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Efficacy of Black Seed (*Nigella sativa*) and Lemon Balm (*Melissa officinalis*) on Non-Alcoholic Fatty Liver Disease: A Randomized Controlled Clinical Trial

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Abstract

Background: There are several therapeutic strategies available from the viewpoint of Traditional Persian Medicine (TPM) to treat hepatic diseases.
Objectives: This study aimed at assessing the efficacy and safety of *Nigella sativa* and *Melissa officinalis* in patients with Non-Alcoholic Fatty Liver Disease (NAFLD).
Methods: From November 2014 to May 2016, in an open-label, simple-blocked, randomized controlled clinical trial, the researchers evaluated the efficacy of *Nigella sativa* and *Melissa officinalis* compared with Orlistat capsule on the grade of fatty liver and the serum levels of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) in 50 patients with NAFLD in Iran.
Results: Regarding within-group changes, a significant decrease was observed in the serum level of AST, ALT, body mass index, and grade of fatty liver in both groups after the intervention compared with baseline ($P < 0.001$). Repeated measures logistic regression analysis showed that there was a more significant reduction in the grade of fatty liver over the study period in the intervention group compared with the control group (0.58 ± 0.50 versus 1.51 ± 0.54, $P < 0.001$).
Conclusion: Traditional Persian Medicine-based preparations of *Nigella sativa* and *Melissa officinalis* could reduce body weight and liver enzymes and improves the grade of fatty liver in Non-Alcoholic Fatty Liver Disease.

Keywords: Disease, Fatty Liver, Lemon Balm, *Melissa officinalis*, Medicine, *Nigella sativa*, Non-Alcoholic, Traditional

1. Background

Non-Alcoholic Fatty Liver Disease (NAFLD) is a chronic disease that is predicted to become one of the main leading causes of end-stage liver disease around the world (1, 2).

Since the pathogenesis of NAFLD remains unknown in addition to the fact that NAFLD often has an association with other diseases, such as obesity, hypertension, dyslipidemia, and diabetes, the management of this condition is empirical (3). Modification of lifestyle in conjunction with medical therapy has been recommended for the treatment of NAFLD (4-6). Avoiding heavy alcohol consumption, regular exercise, and weight loss are the most important lifestyle modifications for these patients (7, 8). It is important to note that current treatments for NAFLD are inadequate and sometimes associated with side effects.

Over the past decades, there has been increasing interest in the use of complementary and alternative medicine for the treatment of chronic diseases, such as fatty liver (9-12). Traditional Persian Medicine (TPM), as an alternative medical system, has been practiced among Iranian people since ancient times (13, 14). There are several therapeutic strategies available from the viewpoint of TPM to treat hepatic diseases, ranging from lifestyle modification

to herbal therapy (15-18). Black seed or *Nigella sativa* and lemon balm or *Melissa officinalis* are amongst hepatotonic plants that have long been used in TPM to treat overweightness and gastrointestinal disorders. Moreover, several preclinical and clinical studies have shown beneficial effects of these herbs in the treatment of a wide range of clinical settings, such as fatty liver, hyperlipidemia, hypertension, obesity, and diabetes mellitus (19-26). Experimental studies have shown that *Nigella sativa* could protect the liver against hepatic ischemia-reperfusion injury. Also, the protective role of *Nigella sativa* by a decrease in protein oxidation and depleted anti-oxidants rejuvenation of cellular fraction has been investigated. In another animal study, *Nigella sativa* significantly reduced the elevated levels of serum enzymes as well as hepatic malondialdehyde content and significantly increased hepatic non-protein sulfhydryl. Moreover, *Nigella sativa* contributes to inhibition of the enzymes present in the neoglucogenesis pathway in the liver (27). Previous studies on *Melissa officinalis* demonstrated that *Melissa officinalis* reduces high fat diet-induced visceral adipose tissue angiogenesis, visceral obesity, and NAFLD in obese female ovariectomized mice (28). Significant effects of *Melissa officinalis* on controlling blood sugar level and serum lipid profiles that attribute to the antioxidant benefits of flavonoids have been investigated as well (29).

