Effect of Total Anthocyanin-base Standardized Cornus Mas L. Fruit Extract on Hepatic Steatosis and Visceral Adiposity Index in Patients With Non-alcoholic Fatty Liver Disease: a Double-blind Randomized Controlled Trial

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Research

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Abstract

Background:

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide. Experimental evidence has proposed the beneficial effects of *Cornus mas L.* (cornelian cherry) extract, as a rich source of anthocyanins, on steatosis and central obesity. However, very few clinical trials were conducted in this regard. We investigated the effect of total anthocyanin-base standardized cornelian cherry fruit extract on hepatic steatosis index (HSI) and NAFLD liver fat score (NAFLD-LFS), and visceral adiposity index (VAI) in patients with NAFLD.

Methods:

The present study was conducted as a double-blind, randomized controlled clinical trial among 50 subjects suffering from NAFLD. Subjects were randomly assigned to receive cornelian cherry extract (20 cc/d, which provides 320 mg/d total anthocyanin) or placebo for 12 weeks.

Results:

There was no difference between the two groups in HSI, NAFLD-LFS and VAI at the baseline. After 12-week intervention, cornelian cherry extract compared to the placebo demonstrated no significant effect on HSI (change: -0.9 ± 3.5 vs. -1.2 ± 3.2; P = 0.72), NAFLD-LFS (change: 0.2 ± 1.3 vs. 0.1 ± 0.7; P = 0.62), and VAI (change: 0.05 ± 0.7 vs. 0.07 ± 1.0; P = 0.94).

Conclusion:

Cornelian cherry extract (20 cc/d) for 12 weeks has no effect on steatosis and visceral obesity. To better conclude, further trials with longer intervention durations are required.

Trial registration:

The study protocol was registered on 30 September 2018 at Iranian Registry of Clinical Trials under code IRCT2018041903935N1 (https://www.irct.ir/trial/30707).
NAFLD (7). Some validated simple tests such as hepatic steatosis index (HSI), and NAFLD liver fat score (NAFLD-LFS) have been developed to evaluate and estimate the severity of hepatic steatosis based on laboratory markers and anthropometric parameters (8–10). Insulin resistance, dyslipidemia and obesity are the most important causes of NAFLD (11, 12). The visceral adiposity index (VAI) is an accurate and sensitive marker of cardiovascular risk factors such as insulin resistance and central obesity (13).

The recent evidence suggested that the flavonoid compounds with antioxidant and anti-inflammatory properties such as anthocyanins may have beneficial effects on features of NAFLD (14–16). Fruits and vegetables are the main sources of anthocyanins (17). *Cornus mas L.* (cornelian cherry) fruit is one of the richest sources of anthocyanins (18). Some in vitro experiments have demonstrated that anthocyanins via phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and inhibition of lipid biosynthesis as well as triglyceride (TG) accumulation in hepatic cells and promotion of hepatic lipid clearance can attenuate the development of hepatic steatosis (19, 20). In addition, cornelian cherry can ameliorate main risk factors of NAFLD such as insulin resistance, dyslipidemia and oxidative stress in mice (21, 22). Clinical trials investigating the effects of cornelian cherry on features of NAFLD are scarce (23). On the other hand, animal studies have suggested that rich sources of anthocyanins can improve cardiovascular risk factors such as dyslipidemia and obesity (24, 25). The clinical effects of anthocyanins on dyslipidemia and obesity are inconsistent (16, 26–30).

Therefore, due to limited number of clinical trials examining the effect of cornelian cherry on the severity of NAFLD and fat accumulation in patients with NAFLD, and lack of trials evaluating cornelian cherry/anthocyanins on above-mentioned indices, the present study was designed to investigate the effect of total anthocyanin-base standardized cornelian cherry extract on HSI, NAFLD-LFS and VAI in patients with NAFLD.

