Avicenna Journal of Phytomedicine (AJP)

Original Research Article

Protective effects of *Cichorium intybus* and N-acetylcysteine on liver side effects of fingolimod in patients with multiple sclerosis: A randomized clinical trial

Masoud Ghiasian[1], Davoud Sarmadi Khojasteh[1,2], Sara Ataei[2], Dara Dastan[3], Salman Khazaei[4], Parastoo Mirzabeigi[5,*]

[1]*Department of Neurology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
[2]*Department of Clinical Pharmacy, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran*
[3]*Department of Pharmacognosy, School of Pharmacy, Medicinal Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*
[4]*Department of Epidemiology, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran*
[5]*Department of Clinical Pharmacy and Pharmacoeconomics, School of Pharmacy, Iran University of Medical Sciences, Tehran, Iran*

Article history:
Received: Jun 07, 2026
Received in revised form: Oct 27, 2026
Accepted: Oct 27, 2026
Epub ahead of print

**** Corresponding Author:***
pmirzabeygi@gmail.com

Keywords:
Multiple sclerosis
Fingolimod
C. intybus plant
N-acetylcysteine
Liver enzymes

Abstract

Objective: Multiple sclerosis (MS) is an immune-mediated central nervous system disease with limited disease-modifying options. While fingolimod is widely used in relapsing–remitting MS (RRMS), its hepatotoxic potential may compromise long-term adherence. This randomized trial evaluated the efficacy of N-acetylcysteine (NAC) and the hydroalcoholic extract of *Cichorium intybus* (*C. intybus*) from the aerial parts of the plant in mitigating fingolimod-induced hepatotoxicity and clinical disability in patients with RRMS.

Materials and Methods: Sixty-three patients with RRMS receiving fingolimod were randomly assigned to three groups: fingolimod plus placebo, fingolimod plus NAC, or fingolimod plus *C. intybus*. Liver enzymes—alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)—and Expanded Disability Status Scale (EDSS) scores were assessed at baseline and after 4 months of treatment.

Results: After 4 months, both NAC and *C. intybus* showed significantly lower EDSS scores than the placebo group. Compared with *C. intybus*, NAC showed a significant reduction in EDSS. Both interventions significantly reduced ALT, AST, and ALP levels versus placebo. *C. intybus* and NAC had no significant difference in reducing ALT, AST or ALP levels.

Conclusion: Adjunctive treatment with NAC or *C. intybus* significantly attenuated fingolimod-associated hepatotoxicity and it was accompanied by improvements in disability scores. These findings suggest that hepatoprotective supplementation with NAC or *C. intybus* may enhance the safety and therapeutic profile of fingolimod in RRMS.

Please cite this paper as:
Ghiasian M, Sarmadi Khojasteh D, Ataei S, Dastan D, Khazaei S, Mirzabeigi P. Protective effects of Cichorium intybus and N-acetylcysteine on liver side effects of fingolimod in patients with multiple sclerosis: A randomized clinical trial. Avicenna J Phytomed, 2026

Introduction

Multiple sclerosis (MS) is an inflammatory condition of the central nervous system that predominantly impacts adults (McGinley et al. 2021). It is characterized by demyelination and degenerative changes that can cause neurological and mental disorders. This disease presents various phenotypes, the most common of which is Relapsing-remitting MS (RRMS). RRMS begins with clinical relapses followed by partial or complete recovery; however, over time, recovery may become incomplete, and disability often increases (Giussani et al. 2021). The U.S. Food and Drug Administration (FDA) approved the first disease-modifying treatments (DMTs) for MS in 1993 (Tortorice and Antonovich 2020). In 2010, fingolimod was recognized as the first oral DMT approved by the FDA.

Fingolimod works by binding to the sphingosine-1-phosphate receptor and internalizing it, effectively trapping lymphocytes in the lymph nodes (Tanaka 2014). This process stops these immune cells from contributing to inflammation in the central nervous system. Research indicates that oral fingolimod significantly reduces the frequency of annual relapses compared to interferon beta-1a (Cohen et al. 2010). However, it can cause side effects including bradycardia, atrioventricular block upon the first dose, macular edema, and mild elevations in blood pressure (Behjati et al. 2014). Fingolimod can cause mild to moderate increases in liver enzymes in some patients. In rare cases, more serious liver injury can occur, including acute liver failure requiring transplantation (Caliskan et al. 2023). Therefore, preventing serious liver damage and ensuring safe use of fingolimod are essential. The existing evidence indicates that *Cichorium intybus* and N-acetylcysteine could serve as beneficial adjunctive therapies for MS patients on fingolimod (Banu 2024; Keshavarzi et al. 2024; Popescu et al. 2024; Khalatbari Mohseni et al. 2023).