Regarding the traditional use of *Nigella sativa* and *Melissa officinalis* in addition to their known beneficial effects in recent studies, the current researchers decided to design a randomized, controlled clinical trial to evaluate the efficacy of these herbs on the levels of liver enzymes and the grade of fatty liver in patients with Non-Alcoholic Fatty Liver Disease.

2. Materials and Methods

2.1. Trial Design

The researchers designed a randomized, double arm, open label, active-controlled clinical trial. In this trial, the effect of TPM-based diet and herbal formulation of *Nigella sativa* and *Melissa officinalis* on the grade of fatty liver and the levels of liver enzymes in patients with NAFLD was evaluated. No changes occurred in the methods after the trial commencement.

2.2. Participants

Inclusion criteria for participants enrolled in this study were age of 20 to 60 years, the presence of NAFLD (grade 1 to 3) diagnosed by ultrasound imaging, and body mass index greater than 27 kg/m^2. This study was carried out in the nutrition clinic of Ghaem Hospital, a governmental, general, and referral hospital with 924 beds and more than ten wards. This clinic was affiliated to Mashhad University of Medical Sciences, Mashhad, Iran, from November 2014 to May 2016. All participants signed an informed consent form.

Exclusion criteria were affliction with diabetes, hypertension, cardiovascular diseases, familial hyperlipidemia, breastfeeding, pregnancy, drug addiction, alcohol consumption, active or previous infection with hepatitis B or C, acute liver disease, renal stones, gallstones, major surgery during the last 6 months, any surgery on the liver and gallbladder or general anesthesia drugs during the study, rapid weight loss during the last 3 months for any reason, patients undergoing special diet or exercise for weight loss or gain, and losing weight.

2.3. Intervention

After a diagnosis of fatty liver by a radiologist via sonographic assessment and confirmation of the diagnosis by a gastroenterologist, eligible patients were divided into 2-groups. Patients were randomly assigned to receive either a 3-month TPM based diet plus Hepatomelis capsules (10 mg twice per day) as the intervention group, or the low-fat low-calorie diet plus Orlistat capsules (500 mg twice per day) as the control group. Participants in the intervention group received TPM-based dietary commands by a written list of hot- and cold-natured foods and related recipes for a period of 3 months. To better understand the concept of hot- and cold-natured foods, reference could be made to previous studies (13, 30-32). Those in the control group received a low-fat and low-calorie diet for 3 months. Controls were placed on minus 500 kcal per day deficit on daily energy needs. In this diet, participants received 60% carbohydrate, 20% protein, and 20% fat. The participants were prevented from weight change by out-of-the-protocol-study diet and physical activity during the trial period. Consumption of less than 70% of the drugs during the trial was considered as drug intolerance, and the patient was excluded from the trial.

2.4. Preparation of Drugs

Hepatomelis is a type of herbal tea consisting of *Nigella sativa* and *Melissa officinalis*. The intact seeds of *Nigella sativa* and dry leaf of *Melissa officinalis* were purchased from a herbal market in Tehran (Iran) and was authenticated by a botanist (Voucher number: 8029 and 8028 respectively) and kept at the Herbarium of the Faculty of Pharmacy, Shahid Beheshti University of Medicinal Sciences, Tehran, Iran. Five grams of the intact seeds of *Nigella sativa* was added to 5 g of the coarse milled powder of dry

leaf of the *Melissa officinalis* and filled in a tea bag, weighing 10 g. All patients were instructed to infuse each tea bag in 250 ml of boiled water for 10 minutes and drink according to their assigned dose.

Orlistat (Venustat, anti-obesity agent, capsule, 120 mL, Aburaihan Pharmaceutical Co) was prescribed as a routine drug for the treatment of fatty liver and was used in this study for weight loss.