**Methods**

**Recruitment and eligibility screening**

A total of 50 Patients with NAFLD were recruited from gastroenterology clinics affiliated with Diabetes Research Center and Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The inclusion criteria were as follows: age 25–65 years, alanine aminotransferase (ALT) levels of higher than 30 U/L in men and higher than 19 U/L in women, NAFLD diagnosed by a gastroenterologist, resident of Yazd city, and written consent signed by the patient. The exclusion criteria were as follows: history of alcohol abuse (an average daily alcohol consumption of ≥ 10 g for women and ≥ 20 g for men), viral hepatitis, liver cancer, psychological disorders, pregnancy, lactation, taking corticosteroids, non-steroidal anti-inflammatory drugs, hypoglycemic drugs, tamoxifen, sodium valproate, methotrexate, amiodarone, anti-retroviral agents for HIV, probiotics, antioxidant and anti-inflammatory supplements (such as vitamin D, vitamin E, omega-3, and resveratrol) and unwillingness to continue the study.
Study design

This study was conducted as a double-blind placebo-controlled clinical trial for 12 weeks. We investigated the effect of cornelian cherry fruit extract on HSI, NAFLD-LFS and VAI in patients with NAFLD. Before signing the informed written consent, the participant was notified about the risks and benefits of the study. The protocol was approved by the ethical committee of Shahid Sadoughi University of Medical Sciences and Health Services in Yazd (IR.SSU.SPH.REC.1400.020). The registration of the study protocol was performed on 30/09/2018 at the Iranian clinical trials website (http://www.irct.ir), under code: IRCT20180419039359N1 (https://www.irct.ir/trial/30707).

Intervention

The intervention group received 20 cc/d cornelian cherry extract, which provides 320 mg/d total anthocyanin. Preparing placebo in the same appearance, color, and texture as the cornelian cherry extract was performed by the Pharmacy Faculty of Shahid Sadoughi University of Medical Sciences. The cornelian cherry extract and the placebo were packed in containers with the same color, shape, and size. The bottles containing the extract or placebo as were labeled as A or B by a person who was unaware about the trial details.

Preparation of extract

From the forests of Ghazvin, Iran, fresh cornelian cherry fruits were provided in November 2020 and frozen at -18°C. Assessment of the fruits’ authenticity was performed in the Department of Pharmacognosy, School of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, based on their voucher number (SSU0029). Preparation of the cornelian cherry extract was performed based on the standard protocols (31). Determining the total anthocyanin content of the obtained extract was performed based on pH differential method (32). The microbiological assessments for the final extract were done. The placebo was provided using purified water and red color such as carmoisine color similar to the extract in a similar container.

Randomization and blinding

Participants were divided and stratified based on age, gender, body mass index (BMI) and grade of fatty liver into two groups including intervention (cornelian cherry fruit extract, n = 25) and control (placebo, n = 25) by a person who was not involved in the study, using the computer-generated random numbers (33). The participants, investigators and laboratory staff were blinded to the treatment allocation. Randomization codes were unlocked only after all individuals completed the study.
**Compliance rate**

Cornelian cherry extract and placebo bottles were given to the participants every 2 weeks. Participants were asked to return the bottles with their remaining contents at the end of each 2 weeks. To evaluate the compliance rate, the consumption of cornelian cherry extract and placebo was monitored at the end of each 2 weeks of intervention. At the end of the intervention, the remaining contents of bottles were recorded for each participant. Consuming less than 80% of the administered extract or placebo was defined as poor compliance. Participants with poor compliance were excluded from the study and their data was not analyzed at the end of the study.

**Dietary intake, physical activity and anthropometric evaluations**

To evaluate dietary intake of subjects, a 3-day (1 weekend day and 2 nonconsecutive weekdays) food record was used at weeks 0 and 12. In addition, the short form of International Physical Activity Questionnaire (IPAQ) was utilized for assessment of physical activity at weeks 0 and 12. Height was measured in the standing position at weeks 0 and 12 using a Seca stadiometer with an accuracy of 0.5 cm. Measuring weight, and waist circumference (WC) as the important confounding factors was performed based on standard protocols with light clothes and without shoes by a Seca scale with an accuracy of 100 g. Using the following formula, BMI was calculated: weight (kg)/height (m)$^2$.

