

ORIGINAL RESEARCH

Investigating the effect of 8 weeks of aerobic training and Borage extract on the serum concentration of liver enzymes and lipid profile in rats with non-alcoholic fatty liver disease (NAFLD)

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Abstract

Objective: Non-alcoholic fatty liver disease is known as one of the chronic liver diseases that is closely related to obesity and metabolic disorders, and borage improves oxidative indices due to its antioxidant properties. The aim of this study is to investigate the effect of a part of aerobic exercise with borage extract on the serum concentration of liver enzymes and lipid profile in rats with non-alcoholic fatty liver disease.

Materials and Methods: In this experimental study, 40 male Wistar rats weighing 250-300 grams were divided into 4 groups (N: 10). The groups were: Control group (suffering from fatty liver and receiving enough water and food/without exercise and supplements) supplement group (suffering from fatty liver and receiving 200 mg/kg borage extract) exercise group (suffering from fatty liver and doing aerobic exercise daily for 8 weeks) exercise + supplement group (suffering from fatty liver and receiving 200 mg/kg borage supplement and performing aerobic exercise for 8 weeks). At the end, blood was drawn from all the animals, then the serum lipid profile, including total cholesterol and triglycerides, as well as liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by ELISA method. They were analyzed with SPSS and a significance level of <0.05 .

Results: The findings of the present study showed that in the comparison of total cholesterol serum level changes during the study period after poisoning, there was a significant decrease in the control groups ($P=0.004$) and supplement (borage extract) + exercise ($P=0.047$). Nevertheless, the training and supplementation groups showed a significant increase in serum total cholesterol levels during the study period. In addition, comparing the changes in serum triglyceride levels during the study period after poisoning, only a significant decrease was observed in the supplemented group ($P=0.002$). Also, comparing the changes of serum ALT level during the study period after poisoning, a significant increase in serum ALT level was observed in the control ($P=0.045$) and exercise ($P=0.002$) groups. The supplement group also showed a significant decrease in ALT serum level ($P<0.001$). In comparing the changes of AST serum level during the study period after poisoning, a significant decrease was observed in the control ($P=0.003$) and supplement ($P=0.006$) groups.

Conclusion: The findings of the present study showed that 8 weeks of supplementing with borage extract alone reduced serum triglyceride, AST, and ALT levels, and 8 weeks of aerobic exercise with borage extract reduced total cholesterol levels in rats with non-alcoholic fatty liver disease.

Keywords: Non-alcoholic fatty liver (NAFLD), Aerobic exercise, Borage extract, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lipid profile

Introduction

Non-alcoholic fatty liver disease (NAFLD) is known as one of the chronic liver diseases. Metabolic syndrome is a strong prognosis for this disease, which occurs along with obesity, type 2 diabetes, dyslipidemia, and hypertension. A high body mass index (BMI), increased triglyceride levels, decreased high-density lipoprotein (HDL) levels, and increased hemoglobin A1C levels clearly indicate non-alcoholic fatty liver disease (1). The prevalence of this disease in Iran is reported to be 2.9-1.7% in the population (2).

Research show that the lifestyle change intervention, including diet change and regular exercise, is effective in controlling and treating patients with non-alcoholic fatty liver disease (3).

The exercise program not only leads to an increase in energy consumption and weight loss, but also increases fat metabolism, decreases blood fat, decreases liver fat, and increases muscle volume and strength, followed by an increase in muscle metabolism. Based on this, exercise is the foundation stone. Control and management of non-alcoholic fatty liver (4).

In general, exercise interventions reduce lipid synthesis by increasing the activity of AMPK (AMP-activating protein kinase) in the liver and by deactivating hepatic Acetyl-CoA carboxylase. In addition, exercise reduces weight and improves body composition, increases adiponectin, and regulates leptin level, and inflammatory and pro-inflammatory indicators, which in turn increases AMPK and reduces liver fat (5). Also, aerobic exercise improves triglyceride levels and plasma non-esterified fatty acids concentration and insulin resistance. It reduces the concentration of liver cytokines such as α IL-10, TNF, IL-6-MCP-1 and reduces inflammation by increasing the regulation of Ampk level and the expression of α PPAR- protein. Finally, it has been proven that aerobic exercise improves hepatic steatosis

and inflammation through the Ampk-PPAR- α signaling pathway (6).

As a result, the results of human studies show that regular exercise reduces liver fat (7).