2.5. Outcome Measures

The primary outcome measure in this trial was changes in the level of liver enzymes: Serum Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST). Blood samples were taken after 12 to 14 hours of overnight fasting at the baseline, sixth week, and 3 months after the intervention in 2 groups by using the International Federation of Clinical Chemistry (IFCC) approved method.

Secondary outcome measures were changes in the grade of fatty liver (fatty tissue infiltration in the liver by using ultrasound imaging) and changes in the patients' body mass index. The Sonographic assessment was done with the device model Siemens 40 with the Acuson 15 L8 transducer after 8 hours of fasting by a radiologist. Any observed adverse event was also considered as the secondary outcome. No changes were made to trial outcomes after the trial commenced.

2.6. Sample Size

The sample size was calculated as 25 patients in each group with a total of 50 patients based on the difference in mean enzyme level of ALT obtained from previous studies, and by taking in account 2-sided significance level of 0.05, and a power of 80% (33, 34).

2.7. Safety Assessment

In order to detect possible patient complaints, all patients were followed by physicians every 2 weeks. Moreover, weight and blood pressure measurements were also carried out by the mentioned physicians.

2.8. Randomization

Fifty eligible patients were randomized to 2 parallel groups. A statistician generated a randomized list by using NCSS (statistical software) with the simple block randomization method. Then, the eligible patients were assigned to 2 groups by the secretary of the clinic, according to the randomized list. Only the statisticians were blind to the allocation of the patients.

2.9. Ethical Issues

The trial was in compliance with the Declaration of Helsinki (1989 revision), and also was reviewed, approved, and monitored by the Ethics committee of Mashhad University of Medical Sciences. The trial was registered by the Iranian Registry of Clinical Trials with the following code: IRCT2014081518807N1. All the participants signed an informed consent form prior to enrollment in the study.

2.10. Statistical Methods

All data were analyzed using SPSS statistics for Windows, version 15.0 (SPSS Inc., Chicago, IL., USA). All data were described by mean ± standard deviation (35) or number (percentage). Chi-square and Mann–Whitney U tests were used for statistical comparison of baseline characteristics. Repeated measurement Analysis of Variance (ANOVA) was used to determine changes in outcomes between the two groups of the study. P-values of less than 0.05 were considered significant.

3. Results

From November 2014 to May 2016, 78 volunteers were assessed for eligibility. Fifty patients, who met the inclusion criteria and agreed to participate in the study, were divided to 2 groups. Twenty-five patients were assigned to the intervention group and 25 patients to the control group. Figure 1 is a flowchart of the groups' distribution, recruitment, intervention, follow up, and analysis.

Baseline demographic data of the study groups (age, gender, marital status body mass index, the serum level of AST and ALT, and sonographic grade of fatty liver) are shown in Table 1. The mean age of participants was 46.33 (± 10.82) and 40.35 (± 11.96) years in the intervention and control groups, respectively. As shown in Table 1, no significant differences were observed in baseline demographic data between the 2 groups of the study, except a significant difference that was observed between the study groups in terms of baseline ALT (39.46 ± 27.26 versus 60.30 ± 48.86, P value = 0.021) and Fasting Blood Sugar (FBS) (102.52 ± 22.13 versus 92.62 ± 9.77, P value = 0.052).

Regarding within-group changes, a significant decrease was observed in the the serum level of AST and ALT, body mass index, and grade of fatty acid in both groups after the intervention compared to baseline (Table 2). The trend of changes in outcome measures is shown in Figure 2.

