ROLE OF FLAXSEED OIL AND OLIVE OIL IN MANAGEMENT OF FATTY LIVER DISEASES WITH DYSLIPIDEMIA

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disease. Lifestyle changes are the mainstay of treatment, but adherence is often poor. Whether olive oil or flaxseed oil could improve NAFLD is unknown. **Objective**: To investigate the effects of flaxseed oil, olive oil, and combined therapy on hepatic steatosis, fibrosis, lipid profiles, and cytokeratin 18 fragments (CK18) in patients with non-alcoholic fatty liver disease (NAFLD). **Methods:** In this 12-week, randomized, parallel, placebo-controlled trial, we assigned 119 adults with NAFLD to flaxseed oil, olive oil, a combination of both oils, or placebo. The primary outcome was the change in the controlled attenuation parameter (CAP) measured by fibroscan. Secondary outcomes included changes in lipid profiles. **Results**: After 12 weeks of treatment, Steatosis scores were significantly reduced with flaxseed ($p=0.014$), but not olive oil or the combined treatment. Flaxseed was the only treatment associated with a significant reduction in triglycerides ($p<0.001$). Both flaxseed and olive oil significantly increased HDL ($p=0.017$, 0.007, respectively), while combination therapy resulted in non-significant changes. All treatment arms resulted in a significant reduction ($p<0.05$) of both total cholesterol and low-density lipoprotein (LDL). **Conclusions**: Among patients with NAFLD, olive oil preferentially improved hepatic steatosis and flaxseed oil improved triglycerides. The oils had favorable effects on select metabolic parameters in NAFLD but did not impact fibrosis.

Keywords: NAFLD, CK18, Steatosis, Flaxseed, Olive oil,

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INTRODUCTION

The prevalence of non-alcoholic fatty liver disease (**NAFLD**) globally is 25% with wide geographical variation across the world. The highest prevalence rates have been reported from Middle East and South American countries (around 30%) whereas the limited number of studies from Africa reports a much lower prevalence (13%) [1]

It should be noted that NAFLD, is associated with the risk of cardiovascular diseases and metabolic abnormalities, and the progression of the disease can lead to hepatic failure and cirrhosis [2]

Flax seeds are a common food and fiber crop typically grown in cooler climates. It typically consists of 41% fat, 20% protein, and 28% fiber [3]

Flax seeds are specifically high in omega-3 polyunsaturated fatty acids (**ω-3 PUFA**), which are reported to have significant hepato-protective properties [4]

Improvement of dyslipidemia in response to supplementation with flaxseed was achieved; as plasma levels of triglyceride (TG), total cholesterol (TC), and low-density lipoprotein (LDL) decreased significantly during 12 weeks. HDL levels also increased significantly in the flaxseed group [5]

Olive oil, as part of the Mediterranean diet, is associated with benefits on human health especially regarding the cardiovascular system, obesity, diabetes and related metabolic disorders. Also, it has a beneficial effect in the prevention and treatment of NAFLD which may be due to its anti-oxidant and anti-inflammatory effects [6]

Olive oil improves insulin resistance, increases the release of triglyceride (TG) from the liver and decreases the flux of free fatty acids from peripheral adipose tissue back to the liver [7]

Cytokeratin 18 (CK-18) is the main intermediate filament protein in hepatocytes and is released upon the initiation of cell death. The association between elevated CK-18 levels and cell death in the liver has made circulating CK-18 a candidate marker for detecting NAFLD and fibrosis [8]

While olive oil and flaxseed oil have demonstrated potential benefits in NAFLD, sparse studies have directly compared their effects on hepatic steatosis, fibrosis in adult patients with NAFLD. Moreover, the treatment effects on CK18 in this patient population are unclear. Therefore, the aim of this study was to investigate and compare the effects of 12 weeks of olive oil, flaxseed oil, and combined olive oil and flaxseed oil supplementation versus placebo on hepatic steatosis measured by fibroscan controlled attenuation parameter, liver fibrosis, and lipid parameters in patients with NAFLD. Additionally, this study aimed to characterize the longitudinal changes of CK18 in response to the administered oils.