Herbal medicines are also a rich source of biologically active substances in Chinese medicine, which are prescribed in the prevention of diseases. Many studies have been conducted on the effectiveness of herbal extracts or natural products on the treatment of non-alcoholic fatty liver (8).

Using antioxidants and doing physical activity is one of the most important factors that has attracted the opinion of many researchers in controlling non-alcoholic fatty liver disease (9). Borage flower (*Broage officinalis*) is a native plant of the Mediterranean region in North Africa and the Middle East, which is known as star flower or borage flower (10).

Borage flower is a good source of W3 fatty acids, which contains α -linolenic acid (ALA), which is especially converted to EPA (4-15%). Most types of borage contain w6 PUFA, which is a rich source of w6 linoleic acid. Linoleic acid is converted into the long active form of PUFA chain by FADS2. Although active forms derived from linoleic acid have pro-inflammatory and anti-inflammatory properties (11). Borage flower extract also contains 95% triacylglycerol, which includes C14-C20 fatty acids. 5% contains tocopherol, flavonoid, phospholipid, sterol, free fatty acid, mono and di acyl glycerol. Most of these compounds have an antioxidant role. Borage is the richest plant source of gamma linolenic acid (GLA), which is used as a food supplement to prevent and treat degenerative pathological diseases such as osteoporosis, diabetes, and cancer. Also, GLA for inhibiting tumor growth in the laboratory, improving the oxygenation status, anti-inflammatory activity and beneficial effects in the early stages of infection (12).

In a study conducted in 2016 by Lorestani et al. on the effect of 8 weeks of aerobic exercise on the levels of enzymes related to non-alcoholic

fatty liver disease in obese children, it was observed.

Aerobic exercise was performed for 8 weeks and 4 sessions per week with a duration of 30 minutes and maximum oxygen consumption of 50-65%. The result showed that aerobic exercise for 8 weeks in the form of sessions 4 times a week for 30 minutes each time caused There is a significant decrease in the level of liver enzymes ALT/AST/ALP, and it was proposed as an effective non-pharmacological solution (13).

In a study conducted in 2020 by Tiego Aparceido et al. on the effect of aerobic exercise in improving non-alcoholic fatty liver markers and insulin resistance in the α Ampk-PPAR signaling pathway in obese rats. In this study, aerobic exercise was used as a non-pharmacological method to improve hepatic steatosis. In this research, new mice were fed with 2 standard diets and high fat for 12 weeks, the other group was subjected to 7-8 weeks of fatty food and aerobic exercise with a maximum intensity of 60%. Finally, body composition parameters, expression of inflammatory genes and metabolic mediators were measured. It was found that aerobic exercise is effective on weight gain and not on fat accumulation in the body. Aerobic exercise improves the level of triglycerides and the concentration of non-esterified fatty acids in plasma and insulin resistance. It reduces the concentration of liver cytokines such as α IL-10, TNF, IL-6-MCP-1 and reduces inflammation by increasing the regulation of Ampk level and the expression of α PPAR-protein. Finally, it was proved that aerobic exercise improves hepatic steatosis and inflammation through the Ampk-PPAR- α signaling pathway (6).

In a meta-analysis study conducted by Elisa Baron et al. in 2023 under the title of physical activity protocol in the management and control of non-alcoholic fatty liver disease, it was found that both aerobic exercise and resistance training have beneficial effects in patients with non-alcoholic fatty liver disease. The effectiveness of these exercises on ALT liver enzymes is also evident. Aerobic exercise is more effective in improving lipid profile than resistance exercise, especially in total cholesterol, HDL, LDL and weight loss after 8

weeks of exercise program. Of course, it was found that No difference in reducing liver fat content, abdominal visceral fat, or improving insulin resistance after 12 weeks of exercise between resistance and aerobic exercise in patients with non-alcoholic fatty liver disease (14).

In the study of Mohamad ALHILAL et al. titled the effect of borage oil on high-fat diet leading to liver damage in male Syrian golden rats. In this study, which was conducted with the aim of investigating the effect of borage flower supplement (GLA: gamma linolenic acid) on rat liver damage. The results of the study showed that fat accumulation in hepatocytes increases the level of GGT, LDH, ALP, AST, ALT, and CK enzymes. The group consuming borage oil did not have these negative effects (15).

Cheraghi et al.'s study was conducted on the effect of hydro alcoholic extract of borage extract on serum lipid profile in mice and its comparison with lovastatin. The results of the research showed that the serum concentration of total cholesterol, LDL, and TG decreased significantly in the extract and lovastatin group. In the animals that consumed a high cholesterol diet, consumption of 3 mg/kg of borage extract caused a significant decrease in the concentration of LDL, VLDL, and TG (16).

