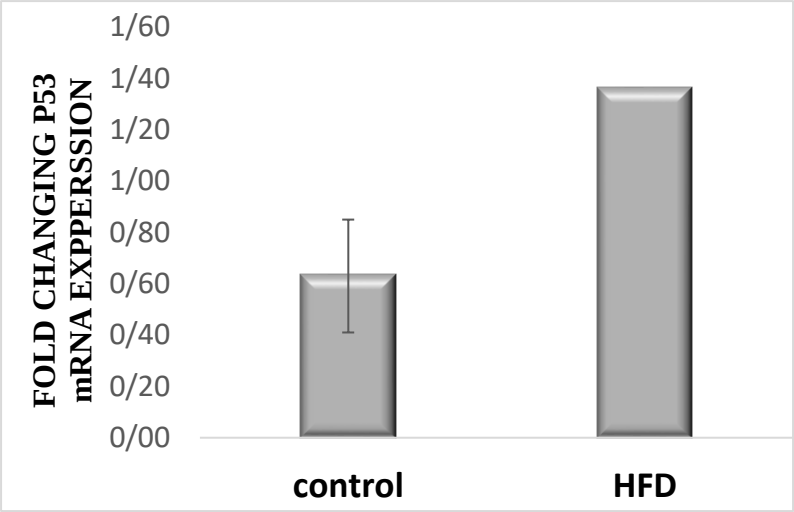
3. Results

Statistical method

Data are presented as mean ± standard deviation. The normality of the data was confirmed by the Shapiro-Wilk test. To determine the effect of a high-fat diet on the studied outcomes, the control group fed a normal diet and the control group fed a high-fat diet were compared through an independent T-test. To pinpoint the location of any differences, the groups were compared pairwise using the Bonferroni test. For each pairwise comparison, the effect size was calculated by Cohen's d. To test the interaction between aerobic exercise and cannabis supplementation on the studied outcomes, a two-way analysis of variance (ANOVA) for independent groups was performed to analyze the results. Data were analyzed using SPSS software version 26, and the significance level

Based on the t-test, a significant difference was observed in the expression of the P53 gene in liver tissue between the control group fed a normal diet and the control group fed a high-fat diet (P = 0.001, d = 11). Consumption of a high-fat diet significantly increased P53 gene expression in liver tissue.

Figure 1. Comparison of P53 gene expression in liver tissue between the control group fed a normal diet and the control group fed a high-fat diet.



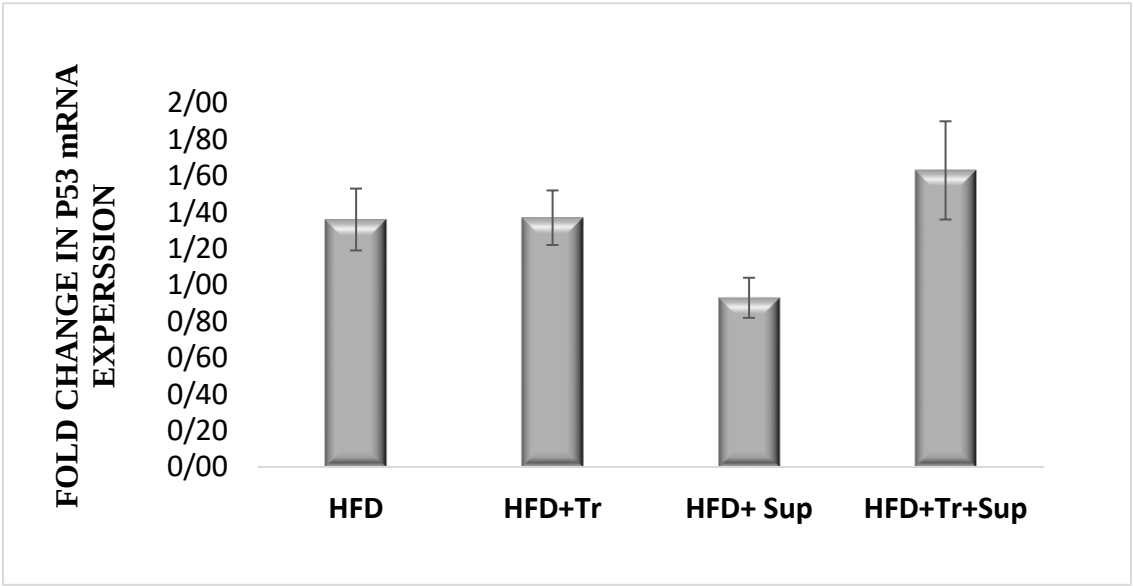
According to the results of the two-way analysis of variance, aerobic exercise did not have a significant effect on the expression of the P53 gene in liver tissue (F = 3.656, P = 0.065, η^2 = 0.147). However, cannabis supplementation did have a significant effect on the expression of the P53 gene in liver tissue (F = 5.695, P = 0.023, η^2 = 0.100), while the interaction between aerobic exercise and cannabis supplementation was not significant (F = 1.274, P = 0.267, η^2 = 0.037).

Bonferroni test results showed that at the end of the study period, P53 gene expression in liver tissue in the aerobic exercise group was not significantly different from that in the control–high-fat diet group (P = 1.000). However, in the supplement group compared to the control–high-fat diet group, the difference was significant (P = 0.001). In the aerobic exercise supplement group compared to the control–high-fat diet group, the difference was not significant (P = 0.245).

A significant difference was observed in P53 gene expression between the aerobic exercise group and the cannabis supplement group (P = 0.002), and a significant difference was also found between the aerobic exercise group and the aerobic exercise–cannabis supplement group (P = 0.001).