Cichory is a medicinal and herbal plant (scientific name *C. intybus*), a plant from the Asteraceae family (Street et al. 2013). It contains many chemical compounds, and citric acid is the main component of its extract (Nwafor et al. 2017). In addition, *C. intybus* extract provides a potential protective effect against alcohol-induced liver damage by preventing antioxidant stress and fat accumulation through ADH (alcohol dehydrogenases) and CYP2E1 (Cytochrome P450 2E1) pathways (Kim et al. 2021). These varied phytochemical compounds in the plant increase appetite and digestion while also providing anti-inflammatory and antibacterial effects (Janda et al. 2021). *C. intybus* is of particular interest as a hepatoprotective agent, reducing liver toxicity and inflammation in a recent preclinical study (Moloudi et al. 2021). *C. intybus* washes the liver and kidneys, builds blood, and strengthens the liver (Gazwi et al. 2022).

N-acetylcysteine (NAC) is an acetylated amino acid commonly used as an antidote for acetaminophen poisoning (Raghu et al. 2021). Due to its ability to support antioxidant activity, NAC enhances the nitric oxide system (Zheng et al. 2024). As a rich source of sulfhydryl groups, which effectively neutralize free radicals (Pedre et al. 2021), NAC aids in safeguarding liver cells from injury. NAC has also demonstrated potential to reduce liver damage in various situations, including liver transplant patients and cases of drug-induced liver injury (Sanabria-Cabrera et al. 2022; Singh et al. 2018; Sukumaran et al. 2023). The use of NAC has also been confirmed in various diseases, including pneumonia, bronchitis, and cystic fibrosis, in patients with tracheostomies, and for pulmonary complications following surgery (Li et al. 2018). It is particularly helpful in relieving mucus blockage in these conditions. However, oral administration of NAC may lead to side effects such as nausea, vomiting, diarrhea, and gastric reflux (Patel et al. 2024). As well, in patients with preexisting

gastrointestinal ulcers or varices, there is a concern about the potential risk of inducing gastrointestinal bleeding with oral NAC (Atefi et al. 2020).

In this study, we hypothesized that *C. intybus* and NAC, by targeting oxidative stress and liver damage, may help reduce some potential side effects of fingolimod treatment in RRMS patients.

Materials and Methods

Study design

This study was a double-blind, randomized clinical trial involving 63 patients with RRMS who received fingolimod (0.5 mg once daily) and were referred to the Neurology Clinic in Hamedan, Iran, between June 2024 and March 2025 (Figure 1). After receiving ethics approval from the Ethics Committee of Hamedan University of Medical Sciences (IR.UMSHA.REC.1401.735) on November 27, 2021, this study was registered in the Iranian Clinical Trials Registration Center under the code IRCT20120215009014N410. The study was conducted in accordance with the ethical standards outlined in the 1964 Declaration of Helsinki and its subsequent amendments, or with comparable ethical standards.