Ranjbar et al.'s study was conducted on the comparative and combined effect of the borage extract and chicory root on the liver of Wistar rats. In this study, the concentrations of 40, 400 and 1000 mg/kg of the aqueous and hydro alcoholic extracts of both substances on the enzyme Liver enzymes ALT, AST, ALP, GGT, MDA and bilirubin level were investigated. The results of this study showed that the concentration of liver enzymes such as ALT and AST increase with increasing the dose of the extract. The consumption of 200mg/kg of the borage extract reduces steatosis. Hepatic and improves inflammation in the liver tissue (17).

The simultaneous effect of aerobic exercise and borage extract was not observed in any study, so the aim of this study is to investigate the simultaneous effect of aerobic exercise and borage extract according to the characteristics and effects of each of them on some non-alcoholic fatty liver parameters (level serum liver enzymes and lipid profile).

Materials and Methods

The current research is fundamental, and its method is experimental. This research was conducted with the approval of the ethics committee at Islamic Azad University, Research Sciences Unit. This research was conducted on 32 male Wistar rats aged 8 to 10 weeks with a weight range of 230 to 300 grams at the Animal Studies Center of Baqiyatullah University of Medical Sciences. The animals were kept in standard laboratory conditions (12 hours of light-12 hours of darkness) with a temperature of 21-23 degrees Celsius. They had free access to water and food. All tests and weighing were done one week after maintenance and adaptation to the new environment conditions. Then, for 4 weeks, all the rats were subjected to a high-fat HFD diet with 60% fat, 40% carbohydrate, and about 10% protein, and in the fifth week, oral tetracycline at a dose of 140 mg per kilogram of body weight (as a solution in 2 ml of distilled water) was fed for 7 days by gavage method by a veterinarian and following the principles of working with animals. Fatty liver (steatosis) was confirmed by measuring liver enzymes and lipid profile, frozen section and hematoxylin eosinophil staining. Therefore, after this, an increase in the serum concentration of liver enzymes was observed in all groups.

Then the male rats were randomly divided into 4 groups of 8. The groups were the control group (suffering from fatty liver and receiving enough water and food), the supplement group (suffering from fatty liver and receiving borage extract) and the training group (suffering from fatty liver and doing aerobic exercise on a daily basis) exercise supplement group (suffering from fatty liver and receiving supplements and doing aerobic exercise on a daily basis) All animals were weighed at the beginning and weekly using a digital scale. Serum concentrations of liver enzymes were checked at the beginning, during and at the end of the study. Blood was taken from the vein at all stages and 0.5 cc of blood was taken from the mice. After the blood collection stage, anesthesia with ketamine solution Cholera was administered at a dose of 5 mg/kg and xylazine at a dose of 2% at a dose of 10 mg/kg, then the liver tissue of the rats was sampled and after washing with physiological serum, it was placed in 10% formalin for fixation. From the liver tissue samples, slices Tissue sections were

prepared with a thickness of 5 microns and stained with hematoxylin-eosin. The stained slides were examined by a pathologist using an optical microscope, and all the slides of the liver tissue sections were recorded and reported for histomorphological changes.

In addition, the extract of borage has been prepared by the percolation method in the Research Institute of Plant and Pharmaceutical Sciences. The ratio of solvent to plant is 1.5. First, the desired plants were ground and then passed through 18 to 35 meshes. Then the desired amount was weighed and soaked with the corresponding solvent for 2 hours. After that, put the lid of the decanter and let the solvent and the plant mix together for 24 hours. After 24 hours, open the tap of the decanter until The liquid extract should come out drop by drop. After the extraction is finished, we transfer them to the balloon for concentration and connect it to the rotary device and depending on the amount of extracts and their concentration, the concentration process may take from several hours to several days.

Then, 0.5 cc borage extract was fed to mice by gavage for 5 days a week. Exercise protocol:

In the first week after the induction of non-alcoholic fatty liver, the rats entered the adaptation phase for the implementation of the research project and were introduced to aerobic exercise 5 days a week. Aerobic training in the acclimatization phase consisted of 30 minutes of running on a treadmill with zero incline at a speed of 15 meters per minute. The first 5 minutes were warm-up and the last 5 minutes were cooling down.