**Steatosis and visceral adiposity indices assessment**

HSI, NAFLD-LFS and VAI were calculated for each participant at weeks 0 and 12, based on the following formulas:

\[
\text{HSI} = 8 \times (\text{ALT/AST ratio}) + \text{BMI} + 2 \text{ (if female)} + 2 \text{ (if diabetes mellitus)} \quad (10).
\]

\[
\text{NAFLD-LFS} = -2.89 + 1.18 \times \text{metabolic syndrome (yes = 1; no = 0)} + 0.45 \times \text{type 2 diabetes (yes = 2; no = 0)} + 0.15 \times \text{fasting insulin (mU/L)} + 0.04 \times \text{AST (U/L)} - 0.94 \times \text{AST/ALT} \quad (8).
\]

\[
\text{VAI}_{\text{men}} = \left[ \frac{\text{WC}}{39.68} + (1.88 \times \text{BMI}) \right] \times \left( \frac{\text{TG}}{1.03} \right) \times \left( \frac{1.52}{\text{HDL-c}} \right) \quad (13).
\]

\[
\text{VAI}_{\text{women}} = \left[ \frac{\text{WC}}{36.58} + (1.89 \times \text{BMI}) \right] \times \left( \frac{\text{TG}}{0.81} \right) \times \left( \frac{1.31}{\text{HDL-c}} \right) \quad (13).
\]

**Laboratory assessments**

Laboratory assessments was performed at weeks 0 and 12. 10 cc blood was drawn after 12 hours fasting and centrifuged for 10 minutes at a speed of 3600 rpm. Serum samples poured into the microtubes were immediately frozen at -75° C. ALT, aspartate aminotransferase (AST), total cholesterol
(TC), TG, low density lipoprotein-cholesterol (LDL-c) and high density lipoprotein-cholesterol (HDL-c) were measured by routine enzymatic assays with Pars Azmoon, Iran, kits using an autoanalyzer. Measuring insulin was performed by ELISA reader (Epoch, Bio Teck, USA) utilizing Monobind, USA, kit. Measurements were performed based on standard methods in laboratory of Nutrition Department, Yazd, Iran.

**Statistical analysis**

Based on the study of Sangsefidi et al. (23), with \( \alpha = 0.05 \), power = 80%, and considering 10% drop-out rate, the optimal sample size was estimated to be 25 per group. SPSS version 24 (SPSS, Inc.) was used for data analysis. Using Kolmogorov–Smirnov test, the normal distribution of variables was assessed. Comparing the qualitative variables between two groups was performed using Chi-Squared test. To compare the means of normal variables at baseline, at the end of study, and compare the mean changes of normal data between two groups, an independent t-test was utilized. Mann-Whitney U test was used to compare abnormal data between the two groups at baseline and after the intervention as well as comparing the mean changes of abnormal data between two groups. Paired t-test was utilized to compare the normal variables in each group, and Wilcoxon test was utilized to compare the abnormal data in each group. P value < 0.05 was considered significant.

**Results**

**Characteristics of the participants**

Fifty subjects were assigned into two groups. During the follow-up, ten participants were excluded due to gastrointestinal symptoms such as flatulence (n = 1), immigration (n = 2), participant’s personal decision to discontinue the study (n = 3), performance of surgery (n = 1), and incidence of corona virus (Covid 19) pandemic (n = 3). Finally, 40 subjects completed the study (cornelian cherry group: n = 22, placebo group: n = 18). No serious adverse events were reported by the participants. However, only one participant in the cornelian cherry group reported flatulence; this subjects had a history of gastrointestinal disorder, namely flatulence (Fig. 1). There was no significant difference between the two groups in the baseline variables (Table 1). In addition, at baseline as well as the end of the study, there was no significant difference between the two groups in confounding factors such as dietary intakes and physical activity (Table 2).
Table 1  
Baseline characteristics of patients with NAFLD