PATIENTS AND METHODS

Study Design

This was a 12-week, parallel-group, randomized, placebo-controlled trial conducted at the hepatogastroenetrology Department of Alazhar University Hospitals and Hepatology & Gastroenterology Department of Theodor Bilharz Research Institute in Egypt during the period from May 2021 to April 2023. The study was approved by the institutional ethics review board. Written informed consent was obtained from all participants.

Patients

Adult patients of both genders aged 18-65 years were recruited from hepatology clinics if they met the inclusion criteria of hepatic steatosis diagnosed by fibroscan and dyslipidemia defined as triglycerides ≥150 mg/dL, HDL cholesterol <40 mg/dL in men or <50 mg/dL in women, or LDL cholesterol ≥100 mg/dL. Exclusion criteria were age <18 or >65 years, pregnancy, chronic diarrhea, hepatitis B or C infection.

Eligible participants were randomly allocated in a 1:1:1:1 ratio to one of four arms: olive oil, flaxseed oil, olive oil + flaxseed oil, or placebo. The randomization process was adopted using computer-assisted random sequence generation. The olive oil arm (Group A) received 22 ml/day first cold pressed virgin olive oil. The flaxseed oil arm (Group B) received 45 ml/day first cold pressed virgin flaxseed oil. The combination arm received 22 ml/day of olive oil plus 45 ml/day of flaxseed oil (Group C). The placebo arm received an identically packaged decaffeinated green tea beverage (Group D). All oils and placebo were purchased from the same supplier, Imtenan Health Shop for healthy and functional foods, Obour, Egypt. Participants were instructed to ingest their assigned oil or placebo in two divided doses with meals. All arms received identical general lifestyle modification advice on diet and exercise. Allocation was concealed using sealed opaque envelopes. Participants and outcome assessors were blinded to treatment assignment.

Data collection

Demographic and medical history data collected were age, gender, ethnicity, relevant medical history, family history, and medications. We also recorded intervention details such as assignment to study arms, compliance with the assigned oil or placebo, and any adverse events. Clinical assessments documented were vital signs at each visit, anthropometric measurements (height, weight, BMI, waist circumference), and abdominal exam findings. Laboratory investigations captured included complete blood counts, liver function tests, lipid profile, inflammatory markers, and cytokeratin-18 levels. Imaging data collected were ultrasound findings and fibroscan results including CAP score and liver stiffness measurements. Follow-up data gathered were any changes to medications, supplements, dietary or exercise habits, and hospitalizations or other clinical outcomes.

Sample Collection and Assays

Serum samples were collected at baseline and 12 weeks and stored at -20°C or -80°C until analysis. Complete blood counts were performed using standard hematology analyzers. Liver enzymes (ALT, AST, ALP, GGT), lipid profiles (total cholesterol, LDL-C, HDL-C, triglycerides), C-reactive protein (CRP), and cytokeratin-18 were measured using automated clinical chemistry analyzers (Roche Diagnostics, Mannheim, Germany) with calibration as per manufacturer protocols.

Hepatic steatosis was quantified using fibro scan 502 (Echosens, France) with the M+ and XL+ probes to obtain controlled attenuation parameter (CAP) values in dB/m. Hepatic fibrosis was evaluated with the same fibroscan device to obtain liver stiffness measurements in kPa. The real-time machine with a transducer of 3.5 MHz was used.

Serum cytokeratin-18 was measured by enzyme-linked immunosorbent assay (Human Cytokeratin 18 ELISA kit, EIAab, Wuhan, China) as per the manufacturer's protocol. Optical densities were read at 450 nm using an automated microplate reader (Infinite F50, Tecan, Switzerland). Cytokeratin-18 concentrations were

calculated from the standard curve and expressed as pg/mL. The detection range of the assay was between 100 - 2400 pg/mL. No significant cross-reactivity or interference was observed.

All assays were performed by experienced technicians blinded to treatment allocation. Inter- and intra-assay coefficients of variation were within acceptable limits for all parameters (<10%).