In the first week, 30 minutes of running on the treadmill at a speed of 27 meters per minute and vo2MAX 50-60% (aerobic exercise with moderate intensity) were performed, the first 5 minutes were for warming up and the last 5 minutes were for cooling down (at a speed of 15 meters per minute). (and gradually during 8 weeks the speed increased and reached 32 m/min in the 8th week. In general, 8 weeks of training / 5 sessions of 30 minutes each week were done (18).

Mean (standard deviation) or median (interquartile range) was used to describe quantitative variables according to conditions, and frequency (percentage) was used for qualitative variables. Kolmogorov-Smirnov test is used to check the normality of data distribution. To compare the mean of the

quantitative results between the two studied groups, use the independent t-test or its non-parametric equivalent, i.e. the Mann-Whitney test, and compare the results between before and after the intervention within each group using the paired t-test or its non-parametric equivalent, i.e. the Wilcoxon test. used. Chi-square test or Fisher's exact test was used to compare qualitative factors between two groups. One-way analysis of variance was used to evaluate the difference in quantitative variables. In the comparison of the results between the two study groups, appropriate regression models were used if there was a need to control the baseline variables according to the type of variable. SPSS version 25 software was used for data analysis and P-value <0.05 was considered statistically significant.

Results

In Table 1, the mean of total cholesterol and triglycerides at different times are compared between 4 groups. At all-time points of the study, there was a significant difference between the 4 groups in terms of total cholesterol serum level. This difference remained significant after adjustment for weight and initial levels of total cholesterol. In the intra-group comparison between the beginning of the study and after poisoning, a significant increase in total cholesterol serum levels was observed only in the control group ($P=0.011$). Also, in the comparison of total cholesterol serum level changes during the study period after poisoning, a significant decrease was observed in the control ($P=0.004$) and supplement + exercise ($P=0.047$) groups. Nevertheless, the training and supplementation groups showed a significant increase in serum total cholesterol levels during the study period. In addition, at the beginning of the study, there was no significant difference between the 4 groups in terms of serum triglyceride levels ($P=0.240$). After poisoning, the serum level of triglyceride in the group receiving the supplement was significantly higher than the other groups ($P=0.002$). In the 4th week, there was no significant difference between the groups in terms of this variable ($P=0.395$). At the end of the study, the control group had a lower serum triglyceride level than the other groups ($P=0.020$). These differences remained

significant after adjusting the effect of weight and initial level of serum triglyceride in the ANCOVA model. As can be seen, in this table, the intra-group difference after poisoning compared to before using the paired sample t-test and at the end of the intervention compared to before the intervention (after poisoning) using the repeated measure ANOVA test has been compared. After poisoning compared to zero day, only a significant decrease in the serum triglyceride level of the supplement + training group was observed ($P=0.042$). In the comparison of changes in serum triglyceride level during the study period after poisoning, only a significant decrease was observed in the supplement group ($P=0.002$).

Table 2 shows the average changes of liver enzymes at different times between 4 groups. At the beginning of the study, there was a significant difference between the 4 groups in terms of serum levels of ALT ($P=0.049$) and AST ($P=0.003$). After poisoning, the four studied groups had significant differences in serum levels of ALT ($P=0.002$) and AST ($P=0.048$). However, in the ANCOVA model, after adjusting the effect of weight and the initial level of ALT, the difference between the groups in the time after poisoning in terms of the level of this variable disappeared. In 4th and 8th week, there were significant differences in serum ALT levels from the 4 groups. In 4th week, the supplement + exercise group had the lowest serum ALT level, and in week 8, the supplement group had the lowest serum ALT level. In 4th and 8th week, there was no difference between the groups in terms of AST serum level. In the intra-group comparison, only a significant increase in the mean ALT of the supplemented group was observed after poisoning compared to 1st day ($P=0.002$). In the comparison of changes in serum ALT level during the study period after poisoning, a significant increase in serum ALT level was observed in the control ($P=0.045$) and exercise ($P=0.002$) groups. Also, the supplement group showed a significant decrease in serum ALT level ($P<0.001$). In terms of serum AST level, no significant change was observed in any of the groups at the time of poisoning compared to the beginning of the study. However, in comparing the changes of AST serum level during the study period after poisoning, a

significant decrease was observed in the control (P=0.003) and supplement (P=0.006) groups.