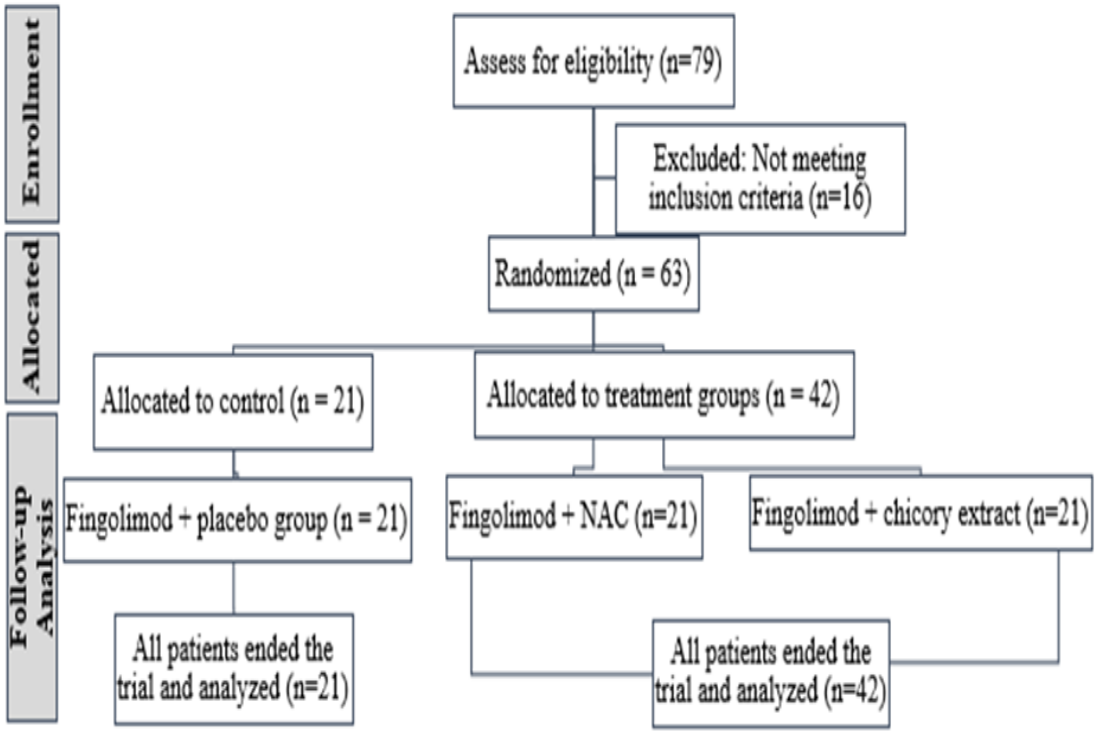

Figure 1. Summary of patient flow

Inclusion and exclusion criteria

The study inclusion criteria included at least three weeks since the onset of the MS relapse and initiation of corticosteroid therapy, showing no liver problems caused by corticosteroids, defined as AST or ALT levels not exceeding twice the upper limit of normal (ULN), being new patients starting fingolimod, and having an EDSS score of less than three. Exclusion criteria included pregnant and lactating women, individuals with sensitivity to *C. intybus* or NAC allergy, and those with a history of chronic liver, kidney, heart, or infectious diseases.

Intervention

After fully explaining the study's aims and protocol, written informed consent was obtained from all participants prior to

enrollment. Following the baseline assessment, RRMS patients were randomly allocated to one of three intervention groups by computer-generated block randomization sequence: A) placebo (at a dose of 250 mg corn starch as an oral capsule once a day) + fingolimod, B) *C. intybus* extract (at a dose of 250 mg as an oral capsule once a day) + fingolimod, and C) NAC (at a dose of 1200 mg once a day) + fingolimod. Patients were randomly assigned to three parallel groups (1:1:1 ratio) using a computer-generated block randomization sequence (fixed block size of 6), prepared by an independent statistician, with allocation concealment maintained by restricting access to the randomization list. The treatment effectiveness was evaluated after four months. The study was conducted using a blinded design. The placebo and *C. intybus* capsules were identical in appearance, form, color, and size (white capsules), while NAC was administered as an effervescent tablet. Participants received only their assigned intervention and were not informed of the treatment allocation of other participants. An independent statistician created the randomization list and kept it concealed from the investigators and participants to maintain blinding. Neither the participants nor the investigators responsible for assessing the patients, nor the outcome assessor, knew the group assignments.

Data collection and follow-up

Demographic and clinical data were recorded using the questionnaire or Case Report Form (CRF). Venous blood samples were collected at baseline and after 4 months of intervention to assess serum liver enzymes (ALT, AST, and ALP) using standard commercial enzymatic kits on an automated clinical chemistry analyzer. All laboratory values were subsequently documented in the patients' CRFs.

The Expanded Disability Status Scale (EDSS) is the most widely used scale for evaluating the degree of neurological impairment in patients with MS. It is a 20-step scale of disease severity ranging from 0 (normal) to 10 (death due to MS). This scale, ranging from 0 to 3.5, considers functional parameters, while scores from 4 to 10 indicate degrees of mobility in patients (Cao et al. 2013). This test is performed at the beginning and end of the study to check the clinical outcomes. All neurological assessments and EDSS scoring at baseline and 4-month follow-up visit were conducted by the same attending neurologist.