Sample size

The sample size was calculated to detect a 50% difference between the study groups including 16 pairwise possible comparisons. The estimated sample size was 120 patients to be allocated to the study groups would provide 80% power considering two-sided alpha level of 0.05.

Statistical analysis

Descriptive statistics for all variables were represented as mean ± standard deviation (SD) and median interquartile range (IQR) for continuous data, and frequencies (with percentages) for categorical data. The Shapiro-Wilk test was used to check for data normality. Baseline characteristics across groups were compared using one-way analysis of variance (ANOVA) for normally distributed continuous data, Kruskal-Wallis ANOVA for continuous data not normally distributed, and Chi-square or Fisher's exact test for categorical data, as applicable.

The paired t-test (for continuous data) or Wilcoxon signed-rank test (for data not normally distributed) was used to assess the effect of time (pre- vs post-intervention) for continuous outcomes. Continuous variable comparisons between study groups were analyzed using Tukey's post hoc test to adjust P values for multiple comparisons.

In instances where normality assumptions were not met, nonparametric tests were employed. The Wilcoxon signed-rank test was used to compare pre-and post-treatment values within groups for non-normally distributed data. Differences across groups were analyzed using the Kruskal-Wallis test, followed by the Mann-Whitney U test for pairwise comparisons. Analysis of categorical data was performed using the Chi-square test or Fisher's exact test, as appropriate. A P value of <.05 was considered statistically significant in all analyses. All statistical analyses were conducted with SPSS version 26.0 (IBM Inc, New York, USA).

RESULTS

A total of 120 patients were randomized into four groups: olive oil (N=29), flaxseed oil (N=30), combined oils (N=30), and placebo (N=30). Baseline characteristics were comparable between groups except for lower age and hemoglobin in the combined oil group and lower platelet count in the olive oil group (Table 1).

There were no significant differences regarding sex (p=0.8) and diabetes status (p=0.3) among the study groups. Age was significantly lower in the patients who received flaxseed+ olive oil (p=0.003). A summary of baseline differences in demographic and clinical characteristics is provided in (Table 1).

Comparing steatosis before and after treatment within each study group, there was only a significant reduction in steatosis with olive oil (p=0.014). Alternatively, there were non-significant differences in the flaxseed group (p=0.7), combined olive oil+ flaxseed group (p=0.38), and placebo (p=0.22) compared to pretreatment values. Pairwise comparisons suggested that none of the study groups demonstrated significantly different steatosis compared to each other (p>0.5 for each pair of comparisons) (Figure 1).

Comparing fibroscan before and after treatment within each study group, there was only a significant reduction in fibroscan with placebo (p=0.007). Alternatively, there were non-significant differences in the flaxseed group (p=0.39), combined olive oil+ flaxseed group (p=0.14), and olive oil (p=0.71) compared to pretreatment values. Pairwise comparisons suggested that none of the study groups demonstrated significantly different fibroscan compared to each other (p>0.5 for each pair of comparisons) (Figure 2).

Lipid profile parameters demonstrated significant differences among the study groups. There were significant differences in the change from baseline values of triglycerides (p=0.005), total cholesterol (p<0.001), and LDL (p<0.001), with no significant differences in HDL levels (p=0.076) among the study groups (Table 2).

For triglycerides, pairwise comparisons suggested that flaxseed significantly improves triglycerides compared to olive oil (p=0.036), with no additional benefit from the addition of olive oil to flaxseed (p=0.348). The changes within each group demonstrated a significant triglyceride reduction when comparing pre-and post-levels for Flaxseed (p<0.001) and flaxseed + olive oil (p=0.024) (Figure 3).

For total cholesterol, pairwise comparisons suggested that none of the treatment arms provided additional benefits in reducing total cholesterol. The changes within each group demonstrated a significant total cholesterol reduction when comparing pre-and post-levels for Flaxseed (p=0.003), olive oil (p<0.001), flaxseed + olive oil (p<0.001), and placebo (p=0.006) (Figure 4).