Table 1- Comparison of total cholesterol and triglyceride in 4 groups during the study period

Variables	Groups	Control	Exercise (8 weeks of aerobic training)	Supplement (Borage extract)	Exercise + Supplement	P-value ¹	P-value ²
TC (mg/dl)	1 st day	89.42 ± 7.96	72.61 ± 9.39	84.69 ± 11.96	78.55 ± 8.30	0.036	0.041*
	After poisoning (After a high-fat diet)	77.43 ± 8.54	59.18 ± 7.07	83.86 ± 8.20	77.64 ± 7.36	<0.001	0.002
	4 th week	61.04 ± 9.91	74.58 ± 3.70	71.59 ± 6.13	78.81 ± 7.22	0.002	0.003
	8 th week	46.60 ± 20.49	91.33 ± 18.21	111.18 ± 16.25	62.85 ± 15.71	<0.001	0.001
	P-value ³	0.011	0.086	0.934	0.932		
	P-value ⁴	0.004	0.001	<0.001	0.047		
	1 st day	71.03 ± 84.44	69.45 ± 58.26	128.13 ± 65.16	126.98 ± 41.05	0.240	0.372
	After poisoning (After a high-fat diet)	48.92 ± 52.53	83.01 ± 84.69	182.99 ± 33.32	66.66 ± 39.80	0.002	0.024
TG (mg/dl)	4 th week	93.18 ± 26.10	67.46 ± 35.64	57.29 ± 55.13	79.14 ± 22.38	0.395	0.474
	8 th week	59.08 ± 43.11	110.69 ± 43.35	123.87 ± 19.00	98.05 ± 21.89	0.020	0.043
	P-value ³	0.473	0.795	0.203	0.042		
	P-value ⁴	0.172	0.453	0.002	0.280		

TC: Total Cholesterol; TG: Triglyceride. Numbers are shown as mean ± standard deviation (SD).

1. Calculated by one-way ANOVA test
2. Calculated by ANCOVA test adjusting the effect of weight and initial level of total cholesterol/triglycerides (cases marked with * were only adjusted for weight)
3. Calculated by paired sample t-test to compare baseline and post-intoxication levels
4. Calculated with repeated measure ANOVA to compare variable changes during the study compared to the time after poisoning

Table 2- Comparison of liver enzymes in 4 groups during the intervention period

Variables	Groups	Control	Exercise (8 weeks of aerobic training)	Supplement (Borage extract)	Exercise + Supplement	P-value ¹	P-value ²
ALT	1 st day	42.48 ± 27.91	31.94 ± 16.67	31.32 ± 20.83	69.58 ± 29.16	0.049	0.066*
	After poisoning (After a high-fat diet)	72.67 ± 18.59	61.91 ± 23.39	116.34 ± 23.50	78.85 ± 21.64	0.002	0.103
	4 th week	99.57 ± 8.72	113.84 ± 12.28	82.44 ± 54.6	58.22 ± 18.15	0.001	<1
	8 th week	91.84 ± 7.21	68.74 ± 16.21	49.94 ± 12.99	62.26 ± 8.12	0.001	<1
	P-value ³	0.072	0.059	0.002	0.615		
	P-value ⁴	0.045	0.002	<0.001	0.178		
	1 st day	189.72 ± 83.48	125.17 ± 41.10	267.46 ± 39.76	149.77 ± 48.66	0.003	0.005*
	After poisoning (After a high-fat diet)	242.20 ± 51.94	170.42 ± 88.12	207.79 ± 43.63	143.31 ± 43.74	0.048	0.178
AST	4 th week	110.72 ± 11.95	118.38 ± 41.60	135.88 ± 39.78	141.82 ± 42.19	0.042	0.463
	8 th week	117.96 ± 19.55	135.77 ± 44.66	108.37 ± 49.30	144.40 ± 30.49	0.362	0.071
	P-value ³	0.335	0.294	0.062	0.855		
	P-value ⁴	0.003	0.342	0.006	0.993		

ALT: Alanine Transaminase; AST: Aspartate Transaminase.

Numbers are shown as mean ± standard deviation (SD).

1. Calculated by one-way ANOVA test
2. Calculated by ANCOVA test adjusting the effect of weight and initial level of total cholesterol/triglycerides (cases marked with * were only adjusted for weight)
3. Calculated by paired sample t-test to compare baseline and post-intoxication levels
4. Calculated with repeated measure ANOVA to compare variable changes during the study compared to the time after poisoning

Discussion

So far, no definitive treatment for non-alcoholic fatty liver has been reported. The treatment is based on weight loss, elimination of possible toxins and drugs, control of diabetes and fat. Also, studies show that diet is an important factor in the occurrence of this disease (19). The malfunction of the liver is related to the high level of oxidative stress and inflammatory

substances. Fortunately, the liver has the necessary capacity to regenerate the factors that damage the liver. Food components such as the borage extract have an anti-inflammatory role and help the liver in this process (20).