Repeated measures logistic regression analysis showed that there was a more significant reduction in the grade of fatty liver over the study period in the intervention group compared with the control group (P <

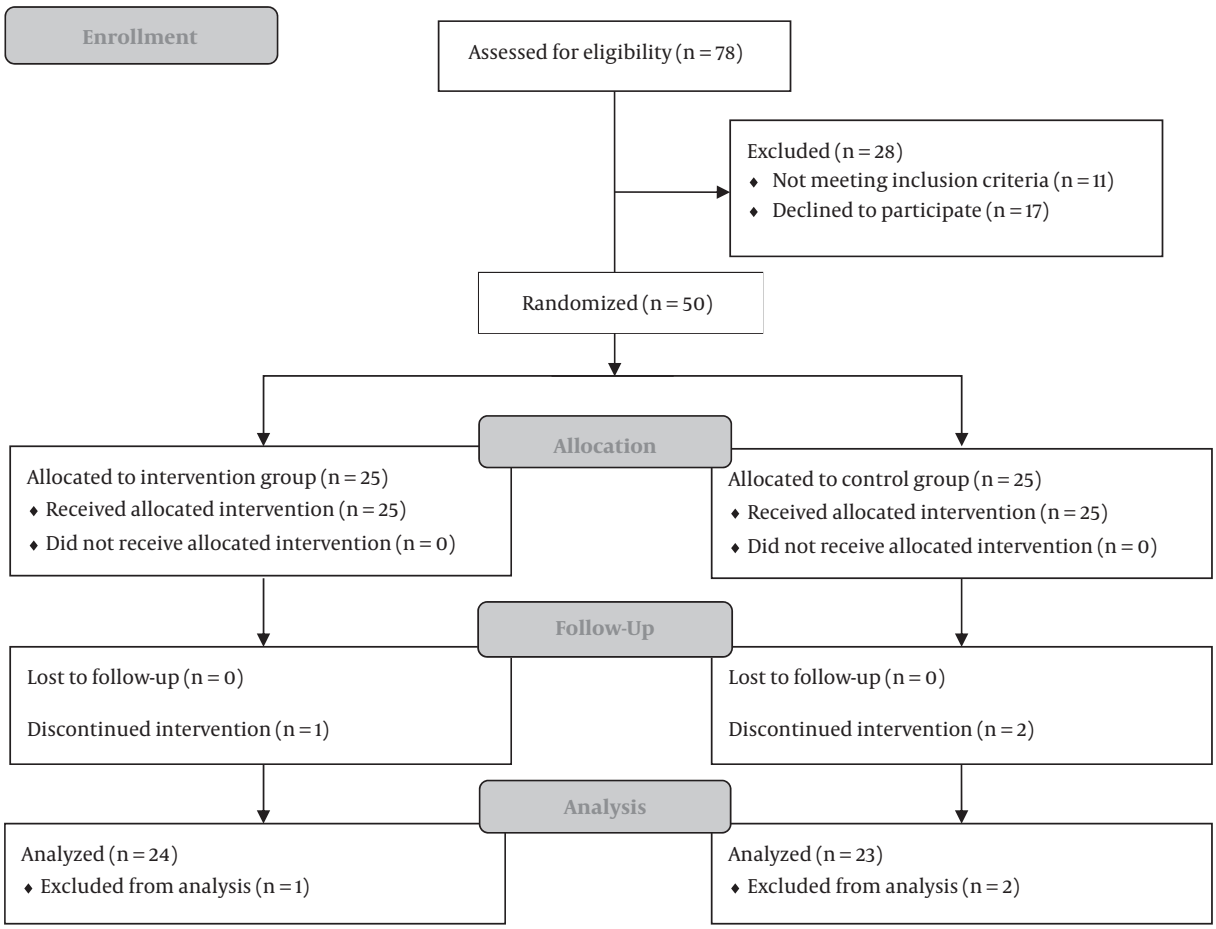

Figure 1. Flow Diagram of the Groups' Allocation, Enrolment, Intervention, Follow-up, and the Analysis in Both Groups of the Study

0.001). Details of the grade of fatty liver change are shown in Table 3.

4. Discussion

Safety and efficacy of *Nigella sativa* and *Melissa officinalis* on NAFLD was evaluated in this study via an open-label randomized controlled clinical trial. Although all of the outcome measures of the study (serum level of AST and ALT, and BMI) improved in both groups, the *Nigella sativa* and *Melissa officinalis* had better effects on reducing sonographic grade of fatty liver in patients with NAFLD, compared with orlistat.