The lowest P53 gene expression in liver tissue was observed in the supplemented group. The interaction between these two interventions was not statistically significant. For clarity, Figure 2 is presented.

Figure 2. Expression of the P53 gene in liver tissue among the studied groups. Data are reported as mean and standard deviation.



4. Discussion

Western countries have a high intake of dietary fats and refined carbohydrates, along with a low consumption of plant fibers (14). Obesity, resulting from energy imbalances and high-fat diets, is associated with excessive fat deposits in the body. This condition is especially prevalent in patients with non-alcoholic Non-alcoholic fatty liver disease (NAFLD) disease (NAFLD), which is one of the most common causes of liver disease globally, particularly in Western countries (15). Lifestyle-related disorders, especially metabolic syndrome, are recognized as major risk factors for liver diseases that can range from hepatic steatosis to hepatocellular carcinoma (HCC). The P53 gene, as a key regulator of metabolism and a tumor suppressor, plays a significant role in maintaining homeostasis and liver disorders. The activity of this gene has dual effects; on the one hand, its overactivity or loss of function may contribute to liver disease, while on the other hand, moderate and temporary induction may be effective in preventing and treating these diseases (8). In this context, non-alcoholic Non-alcoholic fatty liver disease (NAFLD) disease (NAFLD), caused by fat accumulation in the liver, can lead to more serious problems such as hepatic steatosis, non-alcoholic steatohepatitis, liver fibrosis, cirrhosis, and even liver cancer. The role of the P53 gene as a tumor suppressor in NAFLD progression has been widely studied, but its precise relationship to the disease remains controversial. Some studies suggest that P53 plays a crucial role in NAFLD, while others indicate that reduced activity of this gene may exacerbate hepatic steatosis (16). Given that exercise and cannabis supplementation are effective factors in improving and controlling oxidative stress and chronic inflammation, which underlie non-alcoholic Non-alcoholic fatty liver disease (NAFLD) disease (NAFLD), the present study investigates the effects of six weeks of treadmill exercise and cannabis supplementation on the expression of the P53 gene in rats with high-fat diet-induced Non-alcoholic fatty liver disease (NAFLD).

The results of the present study showed that the expression of the P53 gene in the liver tissue of the control group fed a high-fat diet was considerably increased compared to the control group fed a normal diet. Aerobic exercise did not significantly affect P53 gene expression in liver tissue However, cannabis supplementation had a significant effect on P53 gene expression in liver tissue. However, the interaction between aerobic exercise and cannabis supplementation did not significantly influence P53 expression.

Consistent with the present findings, Jiang and colleagues demonstrated that high-fat diets activate the P53 protein, which regulates metabolism and cellular damage in the liver. However, inhibition of P53 can reduce liver injury and control hepatic steatosis (17). Conversely, another study showed that deletion of the P53 gene in mice can lead to increased obesity and fat accumulation in the liver (18).

Given that cannabis supplementation and exercise play critical roles in improving the Non-alcoholic fatty liver disease (NAFLD), cannabis contains bioactive compounds such as cannabinoids that can reduce oxidative stress and inflammation through various signaling pathways (10). CBD consumption alongside a high-fat diet may regulate the expression of genes related to oxidative stress, such as TP53, MDM2, and GADD45a. This finding suggests that cannabis supplements can modulate the detrimental effects of a high-fat diet on liver metabolism (19). Additionally, cannabidiol (CBD) as an adjuvant can increase liver cancer cells' sensitivity to cabozantinib via p53-dependent endoplasmic reticulum stress pathways, indicating promising potential for CBD in combination with other targeted therapies for liver cancer (20). There is substantial evidence that exercise can mitigate the negative effects of high-fat diets on the liver and regulate metabolism (21).

Furthermore, exercise may inhibit JNK activation by regulating P53 and delaying hepatocyte transformation into carcinoma. Cooke and colleagues confirmed that endurance training has beneficial effects on the liver by reducing hepatic steatosis, decreasing de novo lipogenesis, and improving mitochondrial biogenesis, thereby improving non-alcoholic Non-alcoholic fatty liver disease (NAFLD) disease (NAFLD). These exercises also reduce oxidative stress and delay NAFLD progression by suppressing p53 protein and cellular senescence markers such as p22 and p16 (22). However, the results of the present study are not consistent with those of Cooke et al.

۵. Conclusion

The results of the present study indicate that cannabis supplements can effectively help cellular signaling processes by reducing cellular stress. In addition, cannabis supplements can modulate the expression of pro-apoptotic genes such as P53. These findings are particularly significant in liver cancer treatment. They suggest that cannabis can be used as an adjuvant in combination with other targeted therapies. Given cannabis' therapeutic potential, further research is needed to elucidate the precise mechanisms of its effects and investigate its clinical applications. Ultimately, this study may pave the way for new therapeutic strategies for liver cancer patients.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: M.M ,M.Gh , H.P ; Methodology: S M.M ,M.Gh , H.P.; Software: M.M ,M.Gh , H.P; Validation: M.M ,M.Gh , H.P.; Formal analysis: M.M ,M.Gh , H.P; Investigation: S.M.B , K.M , E .A , A. K ; Resources: M.M ,M.Gh , H.P; Data curation: M.M ,M.Gh , H.P; Writing - original draft: M.M ,M.Gh , H.P; Writing - review & editing: M.M ,M.Gh , H.P; Visualization: M.M ,M.Gh , H.P; Supervision: M.M ,M.Gh , H.P; Project administration: M.M ,M.Gh , H.P; Funding acquisition: M.M ,M.Gh , H.P.

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