In a study conducted in 2016 by Lorestani et al. on the effect of 8 weeks of aerobic exercise on the levels of enzymes related to non-alcoholic fatty liver disease in obese children. Aerobic exercise was performed for 8 weeks and 4 sessions per week with a duration of 30 minutes and a maximum oxygen consumption of 50-65%. The result showed that aerobic exercise causes a significant decrease in the level of liver enzymes ALT/AST/ALP and It was proposed as an effective non-pharmacological solution (13).

In the present study, after 8 weeks in the group receiving aerobic exercise, a significant increase in the serum level of cholesterol was seen, but the decrease in the serum level of triglyceride was not significant. The reason for this could be that the training protocol may not have been carefully implemented in the mice, or the desired type of exercise and its time may have been insufficient or inappropriate for the mice. Inconsistent with the present study, Tiego Aparceido's et al. (2020) study on the effect of aerobic exercise in improving non-alcoholic fatty liver markers and insulin resistance in the α AMPK-PPAR signaling pathway in obese rats. In this study, aerobic exercise was used as a non-drug method to improve hepatic steatosis. In this research, new mice were fed with 2 standard diets and high fat for 12 weeks, the other group was subjected to 7-8 weeks of fatty food and aerobic exercise with a maximum intensity of 60%. In this study, it was observed that aerobic exercise improves the level of triglycerides and the concentration of non-sterilized fatty acids in plasma and insulin resistance. Finally, it was proved that aerobic exercise improves hepatic steatosis and inflammation through the AMPK-PPAR- α signaling pathway (6).

The findings of the present study showed that supplementing with borage extract for 8 weeks reduces serum triglyceride, AST, and ALT levels, and performing aerobic exercise with borage extract reduced total cholesterol levels. Also, in some study showed treatment by borage oil alone restored the normal levels of

plasma T.Ch, LDL-Ch, T.Ch/HDL-Ch and liver fat. Fish oil and borage oil were reported to reduce T.Ch, T.Gs and hepatic lipids in mice (10, 21).

Bazzi Allahri et al (2023) showed that, after consuming 25 drops of borage plant extract in combination with 125 ml of water after lunch and dinner for 1 week, the amount of liver enzymes after the intervention was lower than the control group in patients with type 2 diabetes (22).

In a clinical trial study that was conducted in 2012, titled the effect of two plant products, valerian and borage, on liver and kidney function tests in rats. In this study, plant extracts were fed to rats for 7 days and Blood sampling was done on day 8. The changes of AST, ALT and ALKP enzymes were significantly different in the control and control groups (20).

In a study that was conducted in 2018 with the title of the role of borage oil and fish oil in the prevention of cardiovascular disease and fatty liver in mice. In this study, the effect of borage extract and fish oil on dyslipidemia, oxidative stress investigated inflammation and fatty liver as a risk factor for cardiovascular disease. The results showed that the borage extract and fish oil protect against cardiovascular disease and fatty liver to different degrees. The borage extract contains GLA, which is The body is transformed into di homo-gamma-linolenic acid, which is the precursor of prostaglandin and leukotriene. It has anti-inflammatory and anti-thrombosis effects. GLA reduces total cholesterol, triglycerides, and liver fat. It promotes the activity of carnitine palmitoyl transferase and proxymom beta oxidation. The result of which is an increase in beta oxidation of liver fat and a decrease in fat in the liver (10). In a study conducted in 2019 by Mohamad ALHILAL et al. entitled the effect of borage oil on high-fat diet, it led to liver damage in male Syrian golden mice. In this study, which aimed to investigate the effect of borage flower supplement (GLA gamma linolenic acid) on the liver damage of mice in 2 periods. In the first period, it lasted for 4 weeks, and the mice were divided into 4 groups. Group 1: as a control group with a normal diet and groups 2, 3 and 4 with food that contains 80% of normal food and 20% of high-fat food. In the next period, which lasted 4 weeks, group 2 high-fat diet, group 4

gavage of borage flower extract at the rate of gr/kg² was performed. The results of the study showed that fat accumulation in hepatocytes increases the level of GGT, LDH, ALP, AST, ALT, and CK enzymes. The group consuming borage oil did not have these negative effects (15).