Currently, weight loss and calorie restriction is known as the first effective treatment for NAFLD (36). Weight loss causes reduction of hepatic fat content, consequently leading to improved liver function (37). Insulin-sensitizing agents, such as metformin and thiazolidinediones, lipid lowering drugs, such as orlistat and statins, hepatoprotective agents, such as ursodeoxycholic acid, and antioxidants, such as vitamin C and E, are medications that have been used for NAFLD (38, 39).

There are several herbal remedies that reveal probable advantage for management of NAFLD. The most commonly used herbs were *Cassia obtusifolia, Crataegus pinnatifida, Alisma orientalis, Salvia miltiorrhiza, Bupleurum chinense, Rheum p almatum,* and *Astragalus membranaceous* (40). Most of these herbs are used in Traditional Chinese Medicine (TCM) to manage NAFLD by nourish Qi, strengthen the liver and spleen, and clear heat or discharge phlegm based on TCM theories (40). Moreover, the efficacy of some traditional Persian medicinal herbs on hepatic disorders,, such as NAFLD has been evaluated in previous animal and clinical studies. The most commonly used herbs were Rosa *damascena Mill*, *Chlorella vulgaris*, *Camelia sinensis*, *Glycyrrhiza glabra L.*, *Phyllanthus urinaria*, *cuminum*

Table 1. Baseline Demographic Data and Clinical Features of the Trial Participants

Basic Characteristics	Intervention Group (n = 24)	Control Group (n = 23)	P-Value
Mean age, y	46.33 ± 10.82	40.35 ± 11.96	0.86
Sex, N.%			0.557
Male	12 (50)	11 (47.82)	
Female	12 (50)	12 (52.18)	
Marital status, %			0.234
Married	0 (0)	2 (8.6)	
Married	24 (100)	21 (91.4)	
AST	33.92 ± 14.18	47.26 ± 33.58	0.187
ALT	39.46 ± 27.26	60.30 ± 48.86	0.021
Height, cm	166.17±6.90	167.65±10.47	0.567
Weight, kg	81.91±7.17	81.72±10.40	0.942
BMI, kg/m^2	29.60±0.47	28.97±0.88	0.063
FBS, mg/dl	92.62±9.77	102.52±22.13	0.052
Grade of fatty liver			0.814
Grade 1	6 (25.00)	4 (17.40)	
Grade 2	15 (62.50)	16 (69.56)	
Grade 3	3 (12.50)	3 (13.04)	

Table 2. Mean Values (Mean ± SD) for Aspartate Aminotransferase (AST), Alanine Aminotransaminase (ALT), Body Mass Index (BMI) and Grade of Fatty Liver in the Intervention and Control Groups Before and After the Intervention

	Groups		
	Intervention	Control	P Value
AST, units per liter			
Baseline	33.92 ± 14.18	47.26 ± 33.58	0.187
6 weeks	26.96 ± 7.22	32.35 ± 11.04	0.056
12 weeks	24.71 ± 6.64	27.91 ± 7.04	0.116
P value	< 0.001	< 0.001	
ALT, units per liter			
Baseline	39.46 ± 27.26	60.30 ± 48.86	0.021
6 weeks	29.29 ± 11.36	40.4 ± 21.86	0.130
12 weeks	24.63 ± 8.46	27.35 ± 7.77	0.257
P value	< 0.006	< 0.001	
BMI, kg/m^2			
Baseline	29.60 ± 0.47	28.97 ± 0.88	0.063
6 weeks	28.44 ± 0.69	28.07 ± 0.88	0.112
12 weeks	27.60 ± 0.93	27.36 ± 0.85	0.365
P value	< 0.001	< 0.001	
Grade of fatty liver			
Baseline	1.88 ± 0.61	1.96 ± 0.56	0.814
6 weeks	1.21 ± 0.41	1.83 ± 0.49	< 0.0001
12 weeks	0.58 ± 0.50	1.51 ± 0.54	< 0.0001
P value	< 0.001	< 0.001	

cyminum L., *Berberis Vulgaris L.*, and *Cinnamom umzeylanicum* (41-47). According to TPM, changes in the liver temperament toward cold temperament and decrease in hepatic strength could result in chronic diseases, such as what is now known as NAFLD in current medicine (48). Hence, hepatotonic and warm temperament herbs are usually used for management of NAFLD (49).