For LDL, pairwise comparisons suggested that none of the treatment arms provided additional benefits in reducing LDL. The changes within each group demonstrated a significant LDL reduction when comparing pre- and post-levels for Flaxseed ($p<0.001$), olive oil ($p<0.001$), and flaxseed + olive oil ($p=0.005$) (Figure 5).

For HDL, pairwise comparisons suggested that none of the treatment arms provided additional benefits in increasing HDL. The changes within each group demonstrated a significant HDL increase when comparing pre- and post-levels for Flaxseed ($p=0.017$), and olive oil ($p=0.007$), while flaxseed + olive oil ($p=0.09$) or placebo ($p=0.8$) did not change significantly after treatment (Figure 6).

Comparing CK18 levels before and after treatment within each study group, there was a nonsignificant difference in CK18 levels with flaxseed oil ($p=0.49$), olive oil ($p=0.054$), the combined olive oil+ flaxseed group ($p=0.7$) and the placebo group ($p=0.51$) compared to pretreatment values. Pairwise comparisons suggested that none of the flaxseed oil, olive oil, or combined olive oil+ flaxseed group achieved a significant difference in CK18 levels compared to each other ($p>0.5$ for each pair of comparisons) (Figure 7).

Table 1: Comparing baseline demographic, laboratory, and clinical characteristics among the study groups (N=119).

Characteristic	Flaxseed oil (n=30)	Olive oil (n=29)	Olive oil + Flaxseed oil (n=30)	Placebo (n=30)	*p*[1]
Age, years	45.5±6.6	45.7±6.9	39.4±9.0	46.2±6.7	**0.003**
Sex, female	19 (63.3%)	20 (69%)	22 (73.3%)	22 (73.3%)	0.8
Diabetes	7 (23.3%)	6 (20.7%)	7 (23.3%)	2 (6.7%)	0.3
ALT, IU/L	31.0±11.5	30.4±11.6	26.7±9.7	28.5±12.3	0.5
AST, IU/L	28.2±10.6	30.2±11.1	25.8±9.4	25.8±10.7	0.4
ALP, IU/L	72.1±26.9	70.1±22.3	72.9±25.3	89.6±35.7	0.077
GGT, IU/L	27.3±10.9	22.6±8.5	26.9±8.6	27.9±6.9	0.12
CK18, U/L	560.0±633.7	237.4±127.3	577.5±620.2	281.7±227.9	0.10
Triglycerides, mg/dL	178.9±36.4	169.2±45.3	191.7±68.8	189.1±51.8	0.4
Total cholesterol, mg/dL	295.6±50.1	303.5±42.1	288±47.5	277.5±46.3	0.2
Staetosis score, kPa	319.8±42.2	329.8±36.6	339.3 ±36.4	314.1±38.2	0.069
Firoscan	5.0±0.9	5.2±1.7	6.1±3.0	5.0±1.3	0.6
LDL, mg/dL	201.2±51.4	194.8±47.7	214.3±64.5	202.1±51.0	0.5
HDL, mg/dL	40.1±7.7	39.3±8.1	39.4±9.3	45.5±9.6	0.066
Hemoglobin, g/dL	12.4±1.2	13.4±1.6	12.1±1.4	12.9±1.4	**0.003**
WBC count	6.6±2.7	7.6±2.9	7.2±1.8	7.0±2.6	0.5
Platelets	296.9±75.7	251.6±62.7	298.2±54.2	290.7±47.4	**0.024**
CRP, mg/dL	0.9±2.2	1.0±2.7	0.7±2.4	1.0±3.7	0.6
High CRP	5 (17%)	5 (17%)	3 (10%)	2 (7%)	0.6

Data are mean ± SD or n (%)

[1] p-value calculated using Kruskal-Wallis rank sum test.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; CK18, cytokeratin 18; LDL, low density lipoprotein; HDL, high density lipoprotein; WBC, white blood cell; CRP, C-reactive protein.