The results of the present study showed that 8 weeks of supplementing with borage extract alone reduced the serum level of triglyceride, AST and ALT, and also 8 weeks of aerobic exercise with borage extract decreased the total cholesterol serum level in rats. A desenter was diagnosed with non-alcoholic fatty liver disease. The possible mechanism of improving liver function is that the borage extract reduces liver steatosis and improves inflammation in liver tissue due to its antioxidants. Therefore, from this result, it can be concluded that the method of aerobic exercise which is the cornerstone of the control and management of non-alcoholic fatty liver, and the consumption

of borage extract can probably be useful for people with NAFLD, but to obtain more complete and definitive results to more studies and human samples are needed in this field.

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Conflict of interest

Author declares no conflict of interest.

References:

1. Duck-Pil Ok, Kangeun Ko and Ju Yong Bae. Exercise without dietary changes alleviates nonalcoholic fatty liver disease without weight loss benefits. *Lipids in Health and Disease* (2018) 17:207 <https://doi.org/10.1186/s12944-018-0852-z>.
2. Ammar Salehisahlabadi, Samaneh Sadat, Ali Lotfi, Maryam Mohseni, Hussein Jadidi. prevalence-of-non-alcoholic-fatty-liver-disease-in-iran-a-population-based-study. *J Biochem Tech* (2018) Special Issue (2): 54-57 ISSN: 0974-2328.
3. Phunchai Charatcharoenwitthaya, Kraisingh Kuljirattitikal, Ophas Aksornchanya, Kusuma Chaiyasoot, Wimolrak Bandidniyamanon, and Natthinee Charatcharoenwitthaya. Moderate-Intensity Aerobic vs Resistance Exercise and Dietary Modification in Patients With Nonalcoholic Fatty Liver Disease: A Randomized Clinical Trial. *Clinical and Translational Gastroenterology*. 2021.
4. Ahmad Hematfar , Mohammad Ali Samavati Sharif , Yousef Valizadeh , Hojjatollah Siavoshy , Jafar Keihanshokouh. Effect of a Six-week Combined Aerobic and Resistance Exercise Training on Some Liver Function Parameters in Middle-aged Men with Non-alcoholic Fatty Liver Disease. *Scientific Journal of Hamadan University of Medical Sciences* No 85, Volume 24, Issue 3, Autumn 2017, Pages: 206-214. doi: 10.18869/acadpub.ajcm.24.3.206.
5. Ebrahim Banitalebi , Majid Mardaniyan Ghahfarrokhi , Mohammad Faramarzi , Samira Nasiri. Effect of a 10-week combined exercise training on new fatty liver markers in women with type 2 diabetes. *Journal of Shahid Sadoughi University of Medical Sciences*. Vol 26; NO 3 ; June 2018.
6. Tiego Aparecido Diniz, Edson Alvesde Lima Junior, Alexandre AbílioTeixeira, Luana Amorim Biondo, Lucas Ariel Fernandesda Rocha, Iuri Cordeiro Valadão, Loreana Sanches Silveira, CarolCabral-Santos, Camila Oliveirade Souza, José Cesar Rosa Neto. Aerobic training improves NAFLD markers and insulin resistance through AMPK-PPAR- α signaling in obese mice. *Life Sciences*. Volume 266, 1 February 2021, 118868. <https://doi.org/10.1016/j.lfs.2020.118868>.
7. Shelley E Keating, Jacob George, Nathan A Johnson. The benefits of exercise for patients with nonalcoholic fatty liver disease. *Expert Review of Gastroenterology & Hepatology*, 2015, 9:10, 1247-1250, DOI: 10.1586/17474124.2015.1075392 .
8. Hong Yao, Yu-Jie Qiao, Ya-Li Zhao, Xu-Feng Tao, Li-Na Xu, Lian-Hong Yin, Yan Qi, Jin-Yong Peng. Herbal medicines and nonalcoholic fatty liver disease. *World J Gastroenterol* 2016 August 14; 22(30): 6890-6905. DOI: 10.3748/wjg.v22.i30.6890.
9. Amir Haj Ghasem , Parvin Farzanegi , Zohreh Mazaheri. Effects of combined therapy with resveratrol, continuous and interval exercises on apoptosis and lipid profiles in the liver of rats with nonalcoholic fatty liver disease. *Koomesh*. 2019; 21(3): 540-548.
10. Sahar Y. Al-Okbi, Salah M. El-qousy, Samah El-Ghlban, and Hosam F. Moawad. Role of Borage Seed Oil and Fish Oil with or without Turmeric and Alpha- Tocopherol in Prevention of Cardiovascular Disease and Fatty Liver in Rats. *Journal of Oleo Science*. 67, (12) 1551-1562 (2018). doi : 10.5650/jos.ess18064.
11. Swapnil V. Shewale , Elena Boudyguina, Xuewei Zhu , Lulu Shen , Patrick M. Hutchins, Robert M. Barkley , Robert C. Murphy , and John S. Parks. Botanical oils enriched in n-6 and n-3 FADS2 products are equally effective in preventing atherosclerosis and fatty liver. *Journal of Lipid Research* Volume 56, 2015.
12. Hala A.H. Khattab , Inas Z.A. Abdallah , Fatimah M. Yousef and Etimad A. Huwait. EFFICIENCY OF BORAGE SEEDS OIL AGAINST GAMMA IRRADIATION-INDUCED HEPATOTOXICITY IN MALE RATS: POSSIBLE ANTIOXIDANT ACTIVITY. *Afr J Tradit Complement Altern Med.*, (2017) 14 (4): 169-179 <https://doi.org/10.21010/ajtcam.v14i4.20>.
13. Zinvand Lorestani A , Mirnasuri R, Rahmati M. The effect of eight weeks of aerobic training on the levels of enzymes associated with non-alcoholic fatty liver in obese children. *Yafte*. 2018; 20 (2).
14. Elisa Barrón-Cabrera , Raúl Soria-Rodríguez , Fernando Amador-Lara and Erika Martínez-López. Physical Activity Protocols in Non-Alcoholic Fatty Liver Disease Management: A Systematic Review of