The black seed, as a dry and warm temperament herb, is used in TPM for the treatment of NAFLD. A previous study showed that *Nigella sativa* in combination with *Urtica dioica* L decreases lipid per-oxidation and liver enzymes, and increases anti-oxidant defense system activity in carbon tetrachloride-treated rats (50). Also, *Nigella sativa* oil has a favorable effect on reducing serum total cholesterol, low density lipoprotein and triglycerides, and elevating serum high density lipoprotein level in the carbon

Table 3. Frequency Distribution of the Grade of Fatty Liver in the Intervention and Control Groups During the Study Period

Time/ Grade of Fatty Liver	Intervention Group N (%)	Control Group N (%)
Baseline		
Grade 1	6 (25)	4 (17.4)
Grade 2	15 (62.5)	16 (69.6)
Grade 3	3 (12.5)	3 (13)
6 weeks		
Grade 1	19 (79.2)	5 (21.7)
Grade 2	5 (20.8)	17 (73.9)
Grade 3	0 (0)	1 (4.4)
12 weeks		
Grade 0	10 (41.7)	0 (0)
Grade 1	14 (58.3)	17 (73.9)
Grade 2	0 (0)	6 (26.1)

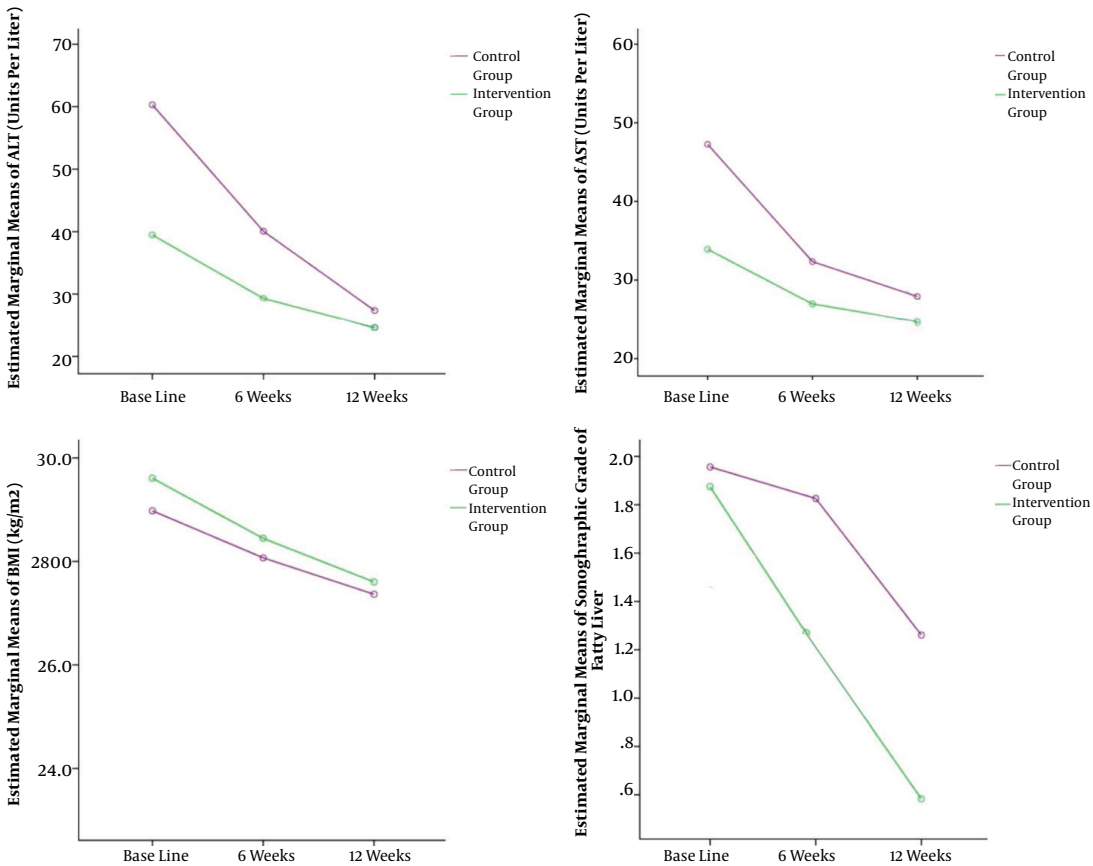

Figure 2. Comparison of Aspartate Aminotransferase, Alanine Aminotransaminase, Body Mass Index and Grade of Fatty Liver Before and After Three Months of Intervention in Both Study Groups

tetrachloride-treated rats (51). In experimentally-induced diabetic rabbits, *Nigella sativa* caused prevention of lipid peroxidation, increased anti-oxidant defense system activity and also prevented liver damage (52). Sahar et al. in their animal study revealed that the crude oil of *Nigella sativa* seed may potentially be used as a dietary supplement for prevention of inflammatory fatty liver. Thymoquinone, as an active constituent of *Nigella sativa* was re-

ported to prevent oxidative stress injury in hepatocytes (22). Vedanarayanan et al., in their study showed that extract of *Nigella sativa* could reduce lipid profile and liver enzymes of rats fed with high-fat diets (53).

