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The therapeutic effects of chicory seed aqueous extract on cardio-metabolic profile and liver enzymes in nonalcoholic fatty liver disease; a double blind randomized clinical trial

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Abstract

Introduction: As diabetes and obesity increase worldwide, non-alcoholic fatty liver disease (NAFLD) becomes a more worrying health threat.
Objectives: This study was designed to assess the impacts of chicory seed aqueous extract on the treatment of NAFLD.
Patients and Methods: Around 60 patients with NAFLD who referred to Zanjan Metabolic Disease Research Center between March 2016 and April 2017 were allocated into treatment and placebo groups, randomly. The participants in the treatment group (n=30) were scheduled to consume 8 ml of chicory seed syrup (made by soaking 100 g of dried seeds in one liter of boiling water) twice daily for 12 weeks. The control group's patients received placebo syrup with an identical figure and flavor and also with the same prescript. The analysis of covariance (ANCOVA) test, chi-square test, independent t-test and paired t test were applied for analysis of data. From the point of statistical view, $P < 0.05$ was considered significant.
Results: The body mass index (BMI), liver enzymes, fasting blood sugar test (FBS), HbA1c, fatty liver grading and all the indices of lipid profile decreased significantly (all P values < 0.001) in the chicory group. The shifting change in fatty liver grading was also striking in treatment group's patients compared to controls.
Conclusion: The findings of this study support the application of chicory seed in the treatment of NAFLD due to the ameliorative effects on the metabolic indices of the disease.
Trial Registration: This clinical trial protocol has been registered in Iranian Registry of Clinical Trial (identifier: IRCT2016061228411N1; https://en.irct.ir/trial/23063, ethical code #IR.ZUMS.REC.1395.195).

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Introduction

As the incidence of diabetes and obesity increases, non-alcoholic fatty liver disease (NAFLD) becomes a more prevalent reason for chronic liver disease leading to a significant number of morbidity and mortality such as cirrhosis, liver cancer and liver mortality (1). NAFLD affects up to 25% of the whole population worldwide mostly associated with male gender, old age, diabetes, metabolic syndrome, hypertension, high serum alanine transaminase (ALT) and hypertriglyceridemia (2). According to Iranian traditional medicine, liver dysfunction can also disrupt the balance in humour, creating a variety of Sū-e-mizaj, such as melancholic, sanguine, phlegmatic or choleric, or abnormal humour, which in turn can lead to a kind of somatic disease (3).

The current therapies for NAFLD are mostly directed to improving insulin sensitivity, whether by means of medications or by lifestyle modifications such as weight reduction, dietary changes and increased physical activity; however, no definitive therapy has been ever established (4). Unfortunately, if these strategies do not respond, liver transplantation is the only remained option for patients affected by advanced liver disease (5). In recent decades, complementary and alternative medicine, especially herbal therapy, has emerged as a therapeutic option for patients with liver diseases (6,7). For centuries, chicory (*Chicorium intybus*), known in Iran as Kasni, has been a universal herb

Key point

This study revealed the positive effects of chicory on cardio-metabolic profile and liver enzymes in patients affected by NAFLD.

used by various nations due to a wide range of therapeutic effects (8). Herbal dietary supplements containing chicory have been also shown to have hepatoprotective effects in rats in different settings (9,10).

Objectives

The rarity of reports studying the effects of chicory in humans with NAFLD and the need to find a more potent treatment provoked our interest to be directed into this field and therefore, this study was designed to evaluate the impacts of chicory seed aqueous extract on liver enzymes and lipid profile in the treatment of NAFLD patients.