During the 4-month treatment period, to assess patient safety, compliance, and potential adverse effects (e.g., gastrointestinal reactions, hypersensitivity reactions, or neurological worsening), patients received biweekly telephone calls from the pharmacist. Also, participants were provided with a 24/7 contact line to report any unexpected medical events to the neurologist. Seven days before the scheduled month 4 follow-up visit, patients were reminded by phone call. Patient compliance was measured through pill counts, patient self-report, and prescription refills; any deviations were recorded.

Preparation of the hydroalcoholic extract of *C. intybus* from aerial parts

The maceration method was used to prepare the *C. intybus* herbal formulation. Dried *C. intybus* was obtained from Jihad Agriculture of Hamadan Province. After the aerial parts were crushed, extraction was conducted using a solvent to plant ratio of 5 L of distilled water per 1 kg of plant material. Because this volume exceeded the capacity of an Erlenmeyer flask, extraction was performed in smaller portions. The mixtures were heated at 70°C for 1 hr under continuous shaking. Then, ethanol was added at a ratio of 2 mL per 200 mL of water (1% v/v) to act as a preservative. The mixture was kept at room temperature for 24 hr. Finally, the *C. intybus* extract was filtered and freeze-dried. The resulting

dried extract was encapsulated into 250 mg capsules.

Sample size and sampling method

Based on the study by Ghiasian et al. 2021, the mean post-intervention ALT levels in the intervention and control groups were approximately 28 IU/L and 40 IU/L, respectively. Assuming equal variances (each equal to 10), a power of 90%, and a significance level of 5%, the calculated sample size for each group was 15. However, since three groups were to be compared, the required sample size was multiplied by √(3–1)—approximately 1.4—yielding a sample size of 21 participants per group.

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (\delta_1^2 + \delta_2^2)}{(\mu_1 - \mu_2)^2}$$

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Assumptions:

         alpha =    0.0500  (two-sided)
         power =    0.9000
            m1 =        40
            m2 =        28
           sd1 =        10
           sd2 =        10
         n2/n1 =      1.00

Estimated required sample sizes:

            n1 =        15
            n2 =        15
```

Statistical analysis

Quantitative variables are presented as mean ± SD, and categorical variables as frequency (percentage). Normality of the distribution of continuous variables was assessed using the Shapiro–Wilk test. Baseline characteristics were compared between the three groups using one-way ANOVA for continuous variables and the chi-square test for categorical variables.

The primary analysis focused on between-group comparisons at month 4. For each outcome (EDSS, ALT, AST, and ALP), a one-way analysis of variance (ANOVA) was performed on the month-4 values, with group allocation as the between-subject factor. Mean differences with 95% confidence intervals were calculated for each active group versus placebo. When the overall ANOVA was statistically significant, Bonferroni-adjusted post hoc tests were applied for the three pairwise comparisons (placebo vs *C. intybus*, placebo vs NAC, and *C. intybus* vs NAC) to control for multiple testing.

Within-group pre–post changes were assessed using paired t-tests and are reported exclusively in Tables 2–5 for descriptive purposes; they are not the primary focus of the analysis. A two-sided p-value < 0.05 was considered statistically significant. All analyses were performed using Stata version 14.2 (StataCorp, College Station, TX, USA).

Results

Demographic outcomes

The demographic and baseline characteristics of the 63 patients are summarized in Table 1. The mean age was 34.19 ± 2.41 years, and 47 patients (74.6%) were women. Most patients were aged 25–35 years (n = 19, 30.2%). Regarding marital status, 40 patients (63.5%) were married and 23 (36.5%) were single. In terms of education, 18 patients (28.6%) were illiterate, 21 (33.3%) had less than a diploma, 19 (30.2%) held a diploma or post-diploma degree, and 5 (7.9%) had a bachelor's degree or higher. Importantly, baseline characteristics were comparable across the three groups (all p>0.05; Table 1).

Disability outcomes

As shown in Table 2, baseline EDSS scores were 2.42 ± 0.33, 2.21 ± 0.26, and 1.96 ± 0.24 in the fingolimod + placebo, fingolimod + *C. intybus*, and fingolimod + NAC groups, respectively. At month 4, both active interventions were associated with significantly lower EDSS scores than placebo: the mean difference (MD) was −0.49 (95% CI: −0.61 to −0.37; p<0.001) for *C. intybus* and −1.01 (95% CI: −1.13 to −0.89; p<0.001) for NAC. The NAC group also showed a significantly greater reduction in EDSS than the *C. intybus* group (MD = −0.52, 95% CI: −0.64 to −0.40; p<0.001).

Table 1. Demographic information of the RRMS patients

Characteristic	Placebo + Fingolimod (n=21)	Chicory + Fingolimod (n=21)	NAC + Fingolimod (n=21)	Total (N=63)	p -value*
Age (years), Mean ± SD	34.52±2.51	33.95±2.38	34.10±2.40	34.19±2.41	0.74[a]
Age Groups, n (%)					0.70[b]
25–35 years	5 (23.8%)	5 (23.8%)	8 (38.1%)	19 (30.2%)	
36–46 years	4 (19.0%)	6 (28.6%)	6 (28.6%)	15 (23.8%)	
47–57 years	6 (28.6%)	7 (33.3%)	4 (19.0%)	17 (27.0%)	
58–68 years	6 (28.6%)	3 (14.3%)	3 (14.3%)	12 (19.0%)	
Sex, n (%)					0.91[b]
Male	5 (23.8%)	5 (23.8%)	4 (19.0%)	16 (25.4%)	
Female	16 (76.2%)	16 (76.2%)	17 (81.0%)	47 (74.6%)	
Marital Status, n (%)					0.93[b]
Single	8 (38.1%)	7 (33.3%)	8 (38.1%)	23 (36.5%)	
Married	13 (61.9%)	14 (66.7%)	13 (61.9%)	40 (63.5%)	
Education Level, n (%)					0.98[b]
Illiterate	5 (23.8%)	6 (28.6%)	7 (33.3%)	18 (28.6%)	
Under diploma	8 (38.1%)	7 (33.3%)	6 (28.6%)	21 (33.3%)	
Diploma and post-diploma	6 (28.6%)	7 (33.3%)	6 (28.6%)	19 (30.2%)	
Bachelor's and above	2 (9.5%)	1 (4.8%)	2 (9.5%)	5 (7.9%)	

Data are presented as mean ± standard deviation (SD) for continuous variables and number (percentage) for categorical variables.[a] One-way Analysis of Variance (ANOVA); [b] Pearson Chi-square test (χ^2). NAC, N-acetylcysteine**.**

Table 2. EDSS scores at baseline and month 4, with between-group comparisons

Group	Baseline EDSS (Mean ± SD)	Month-4 EDSS (Mean ± SD)	Within-group change (Δ)[a]	MD vs placebo (95% CI)	Between-group *p*-value[b]
Placebo + Fingolimod	2.42 ± 0.33	2.08 ± 0.20	−0.34	—	
Chicory + Fingolimod	2.21 ± 0.26	1.59 ± 0.17[*]	−0.62	−0.49 (−0.61, −0.37)	<0.001
NAC + Fingolimod	1.96 ± 0.24	1.07 ± 0.18[*†]	−0.89	−1.01 (−1.13, −0.89)	

EDSS, Expanded Disability Status Scale; MD, mean difference; NAC, N-acetylcysteine. [a] Within-group changes (Δ) are reported for descriptive purposes only and are not the primary focus of the analysis. [b] Between-group comparisons were performed using one-way ANOVA on month-4 values.[*] p<0.001 compared with placebo in Bonferroni-adjusted post hoc test.[†] p<0.001 compared with *C. intybus* in Bonferroni-adjusted post hoc test.