Table (2): Comparing the effects of different oils on liver functions, lipid profile, and inflammatory characteristics of the study groups (N=119).

characteristics	Flaxseed oil			Olive oil			Olive oil + Flaxseed oil			Placebo			$p^{2,3}$
	Pre	Post	p^1	Pre	Post	p^1	Pre	Post	p^1	Pre	Post	p^1	
ALT, IU/L	31.0±11.5	30.5±10.2	0.6	30.4±11.6	30.3±9.5	0.87	26.7±9.7	26.2±9.1	0.51	28.5±12.3	28.7±10.8	0.68	0.9
AST, IU/L	28.2±10.6	28.2±9.9	0.99	30.2±11.1	30.6±10.0	0.52	25.8±9.4	25.1±9.0	0.29	25.8±10.7	26.4±10.8	0.17	0.4
ALP, IU/L	72.1±26.9	73.8±27.0	0.44	70.1±22.3	70.8±22.4	0.75	72.9±25.3	71.1±23.0	0.29	89.6±35.7	89.4±35.2	0.66	0.6
GGT, IU/L	27.3±10.9	28.6±10.5	0.08	22.6±8.5	23.3±8.4	0.43	26.9±8.6	26.3±9.4	0.91	27.9±6.9	26.9±5.8	0.19	0.2
Steatosis score, dB/m	329.8±36.6	325.0±56.2	0.7	339.3±36.4	314.7±44.6	**0.014**	314.1±38.2	310.2±35.6	0.38	319.8±42.2	327.4±33.9	0.22	0.05
Fibroscan, kPa	5.2±1.7	4.9±1.4	0.39	6.1±3.0	5.9±2.7	0.71	5.0±1.3	4.8±1.7	0.14	5.0±0.9	4.5±0.8	**0.007**	0.6
CK18, U/L	560.0±634	432.8±470.7	0.49	237±127	182.2±104.4	0.054	578±620	557.2± 652.1	0.7	282±228	264.82±188.2	0.51	0.14
Triglycerides, mg/dL	178.9±36.4	147.0±33.5	**<0.001**	169.2±45.3	158.0±52.7	0.05	191.7±68.8	165.7±40.9	0.024	189.1±51.8	247.7±347.3	0.2	**0.005**
Cholesterol, mg/dL	295.6±50.1	271.1±63.6	**0.003**	303.5±42.1	263.7±55.7	**<0.001**	288±47.5	260.3±54.1	**<0.001**	277.5±46.3	294.5±38.6	**0.006**	**<0.001**
LDL, mg/dL	201.2±51.4	180.6±52.0	**<0.001**	194.8±47.7	170.0±54.5	**<0.001**	214±64	197.8±60.0	**0.005**	202±51	201.3±53.7	0.69	**<0.001**
HDL, mg/dL	40.1±7.7	43.1±8.3	**0.017**	39.3±8.1	42.9±9.4	**0.007**	39.4±9.3	41.6±9.0	0.09	45.5±9.6	45.4±10.2	0.8	0.076
Hemoglobin, g/dL	12.4±1.2	12.5±1.4	0.18	13.4±1.6	13.1±1.4	0.064	12.1±1.4	12.0±1.5	0.49	12.9±1.4	12.8±1.8	0.89	0.3
WBC (10^3 cell/mm^3)	6.6±2.7	7.3±2.4	**0.048**	7.6±2.9	7.0±1.8	0.16	7.2±1.8	6.8±2.0	0.49	7.0±2.6	6.5±1.9	0.75	0.067
Platelets (10^3 /mm^3)	296.9±75.7	297.6±65.8	0.89	251.6±62.7	253.6±59.5	0.98	298.2±54.2	301.6±51.6	0.69	290.7±47.4	265.7±69.0	**0.028**	0.2

[1] p-value calculated using Wilcoxon signed rank test for paired data.
[2] p-value calculated using Kruskal-Wallis rank sum test.
[2] Global p-value representing the differences among the groups at the end of treatment.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; CK18, cytokeratin 18; LDL, low density lipoprotein; HDL, high density lipoprotein; WBC, white blood cell.