Participants and Methods

Study design and participants

This study was a double blind, placebo-controlled, randomized clinical trial carried out on patients with NAFLD. All patients who were referred to our center (Metabolic Disease Research Center, Vali-e-Asr hospital, Zanjan university of medical sciences, Zanjan, Iran) between March 2016 and April 2017 and met the criteria for inclusion were entered in the trial after reading and then signing an informed consent's form. The NAFLD diagnosis was confirmed by a radiologist observing evidence of hepatic steatosis using ultrasonography and also by lack of secondary causes of fat accumulation (11). Therefore, the inclusion criteria were age 18-65 years, evidence of ultrasonographic fatty liver, body mass index (BMI) between 18-35 kg/m^2, and absence of taking medications affecting fatty liver. Those with history of pregnancy, lactation, alcohol consumption, diabetes mellitus, hypersensitivity to chicory, malignancy, any otherwise acute or chronic liver disease, hepatotoxic drugs' usage during the recent six months, hypo or hyperthyroidism and also those with unwillingness to participate in the study were excluded.

The participants were then randomly distributed into two groups; treatment group and placebo one. The 1:1 randomization was generated by allocation software as a non-stratified list using a block size of four. The participants and researchers were both blind to the treatment and control groups. The participants of treatment group were scheduled to consume 8 mL of herbal syrup before eating breakfast and also at bedtime over a time period of 12 weeks based on the dosage prescribed in "Physicians' Desk Reference (PDR) for herbal medicines" (12). The control patients received identically prepared placebo syrup with the same prescript. None of the participants did know which syrup was prescribed for them. They all continued their previous diet and physical activity during the study. The nature of syrups was also maintained unknown to health-care workers who directly gave medications to participants and the researchers did know nothing about the status of medication delivery.

Study protocol

Dried chicory seeds were bought from a regional market in Tehran, Iran. A botanist identified the herbal sample at the Shahid Beheshti school of pharmacy, Tehran, Iran with a specified voucher sample. The type of herbal drug applied in this study was in the form of syrup originated from *Chicorium intybus* seeds. The production of the *C. intybus* seed syrup and also placebo one was performed in the herbal medicine laboratory of the Shahid Beheshti school of pharmacy, Tehran, Iran. According to the conceptual traditional method, 100 g of dried seeds were soaked in one liter of boiling water and maintained in a closed holder for four hours. The extract was precisely filtered and then concentrated via Ben-Murray method. Around 50% sugary solution was mixed with the mentioned extract in order to make a concentration of 50 mg of the herbal extract in each milliliter of the syrup. In parallel, the placebo was made by applying the pharmacopoeia simple syrup formula including standard colors and flavors and also looked the same as the *C. intybus* seed syrup. Then, both the drug and placebo syrup were coded. It should be noted that *C. intybus* seed syrup's standardization was based on total flavonoid content via gas chromatography–mass spectrometry (GC-MS)using rutin solution and aluminum chloride as standard control and reagent, respectively. The total flavonoid/rutin ratio was 7.13 and total flavonoid content was 0.356 mg/mL of the syrup. In addition, the standardization of syrup was conducted based on the total phenolic content of 2.305 mg/mL.

At the beginning and after 12 weeks, the blood pressure, height and weight of all participants were measured and charted. The measurement of body weight was conducted via a calibrated scale device to the nearest 100 g (Seca, Germany). A standard wall-fixed tape was used to measure the height in standing position. The formula; BMI = weight (kg)/height (m^2) was used to calculate the BMI. The blood samples of the participants were obtained from the antecubital vein after a fasting time period of 10-12 hours, first at baseline and then after 12 weeks, for measurement of liver enzymes, fasting blood sugar (FBS), hemoglobin $A1_C$ ($HbA1_C$), and lipid profile. The serum lipids and liver enzymes were measured by using a well-known enzyme assay (Pars Azmoon Co., Tehran, Iran) via Liasys auto-analyzer. Furthermore, any unfavorable (e.g. allergic) reaction was recorded during each follow-up visit. In order to assess the fatty liver, the same radiologist performed both the baseline and follow-up ultrasonography by means of a Philips IU22 machine at weeks 0 and 12. The grading of fatty liver on visual ultrasonographic analysis was categorized as follows:

- Grade 0: normal liver echogenicity

- Grade 1: increased liver echogenicity
- Grade 2: echogenic hepatic parenchyma hiding the echogenic walls of the portal venous branches
- Grade 3: fatty liver in which the diaphragmatic border is obscure (13).