ALT liver enzyme activity

As shown in Table 3, baseline ALT levels were 22.09 ± 4.81, 30.23 ± 5.14, and 24.77 ± 4.24 IU/L in the fingolimod + placebo, fingolimod + *C. intybus*, and fingolimod + NAC groups, respectively. At month 4, both active interventions were associated with significantly lower ALT levels than placebo: the mean difference (MD) was −34.97 IU/L (95% CI: −39.1 to −30.8; p<0.001) for *C. intybus* and −33.43 IU/L (95% CI: −37.5 to −29.4; p<0.001) for NAC. However, the direct comparison between *C. intybus* and NAC was not statistically significant (MD = −1.54 IU/L; Bonferroni-adjusted p = 0.20).

Table 3. Changes in ALT at baseline and month 4, with between-group comparisons

Group	Baseline ALT, IU/L (Mean ± SD)	Month-4 ALT, IU/L (Mean ± SD)	Within-group change (Δ)[a]	MD vs placebo (95% CI)	Between-group *p*-value[b]
Placebo + Fingolimod	22.09 ± 4.81	53.01 ± 6.75	+30.92	—	
Chicory + Fingolimod	30.23 ± 5.14	18.04 ± 3.39*	−12.19	−34.97 (−39.1, −30.8)	<0.001
NAC + Fingolimod	24.77 ± 4.24	19.58 ± 4.12*	−5.19	−33.43 (−37.5, −29.4)	

ALT, alanine aminotransferase; MD, mean difference; NAC, N-acetylcysteine.[a] Within-group changes (Δ) are reported for descriptive purposes only and are not the primary focus of the analysis. [b] Between-group comparisons were performed using one-way ANOVA on month-4 values. * p<0.001 compared with placebo in Bonferroni-adjusted post hoc test; the difference between the *C. intybus* and NAC groups was not statistically significant (p=0.2).

AST liver enzyme activity

As shown in Table 4, baseline AST levels were 16.95 ± 2.54, 22.24 ± 4.47, and 20.63 ± 3.98 IU/L in the fingolimod + placebo, fingolimod + *C. intybus*, and fingolimod + NAC groups, respectively. At month 4, both active interventions were associated with significantly lower AST levels than placebo: the mean difference (MD) was −35.26 IU/L (95% CI: −39.3 to −31.2; p < 0.001) for *C. intybus* and −37.12 IU/L (95% CI: −41.0 to −33.2; p<0.001) for NAC. However, the direct comparison between *C. intybus* and NAC was not statistically significant (MD = −1.86 IU/L; Bonferroni-adjusted p = 0.08).

ALP liver enzyme activity

As shown in Table 5, baseline ALP levels were 172.64 ± 16.27, 180.43 ± 20.62, and 189.15 ± 22.76 IU/L in the fingolimod + placebo, fingolimod + *C. intybus*, and fingolimod + NAC groups, respectively. At month 4, both active interventions were associated with significantly lower ALP levels than placebo: the mean difference (MD) was −44.89 IU/L (95% CI: −56.5 to −33.3; p<0.001) for *C. intybus* and −40.02 IU/L (95% CI: −51.5 to −28.5; p<0.001) for NAC. However, the direct comparison between *C. intybus* and NAC was not statistically significant (MD= −4.87 IU/L; Bonferroni-adjusted p = 0.37).

Table 4. Changes in AST at baseline and month 4, with between-group comparisons

Group	Baseline AST, IU/L (Mean ± SD)	Month-4 AST, IU/L (Mean ± SD)	Within-group change (Δ)[a]	MD vs placebo (95% CI)	Between-group *p*-value[b]
Placebo + Fingolimod	16.95 ± 2.54	52.47 ± 7.29	+35.52	—	
Chicory + Fingolimod	22.24 ± 4.47	17.21 ± 3.86*	−5.03	−35.26 (−39.3, −31.2)	<0.001
NAC + Fingolimod	20.63 ± 3.98	15.35 ± 2.76*	−5.28	−37.12 (−41.0, −33.2)	

AST, aspartate aminotransferase; MD, mean difference; NAC, N-acetylcysteine. [a] Within-group changes (Δ) are reported for descriptive purposes only. [b] Between-group comparisons were performed using one-way ANOVA on month-4 values.* p<0.001 compared with placebo in Bonferroni-adjusted post hoc test; the difference between the *C. intybus* and NAC groups was not statistically significant (p=0.08).

Table 5. Changes in ALP at baseline and month 4, with between-group comparisons

Group	Baseline ALP, IU/L (Mean ± SD)	Month-4 ALP, IU/L (Mean ± SD)	Within-group change (Δ)[a]	MD vs placebo (95% CI)	Between-group *p*-value[b]
Placebo + Fingolimod	172.64 ± 16.27	202.41 ± 19.88	+29.77	—	
Chicory + Fingolimod	180.43 ± 20.62	157.52 ± 17.59*	−22.91	−44.89 (−56.5, −33.3)	<0.001
NAC + Fingolimod	189.15 ± 22.76	162.39 ± 18.08*	−26.76	−40.02 (−51.5, −28.5)	

ALP, alkaline phosphatase; MD, mean difference; NAC, N-acetylcysteine. [a] Within-group changes (Δ) are reported for descriptive purposes only. [b] Between-group comparisons were performed using one-way ANOVA on month-4 values.* p<0.001 compared with placebo in Bonferroni-adjusted post hoc test; the difference between the *C. intybus* and NAC groups was not statistically significant (p=0.37).

Discussion