|                          | Comelian cherry (n = 25) | Placebo (n = 25) | P     |
|--------------------------|--------------------------|------------------|-------|
| **Age, y**               | 41.4 ± 9.5               | 42.6 ± 9.9       | 0.66  |
| **Gender**               |                          |                  | 0.77* |
| Male, n (%)              | 12 (48)                  | 11 (44)          |       |
| Female, n (%)            | 13 (52)                  | 14 (56)          |       |
| **Height, cm**           | 168.0 ± 11.2             | 164.4 ± 9.6      | 0.23  |
| **Weight, kg**           | 79.5 ± 12.6              | 81.1 ± 12.9      | 0.66  |
| **BMI, kg/m^2**          | 28.1 ± 3.5               | 29.9 ± 4.0       | 0.10  |
| **WC, cm**               | 97.6 ± 9.1               | 101.1 ± 9.3      | 0.18  |
| **HDL-c, mg/dL**         | 39.4 ± 7.3               | 40.5 ± 10.2      | 0.67  |
| **TG, mg/dL**            | 180.8 ± 59.7             | 161.9 ± 66.1     | 0.29  |
| **ALT, U/L**             | 32.9 ± 18.7              | 33.0 ± 32.5      | 0.98  |
| **AST, U/L**             | 22.5 ± 8.6               | 23.1 ± 13.6      | 0.84  |
| **Insulin, mU/L**        | 15.9 ± 5.4               | 16.1 ± 5.7       | 0.85  |
| **HSI**                  | 40.7 ± 5.6               | 41.6 ± 5.9       | 0.56  |
| **NAFLD-LFS**            | 1.8 ± 1.1                | 1.9 ± 1.3        | 0.80  |
| **VAI**                  | 3.49 ± 1.4               | 3.16 ± 1.5       | 0.45  |

P values are computed by independent t-test and data are expressed as mean ± standard deviation (SD).

* Chi square.

NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; WC: waist circumference; HDL-c: high density lipoprotein-cholesterol; TG: triglyceride; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HSI: hepatic steatosis index; NAFLD-LFS: NAFLD liver fat score; VAI: visceral adiposity index.
| variables                     | Cornelian cherry (n = 25) | Placebo (n = 25) | $p^{†}$ |
|------------------------------|---------------------------|-----------------|--------|
| **Energy intake, kcal/d**    |                           |                 |        |
| Baseline                     | 2960.80 ± 885.15          | 2576.24 ± 617.81| 0.11   |
| Week 12                      | 2943.50 ± 758.81          | 2635.78 ± 621.91| 0.16   |
| P                            | 0.85                      | 0.31            |        |
| **Carbohydrates, g/d**       |                           |                 |        |
| Baseline                     | 425.08 ± 185.33           | 375.33 ± 128.80 | 0.32   |
| Week 12                      | 460.46 ± 172.58           | 379.39 ± 132.36 | 0.10   |
| P                            | 0.32                      | 0.80            |        |
| **Proteins, g/d**            |                           |                 |        |
| Baseline                     | 95.25 (83.30 to 120.22)   | 88.36 (67.44 to 99.17) | 0.14*  |
| Week 12                      | 93.99 (81.58 to 134.23)   | 96.34 (74.94 to 113.18) | 0.48*  |
| P                            | 0.83                      | 0.19            |        |
| **Fats, g/d**                |                           |                 |        |
| Baseline                     | 93.18 (54.68 to 156.09)   | 69.37 (53.85 to 106.85) | 0.23*  |
| Week 12                      | 83.46 (59.56 to 99.97)    | 72.12 (61.25 to 100.25) | 0.76*  |
| P                            | 0.30                      | 0.45            |        |
| **Physical activity, (MET/hr/week)** |                   |                 |        |
| Baseline                     | 660.45 (307.12 to 791.25) | 341.5 (0 to 1705.50) | 0.75*  |
| Week 12                      | 850.50 (111.37 to 881.29) | 283.75 (0 to 1234.12) | 0.49*  |
| P                            | 0.31                      | 0.28            |        |

Values of total energy and carbohydrates were presented as mean ± standard deviation (SD), while for proteins, fats and physical activity were presented as median and quartile range.