The lemon balm, as another dry and warm temperament herb, is used in TPM for the treatment of liver diseases. A previous study showed that the administration of *Melissa officinalis* extract reduced total cholesterol, total lipid, ALT and AST levels in the serum, and lipid peroxidation levels in the liver tissue, and increased the glutathione levels in the liver of hyperlipidemic rats (20). Moreover, *Melissa officinalis* essential oil reduces the plasma triglycerides in human apolipoprotein E2 transgenic mice by inhibiting sterol regulatory element-binding protein-1c-dependent fatty acid synthesis (54). Chung et al. in their animal study revealed that *Melissa officinalis* was an efficient hypoglycaemic agent, probably due to enhanced glucose uptake and metabolism in the liver and adipose tissue, and the inhibition of gluconeogenesis in the liver (55). Zarei et al. in 2014 suggested that *Melissa officinalis* extract exerted a hypolipidemic effect similar to atorvastatin and showed a protective effect on the liver of hyperlipidemic rats (23). They continued their research in 2016 and showed that the extract of *Melissa officinalis* reduced levels of liver enzymes, particularly alkaline phosphatase and gamma-glutamyl transferase, and it was effective in improving liver function (56). Jeongjun Kim et al. revealed that *Melissa officinalis* attenuates NAFLD by reducing visceral adipose tissue mass and regulates visceral adipose tissue dysfunction, leading to attenuation of obesity-induced NAFLD and NASH (57).

To the best of the authors' knowledge, this was the first clinical trial that evaluated safety and efficacy of the *Nigella sativa* and *Melissa officinalis*, on NAFLD. However, the current study also had some limitations. Short time duration of the study, lack of a placebo group, lack of more accurate objective indices like liver biopsy for the assessment of patients' grade of fatty liver and the small sample size, were the main limitations of this study. In addition, this study was an open-label study that may possibly have some bias.

4.1. Conclusion

This randomized open-label controlled clinical trial demonstrated that TPM-based preparation of black seed and lemon balm could reduce body weight and liver enzymes and improve grade of fatty liver in NAFLD. However, larger-scale qualified methodological trials with longer duration of the intervention are needed to replicate and expand preliminary findings in this trial.

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