In this randomized clinical study, the effects of NAC and *C. intybus* on the liver enzyme profile of patients with RRMS receiving fingolimod were investigated. The main finding was that serum liver enzyme changes differed between treatment groups during the 4-month follow-up period. While patients receiving fingolimod plus placebo showed increases in ALT, AST, and ALP, patients receiving fingolimod plus either *C. intybus* or NAC showed lower enzyme levels at follow-up. The observed changes are of clinical interest because elevations in hepatic enzymes are a recognized safety consideration with fingolimod treatment and may warrant biochemical monitoring during therapy. In the present study, the pattern of liver enzyme changes was not uniform across the three groups, providing a rationale for further investigation of interventions that may modify hepatic biochemical responses during fingolimod treatment. Furthermore, adjuvant therapy led to significant improvements in neurological disability scores, as evidenced by a meaningful reduction in the Expanded Disability Status Scale (EDSS). These results suggest that natural and pharmacological antioxidant strategies can be effective, hepatoprotective supplements to optimize the safety and tolerability of disease-modifying therapies (DMTs) in patients with RRMS.

The liver, as the primary organ of the digestive system, plays a crucial role in metabolizing and detoxifying both chemical and medicinal substances (Kalra et al. 2021). Key biomarkers for early detection of liver damage include the enzymes AST, ALT, and ALP (Lala et al. 2023). An increase in liver transaminase levels is a common side effect of fingolimod (Joni et al. 2020). In the current study, three groups of participants were assessed before and after treatment. The results indicated that transaminase levels, specifically ALT and AST, increased significantly in the placebo group compared with their pre-treatment levels. Regarding ALP, both the *C. intybus* and NAC groups showed a significant decrease in the fourth month compared with the placebo group, indicating a broad protective effect on hepatic biochemical markers. These findings align with other research (Kappos et al. 2006; Kappos et al. 2010; Cohen et al. 2010), which consistently reported that liver transaminase levels increase in MS patients treated with fingolimod. Furthermore, all three studies noted that discontinuing fingolimod treatment led to the normalization of enzyme levels. Additionally, consistent with other studies (Moloudi et al. 2021), the hydroalcoholic extract of chicory was found to mitigate fingolimod-induced serum biochemical changes, resulting in decreased levels of the liver enzymes ALT, AST, and ALP.

Chicory (*C. intybus* L.) enhances the activity of antioxidant enzymes and reduces liver enzyme levels; furthermore, its antioxidant properties and free-radical scavenging capabilities have been demonstrated *in vitro* (Abduljabbar Ramadhan and Akram Ghareeb 2022). This plant contains high levels of bioactive compounds including chlorogenic acid, chicoric acid, cynarin, luteolin, and other flavonoids. The hepatoprotective effects of chicory are primarily attributed to its chlorogenic acid and cynarin content (Zawirska-Wojtasiak et al. 2014). Clinical studies have also shown that daily supplementation with a mixture of chicory and cinnamon leads to a significant reduction in liver enzymes in patients with non-alcoholic fatty liver disease (NAFLD) (Asl et al. 2014). Moreover, aqueous extracts of chicory seeds and hairy roots exhibit potent antioxidant, anti-inflammatory, and hepatoprotective activities due to their rich content of polyphenolic and flavonoid compounds (Matvieieva et al. 2023). On the other hand, impaired tissue perfusion depletes endogenous antioxidant reserves. Under these conditions, NAC can contribute to the defense mechanism by scavenging free

radicals and replenishing depleted glutathione. Furthermore, this compound exerts a protective effect against hepatic cellular injury caused by hypoxia, toxins, and ischemia (Erdogan et al. 2023). Consistent with the findings of the present study, other animal and clinical studies have also demonstrated its protective role against hepatotoxicity induced by acetaminophen, carboplatin, and isoniazid (Singh et al. 2018).

In this research, both interventions effectively reduced elevations in aminotransferases and ALP, with no statistically significant difference between *C. intybus* and NAC in suppressing liver enzymes; this indicates that the herbal supplement possesses a hepatoprotective effect comparable to that of NAC.