Statistical analysis

Reviewing such previous studies (8) and considering a 0.05 two-sided significance level, 0.8-study power and 1/5 dropout ratio of the entered patients, the sample size was determined to be 30 people in each group using PASS 11 software and the following equation:

$n= (Z_{1-\alpha/2}+Z_{1-\beta})^2(S_1^2+S_2^2)/ (\mu_1-\mu_2)^2$

S_1: Standard deviation in cases

S_2: Standard deviation in controls

μ_1: Mean value in cases

μ_2: Mean value in controls

The gathered data were analyzed by means of SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA). The chi-square test was used to analyze the changes in steatosis grade. For other variables, the independent *t* test and paired *t* test were also used to compare the significant differences of the same variables between or within the case and control groups. To remove the confounding factors' effects, analysis of covariance (ANCOVA) test was applied and adjustment for age, gender, weight, ALT, aspartate transaminase (AST) and the basal value of each parameter was also considered. From the point of statistical view, the $P<0.05$ was noted as being significant.

Results

A total of 60 eligibility criteria-met individuals were divided into chicory group and placebo one via randomization. Of 56 participants who completed the course of treatment, one patient refused to undergo the follow-up lab investigation and finally, data obtained from a total number of 55 patients were collected for statistical analysis (Figure 1). The mean age of total participants was 48.68 ± 6.9 years old with no significant difference in distribution between groups ($P>0.99$). About 46% of the patients were men with an approximately similar distribution in both groups ($P>0.99$).

At the end of the 12-week period, the BMI, LDL-c, total cholesterol, triglycerides, liver enzymes, FBS and HbA1c decreased and the LDL-c reduced in patients treated by chicory seed syrup (all $P<0.001$). In addition, the treatment effect in all variables was statistically significant compared with data from the placebo group (Table 1).

The shifting change in fatty liver grading was also notable in the treatment group compared to the control group (Table 2).

Discussion

This trial revealed the beneficial effects of chicory seed syrup in the treatment of NAFLD and all the physical and serum markers of disturbed metabolic status were improved in this course of herbal medical therapy when comparing control's data. Our results are similar to such previous studies on *C. intybus*, which mostly focused on other parts of the plant and often were conducted in animal models and often discussed the combined effect of