$P$: resulted from comparisons within groups.

$P^{†}$: resulted from comparisons between two groups.

*P values were computed by Mann-Whitney U test.

**P values were computed by Wilcoxon test.

NAFLD: non-alcoholic fatty liver disease.
Outcomes

At the baseline, there was no significant difference between the cornelian cherry extract and placebo groups in the terms of HSI, NAFLD-LFS and VAI. In addition, no significant difference was observed between the two groups in the terms of HSI (P = 0.56), NAFLD-LFS (P = 0.31) and VAI (P = 0.45) (Table 3).

Table 3
Effect of cornelian cherry extract on indices in patients with NAFLD*

| Indices          | Cornelian cherry (n = 25) | Placebo (n = 25) | \( P^† \) |
|------------------|--------------------------|-----------------|----------|
| **HSI**          |                          |                 |          |
| Baseline         | 40.7 ± 5.6               | 41.6 ± 5.9      | 0.56     |
| Week 12          | 39.8 ± 4.8               | 40.4 ± 4.6      | 0.64     |
| \( P \)          | 0.25                     | 0.08            |          |
| Mean change of HSI | -0.9 ± 3.5              | -1.2 ± 3.2      | 0.72     |
| **NAFLD-LFS**    |                          |                 |          |
| Baseline         | 1.8 ± 1.1                | 1.9 ± 1.3       | 0.80     |
| Week 12          | 2.0 ± 1.8                | 2.0 ± 1.5       | 0.98     |
| \( P \)          | 0.49                     | 0.75            |          |
| Mean change of NAFLD-LFS | 0.2 ± 1.3       | 0.1 ± 0.7       | 0.62     |
| **VAI**          |                          |                 |          |
| Baseline         | 3.49 ± 1.4               | 3.16 ± 1.5      | 0.45     |
| Week 12          | 3.54 ± 1.6               | 3.23 ± 1.4      | 0.85     |
| \( P \)          | 0.76                     | 0.74            |          |
| Mean change of VAI | 0.05 ± 0.7              | 0.07 ± 1.0      | 0.94     |

Data are expressed as mean ± standard deviation (SD).

\( P \): resulted from comparisons within groups by paired t-test.

\( P^† \): resulted from comparisons between two groups by independent t-test.

NAFLD: non-alcoholic fatty liver disease; HSI: hepatic steatosis index; NAFLD-LFS: NAFLD liver fat score; VAI: visceral adiposity index.
According to the mean changes after 12-week intervention, the cornelian cherry extract compared to the placebo had no significant impact on HSI (-0.9 ± 3.5 vs. -1.2 ± 3.2; P = 0.72), NAFLD-LFS (0.2 ± 1.3 vs. 0.1 ± 0.7; P = 0.62) and VAI (0.05 ± 0.7 vs. 0.07 ± 1.0; P = 0.94) (Table 3).

Discussion

The worldwide prevalence of NAFLD is increasing, and has become a main problem in the health care (3, 4). Currently, there is no approved pharmacological treatment in this regard (34), and the main strategy to manage NAFLD is lifestyle modification including adherence to the healthy dietary patterns, weight loss, and body exercise (34, 35). Recent evidence suggested that rich sources of anthocyanins due to their antioxidant and anti-inflammatory properties can have a therapeutic effect in the management of NAFLD (14, 15, 21, 25). We investigated the effect of cornelian cherry extract as the one of the richest sources of anthocyanins on steatosis indices (HSI and NAFLD-LFS) and VAI in patients with NAFLD. The present study demonstrated that intake of cornelian cherry extract (20 cc/d, providing 320 mg anthocyanins/d) for 12 weeks has no beneficial effect on HSI, NAFLD-LFS and VAI indices in patients with NAFLD.