In clinical trials, the EDSS is a valuable tool for quantifying and monitoring changes in disability levels over time in patients with MS (Cortesi et al. 2022). The EDSS score ranges from 0 to 10 points, with scores between 1.0 and 4.5 indicating a high degree of ambulatory ability and scores between 5.0 and 9.5 indicating varying degrees of ambulatory loss (Hanaei et al. 2021). Our study found that the RRMS patients had EDSS scores ranging from 1.96 to 2.21 at the beginning of the study. After four months of treatment with NAC and chicory, the patients' EDSS scores decreased. Notably, the largest reduction in scores was observed among patients receiving NAC in combination with fingolimod.

The average age of the patients was 34.19 ± 2.41 years, and the number of female MS patients was three times that of male patients. RRMS patients were between 25 and 35 years old. It has been reported that increasing age is the most significant factor contributing to worsening disability in MS patients (Azevedo et al. 2019). Additionally, aging is linked to a reduction in the central nervous system (CNS) structural reserve and remyelination ability, which are influenced by MS-related mechanisms (Correale and Ysrraelit 2022). Studies have indicated that women with MS experience approximately 20% more clinical relapses than men (Gilli et al. 2020). This difference may be linked to factors such as sex hormones (testosterone, estrogen, and progesterone), sex chromosomes, and gender-related differences in immune responses, as well as relevant genetic and epigenetic factors (Sparaco and Bonavita 2021).

The present study found that co-administration of *C. intybus* extract or NAC with fingolimod significantly reduced elevated serum levels of liver enzymes (AST, ALT, and ALP) following fingolimod administration in patients with RRMS over a 4-month treatment period. While both interventions demonstrated hepatoprotective properties and improved EDSS scores (with the greatest reduction in the NAC group), chicory extract exhibited efficacy comparable to NAC, with favorable tolerability. Therefore, administering *C. intybus* and NAC may be considered potentially useful adjunctive hepatoprotective agents for RRMS patients treated with fingolimod. However, this research suffers from several limitations. We did not measure total bilirubin, and hepatic safety was assessed using only ALT, AST, and ALP. In addition, the generalizability of the findings is limited by the relatively small sample size and single-center design. Larger placebo-controlled studies with longer follow-up periods are necessary to assess and confirm the long-term safety and clinical outcomes of *C. intybus* and NAC.

Acknowledgment

The authors thank all participants and research staff for their contributions

Conflicts of interest

No conflicts of interest are declared by the authors.

Funding

This study was supported by the Vice Chancellor for Research and Technology at Hamadan University of Medical Sciences (Grant No. 140104212838838).

Ethical Considerations

The Ethics Committee of Hamedan University of Medical Sciences approved the study under the code IR.UMSHA.REC.1401.735, on 2021-11-27. This study was registered in the Iranian Clinical Trials Registration Center with the code IRCT20120215009014N410 on 8 December 2021. The study was conducted in accordance with the ethical standards outlined in the 1964 Declaration of Helsinki and its subsequent amendments, or with comparable ethical standards.

Code of Ethics

IRCT20120215009014N410

Authors' Contributions

D.S.: Data Curation and Experiments, Writing - original draft; S.KH.: Formal analysis; D.D.: Advisor, Methodology, Writing - review & editing; S.A.: Investigation, Study design, and write review; M.Gh. and P.M.: Supervise, conceptualize, and write- review & editing; M.Gh, D.S, S.A, D.D, S.KH and PM approved the final version.

Abbreviations

ADH: Alcohol Dehydrogenase
ALP: Alkaline Phosphatase
ALT: Alanine Aminotransferase
ANOVA: Analysis of Variance
AST: Aspartate Aminotransferase
CI: Confidence Interval
CRF: Case Report Form
CYP2E1: Cytochrome P450 2E1
DMTs: Disease-Modifying Therapies
EDSS: Expanded Disability Status Scale
FDA: Food and Drug Administration
IRCT: Iranian Registry of Clinical Trials
IU/L: International Units per Liter
MD: Mean Difference
MS: Multiple Sclerosis
NAC: N-acetylcysteine
RRMS: Relapsing–Remitting Multiple Sclerosis
SD: Standard Deviation
ULN: Upper Limit of Normal

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