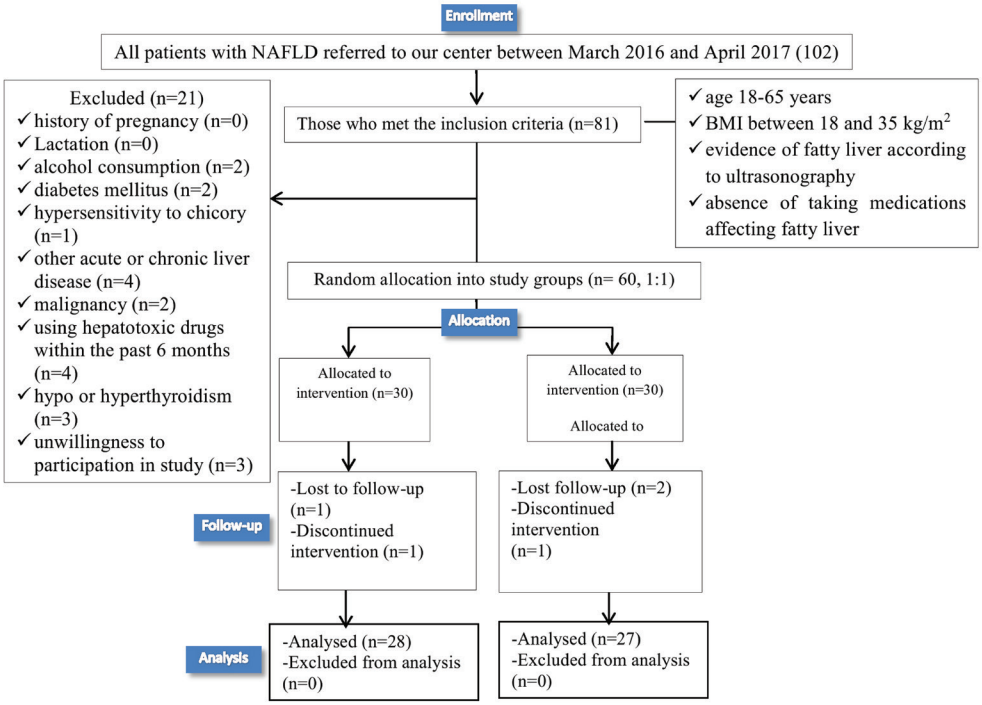

Figure 1. The CONSORT flowchart of step-wised patient selection.

Table 1. The baseline and follow-up measurements of patients in chicory (n=28) and placebo (n = 27) groups after 12 weeks of herbal treatment*

Variable	Group	Before	After	MD (SD)	*P* value
BMI (kg/m²)	Chicory	29.26±3.36	26.10±3.03	-3.16±0.96	0<0.001
	Placebo	27.25±2.68	27.25±2.69	0.007±0.29	0.9
	P value	0.017	0.143	0<0.001	
LDL-c (mg/dL)	Chicory	108.82±33.27	89.54±30.68	-19.28±12.57	0<0.001
	Placebo	99.67±41.48	97.56±40.61	-2.11±7.40	0.150
	P value	0.370	0.414	0<0.001	
HDL-c (mg/dL)	Chicory	42.5±10.84	50.61±12.45	8.10±0.18	0<0.001
	Placebo	37.78±12.76	37.96±11.95	6.03±2.25	0.673
	P value	0.144	0<0.001	0<0.001	
TG (mg/dL)	Chicory	203.39±61.69	188.07±54.58	-15.32±11.23	0<0.001
	Placebo	212.15±76.33	206.37±74.42	-5.78±10.22	0.07
	P value	0.641	0.302	0.002	
Chol (mg/dL)	Chicory	190.75±41.31	163±32.1	-27.75±16.98	0<0.001
	Placebo	185.89±32.11	179.59±29.74	-6.30±18.16	0.083
	P value	0.629	0.052	0<0.001	
ALT (IU/L)	Chicory	79.67±25.01	38.07±12.38	-41.57±16.93	0<0.001
	Placebo	73.93±23.19	73.37±21.99	-0.55±4.49	0.526
	P value	0.384	0<0.001	0<0.001	
AST (IU/L)	Chicory	45.71±15.97	24.43±7.52	-21.28±11.97	0<0.001
	Placebo	41.22±15.04	40.11±14.66	-1.16±3.08	0.072
	P value	0.288	0<0.001	0<0.001	
ALK (IU/L)	Chicory	207.89±61.65	157±41.44	-50.89±34.30	0<0.001
	Placebo	177.48±45.11	145.19±43.61	-2.30±7.47	0.122
	P value	0.042	0.119	0<0.001	
FBS (mg/dL)	Chicory	94.46±5.61	84.5±4.11	-9.96±5.71	0<0.001
	Placebo	91.19±5.8	89.93±4.82	-1.26±3.47	0.071
	P value	0.38	0<0.001	0<0.001	
HbA1c (%)	Chicory	5.17±0.34	4.67±0.34	-0.50±0.21	0<0.001
	Placebo	4.89±0.43	4.86±0.45	-0.02±0.09	0.148
	P value	0.009	0.075	0<0.001	

Abbreviations: ALK, Alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; Chol, Cholesterol; CI: confidence interval, FBS, fasting blood sugar; HbA1c, hemoglobin A_1C; HDL, high-density lipoprotein; IU/L, international unit per liter; kg/m², kilogram per squared meters; LDL, low-density lipoprotein; TG, triglycerdies.

Data were expressed as mean ± SD. * Applied tests: ANCOVA, independent and paired t-tests.