As mentioned, liver biopsy as the gold standard method to detect the severity of NAFLD is an invasive test (6). On the other hand, non-invasive methods like magnetic resonance imaging and ultrasonography are expensive (7, 36, 37). Therefore, investigators designed and developed valid, non-invasive and inexpensive tools like HSI and NAFLD-LFS indices to estimate and evaluate the severity of NAFLD (7, 8, 10). There are limited number of clinical trials examining the effect of cornelian cherry on hepatic steatosis. Consistent with our findings, the study of Sangsefidi et al. (23) reported that intake of cornelian cherry extract for 3 months has no therapeutic effect on hepatic steatosis, and levels of liver enzymes such as ALT and AST; however, compared to the cornelian cherry group the fibrosis score significantly increased in the placebo group. Another study that conducted among hyperlipidemic adult patients demonstrated that intake of Vaccinium arctostaphylos L. fruit, as a rich source of anthocyanins, had no significant effect on ALT and AST levels (38). In addition, clinical trials evaluating the effects of other sources of anthocyanins such as Hibiscus sabdariffa extract (27) and barberry juice (39) on liver enzymes in patients with NAFLD showed findings similar to our results. Furthermore, Qin et al. (28), and Yang et al. (40) found that pure anthocyanins has no significant effect on liver enzymes in dyslipidemic and prediabetic subjects, respectively. On the other hand, the trial of Zhang et al. (41) that conducted among patients with NAFLD demonstrated that fibrosis score was higher in the placebo group compared with who consumed pure anthocyanins for 12 weeks. In addition, the study of Zhang et al. (41) showed that pure anthocyanins supplementation can decrease levels of ALT; however, AST remained without significant change. Moreover, based on the study of Stote et al. (26) that conducted among patients with NAFLD, intake of freeze-dried blueberries, as a rich source of anthocyanins can improve levels of liver enzymes. Some discrepancies in the results can be due to the differences in the health status of participants, duration of the study, and sources as well as dosages of anthocyanins. The exact mechanisms of cornelian cherry/anthocyanins in this field are still unclear. The early evidence suggested that anthocyanins by inhibiting nuclear factor-κ-B (NF-κB) signaling pathways, modulating gene expression of inflammatory markers such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) can
reduce cellular inflammation as an important factor involved in the pathogenesis of NAFLD (15, 39, 42, 43). On the other hand, anthocyanins by reducing reactive oxygen species and hepatic lipid peroxidation, as well as increasing the activity of antioxidant enzymes can improve oxidative stress (15, 38, 44). Generally, the therapeutic effects of cornelian cherry are attributed to their anti-inflammatory and antioxidant properties (15, 45). Inflammation and oxidative stress are the main contributors in the progression of steatosis to fibrosis (12, 46, 47). Based on the mentioned mechanisms as well as the clinical evidence, anthocyanins may have a beneficial effect on fibrosis, but not steatosis. However, further clinical trials in this field are required to make a correct conclusion.