- Randomized Clinical Trials and Animal Models. *Healthcare* 2023, 11, 1992. <https://doi.org/10.3390/healthcare11141992>.
15. Mohammad ALHILAL, Suzan ALHILAL, Thana ALBAZ. The Effects of Borage Oil on Hyperlipidemic Diet-induced Liver Damage in Male Golden Syrian Hamsters. *Araştırma Makalesi*, 2019; 14(3): 252-262. DOI: 10.17094/ataunivbd.505675.
16. Cheraghi J, Kimiagar M, Pilevarian A. The Effects of Hydroalcoholic Borage Extract on Serum Lipid Profile in Mice and Comparison with Lovastatin. *Scientific Journal of Ilam University of Medical Sciences*. 2016; 24(3).
17. Monireh Ranjbar and Nilufar Motiee. Comparative and Combined Effects of Echium amoenum and Cichorium intybus Extracts on the Live of Wista Rats. *Journal of Medicinal Plants and By-products*. *Journal of Medicinal Plants and By-products* (2023) 4: 495-503. <https://doi.org/10.22092/jmpb.2022.356065.1403>.
18. Pooya Shokati, Hoseyn Fatollahi. The effect of six weeks of aerobic exercise with pomegranate peel extract on liver enzymes of obese female rats. *Journal of Torbat Heydariyeh University of Medical Sciences* 2022, Volume 10, No. 2: 13-24.
19. Labenz C., Huber Y., Kalliga E., Nagel M., Ruckes C., Straub B.K., Galle P.R., Wörns M.A., Anstee Q.M., Schuppan D., et al. Predictors of Advanced Fibrosis in Non-Cirrhotic Non-Alcoholic Fatty Liver Disease in Germany. *Aliment. Pharmacol. Ther.* 2018;48:1109–1116. doi: 10.1111/apt.14976.
20. Monika Michalak, Martyna Zagórska-Dziok, Marta Klimek-Szczykutowicz, and Agnieszka Szopa. Phenolic Profile and Comparison of the Antioxidant, Anti-Ageing, Anti-Inflammatory, and Protective Activities of Borago officinalis Extracts on Skin Cells. *Molecules*. 2023 Jan; 28(2): 868. doi: 10.3390/molecules28020868.
21. Shewale, S.V.; Brown, A.L.; Bi, X.; Boudyguina, E.; Sawyer, J.K.; Alexander-Miller, M.A.; Parks J.S. In vivo activation of leukocyte GPR120/FFAR4 by PUFAs has minimal impact on atherosclerosis in LDL receptor knockout mice. *J. Lipid Res.* 58, 236-246 (2017).
22. Alireza Bazzi Allahri, somayeh Bagheri, Fatemeh Bazzi Allahri, Raheleh Rahbarian, Mohammadreza Shadan, Fahimeh K. houshabi. The Effect Of Borage Plant Extract On Liver Enzyme Levels In Patients With Type 2 Diabetes. *Journal of population therapeutics & clinical pharmacology (JPTCP)*. Vol 30 No.18(2023): (270-283).