Table 2. The baseline and follow-up ultrasonographic grades of fatty liver*

	Group	Fatty liver grade			
		0	1	2	3
Baseline	Chicory (n=28)	0	0	17 (60.71%)	11(39.28%)
	Placebo (n=27)	0	1 (3.70%)	16 (59.26)	10 (37.03%)
Follow-up	Chicory (n=28)	7 (25%)	14 (50%)	7 (25%)	0
	Placebo (n=27)	1 (3.70%)	2 (7.41%)	14 (51.85%)	10 (37.03%)

*Applied test: chi-square test.

chicory with other plants.

Recently, the different derivatives of chicory plant have been used in the treatment of liver disease. At first, Lepczyński et al (14), showed that diet enrichment with inulin or dried chicory root contributed to important changes in the level of hepatic cytoskeletal proteins in pigs and noted that cytokeratin 18 (i.e. an acute phase protein of liver) downregulation could increase the inulin-type fructans' anti-inflammatory effects. In another animal study, chicory polysaccharides relieved the metabolic pathway involved in the metabolism of amino acids, production of fatty acids and also beta-oxidation in rats affected by NAFLD (15). Similar studies in high-fat diet-induced NAFLD rats revealed that fractionated chicory polysaccharides taken from plant roots could attenuate NAFLD via adenosine monophosphate-activated protein kinase activation. They also decreased the body weight, liver enzymes, blood sugar and serum triglycerides and cholesterol level (16,17).

One of the remedial properties of chicory is its antioxidant effect. Seed extract of this plant has strong antioxidant activities (is rich in flavonoid compounds)

(18). Additionally, Huang et al showed the photoprotective properties of chicory in the mitochondria of rat liver cell (19). Chicory is an important hepatoprotective plant in Greece medicine. It is effective against hepatitis and jaundice (20). Furthermore, the study by Hassan et al, showed that chicory precursors in animals resulted in a striking decrease in liver thiobarbituric acid reactive species and lipid profile, total bilirubin, and hepatic enzyme activity in both hepatic parenchyma and serum (21).

This study revealed that chicory seed syrup could improve lipid profile, blood sugar indices, anthropometric parameters including BMI and also ultrasonographic characteristics in NAFLD. Despite such a wide acceptable therapeutic effect, now in modern complementary and herbal medicine, there is still a need for more studies on chicory in the treatment of NAFLD. The most favorable and potent part of the plant should be investigated in a setting of comparative study. In addition, the more useful chemical component of each part should be further investigated in order to extract a probable new herbal drug.

Conclusion

The findings of this trial presented the positive effects of chicory seed syrup on cardiometabolic profile and liver enzymes in patients affected by NAFLD and may be considered as a future therapeutic option. Much more studies with relatively larger sample sizes are required to better clarify and validate the results of the current investigation.

Limitations of the study

Despite the above-mentioned advantages of the present trial about a rarely studied part of chicory in NAFLD, this study has some limitations. One limitation is the small sample size due to the nature of a follow-up study and also excluding comorbidities and confounding factors predetermined to enhance the study power. In addition, very young or very old individuals were not enrolled in this study and so, the results cannot be generalized to these two extremes of age. Moreover, the treatment course was only 12 weeks and we did not study the longer beneficial or harmful effects.

Authors' contribution

MM and MT participated in the design and conduct of the study. MJ and MB preparing the manuscript draft. MK, MHM and NK participated in describing the methodology of the study and statistical analysis of the data and amending the manuscript draft of the article. All authors read and approved the final manuscript.

Conflicts of interest

The authors declared no competing interests.

Ethical issues

This work followed the principles of the Declaration of Helsinki and was extracted from the Ph.D., thesis by Maral Marzban (Thesis#A-12-500-19) at the Research Center for Traditional Medicine and History of Medicine, School of medicine, Zanjan University of Medical Sciences. The study was also registered in the Iranian Registry of Clinical Trials (identifier: IRCT2016061228411N1; https://en.irct.ir/trial/23063) and was approved by the ethics' committee of the Zanjan University of Medical Sciences (#IR. ZUMS.REC.1395.195). First, the study process was exactly explained to the participants then informed consent was signed by all of them. Additionally, ethical issues including plagiarism, double publication, and redundancy have been completely observed by the authors.

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