Our study demonstrated that intake of cornelian cherry extract has no impact on VAI. VAI is an accurate and sensitive indicator of cardiovascular risk factors such as insulin resistance and visceral obesity (13). There is no clinical trial evaluating the effect of cornelian cherry/anthocyanins on VAI. This index is based on anthropometric variables and lipid profile (13). Results of the studies that investigated the effect of cornelian cherry/anthocyanins on anthropometric variables and lipid profile are not integrated. The study of Asgary et al. (30) demonstrated that intake of cornelian cherry for 6 weeks has no significant effect on lipid profile in subjects with dyslipidemia. In addition, the study of Lee et al. (48) reported that 12-week consumption of cranberry can't reduce WC in patients with T2DM. However, two studies found that supplementation with anthocyanins for 24 weeks showed some lipid-modifying effects in subjects with dyslipidemia (44, 49). It seems, the main reason of this inconsistency between findings is difference in the duration of follow-up. Studies with longer-term interventions showed beneficial effect of anthocyanins on dyslipidemia. Anthocyanins may improve dyslipidemia by mechanisms such as modulation of β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase activity, inhibition of cholesteryl ester transfer protein (CEPT), and reduction of the apolipoprotein B and apolipoprotein C-III–lipoprotein, which are TG transporters (15, 16, 28, 42). On the other hand, Asgari et al. (30) demonstrated that 6-week intake of cornelian cherry has no effect on body composition. In addition, based on the study of Yarahmadi et al. (50), 6-week anthocyanin supplementation can't decrease percent body fat. However, the studies of Basu et al. (51), and Gholamrezayi et al. (29) found a significant decrease in WC of their intervention groups after intake of cranberry juice and cornelian cherry, respectively. Based on the experimental studies, anthocyanins can increase the expression of peroxisome proliferator-activated receptor (PPAR) alpha and delta, regulate the adipose tissue, modulate the gene expression of adipocytokines, and increase levels of adiponectin (14). In addition, anthocyanins by inhibiting the activity of the pancreatic lipase, and decreasing intestinal fat absorption can reduce visceral fat accumulation (14, 19).

The present study had some strengths. For the first time we used accurate, simple and inexpensive indices such as HSI and NAFLD-LFS to evaluate the effect of cornelian cherry extract on hepatic steatosis in patients with NAFLD. In addition, the cornelian cherry extract was standardized based on its total anthocyanin content. Moreover, diagnose of NAFLD was performed by fibroscan, which has higher accuracy than ultrasonography. Furthermore, several confounding variables such as anthropometric indices, dietary intakes, physical activity, and baseline values of variables were controlled. However, the present trial had some limitations such as small sample size, and short duration of follow-up.
Conclusion

In conclusion, we found that intake of 20 cc/d cornelian cherry extract for 12 weeks has no effect on steatosis and cardiovascular risk. To perform a correct as well as comprehensive conclusion, longer-term interventions investigating the effect of cornelian cherry on features of NAFLD are needed.

Abbreviations

ALT: alanine aminotransferase; AMPK: adenosine monophosphate-activated protein kinase; AST: aspartate aminotransferase; BMI: body mass index; CEPT: cholesteryl ester transfer protein; CVD: cardiovascular disease; GGT: γ-glutamyltransferase; HDL-c: high density lipoprotein-cholesterol; HMG-CoA: β-Hydroxy β-methylglutaryl-CoA; HSI: hepatic steatosis index; IL-6: interleukin-6; IPAQ: international physical activity questionnaire; LDL-c: low density lipoprotein-cholesterol; MET-h: metabolic equivalent task hours; NAFLD: non-alcoholic fatty liver disease; NAFLD-LFS: NAFLD liver fat score; NASH: non-alcoholic steatohepatitis; NF-κB: nuclear factor κ-B; PPAR: peroxisome proliferator-activated receptor; T2DM: type 2 diabetes mellitus; TG: triglyceride; TNF-α: tumor necrosis factor-α; VAI: visceral adiposity index; VLDL: very low density lipoprotein; WC: waist circumference.

Declarations

Ethics approval and consent to participate

The research council of Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences and Health Services approved the study protocol. The ethical committee of Shahid Sadoughi University of Medical Sciences and Health Services in Yazd approved the written informed consent (IR.SSU.SPH.REC.1400.020).

Consent to publish

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors have declared no competing interests.
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Authors' contributions

H.M-Kh, M.H, M.A-M and A.R: designed the study; A.A, Z.S and F.Y: conducted the research; F.M and A.S: analyzed the data; A.S: wrote the manuscript; H.M-Kh: critically revised the manuscript. All authors approved the final manuscript.

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Figures

Figure 1
flow chart of eligibility, screening, and follow-up.

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