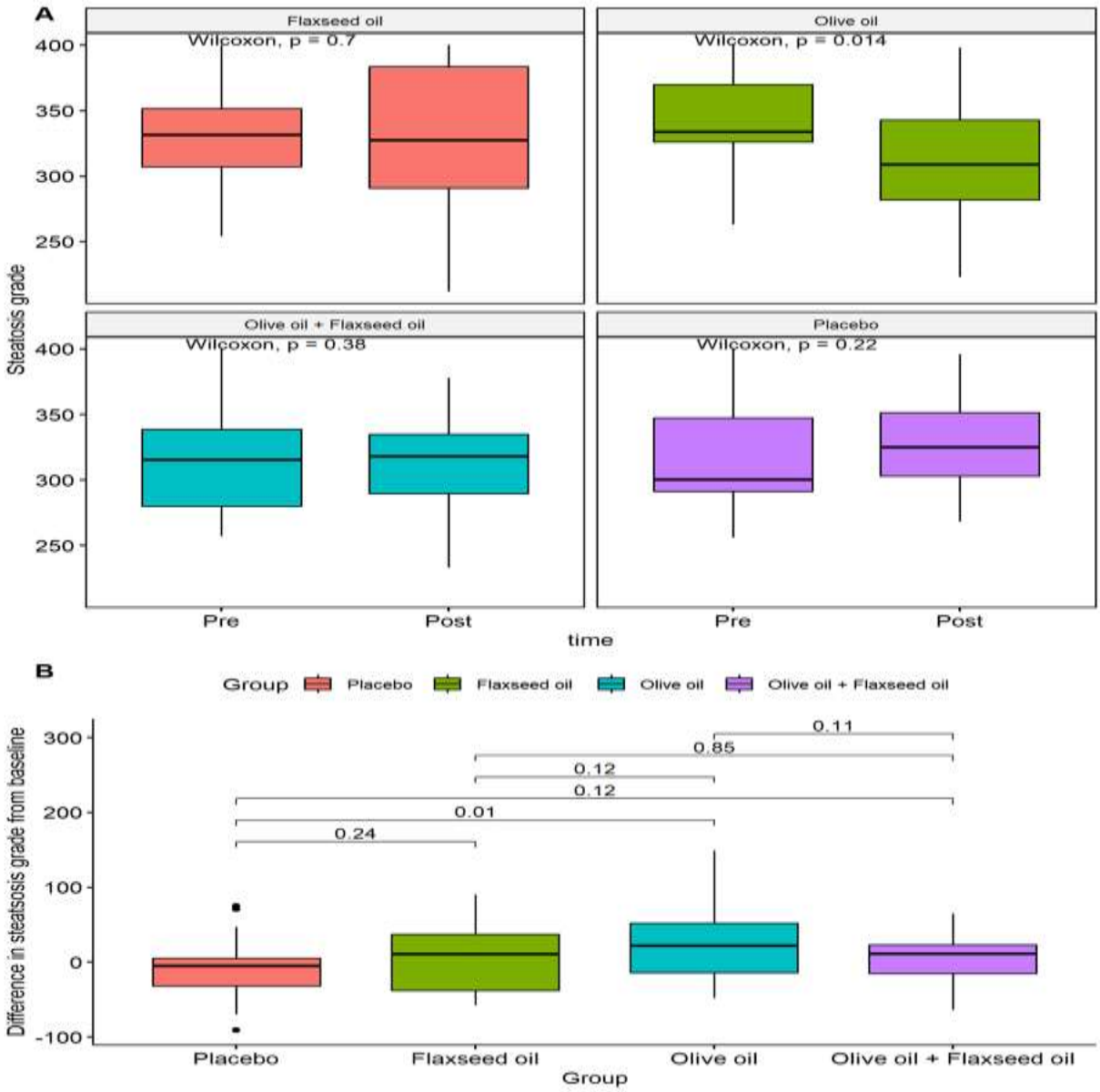

Figure 1: Comparing steatosis among the study groups. A. Boxplots demonstrating the steatosis among the study groups before and after treatment. B. Boxplots demonstrating the differences in steatosis over follow-up time among the study groups with pairwise comparison comparing each group with the others. The calculated p-values for each pairwise comparison were corrected using Bonferroni correction.

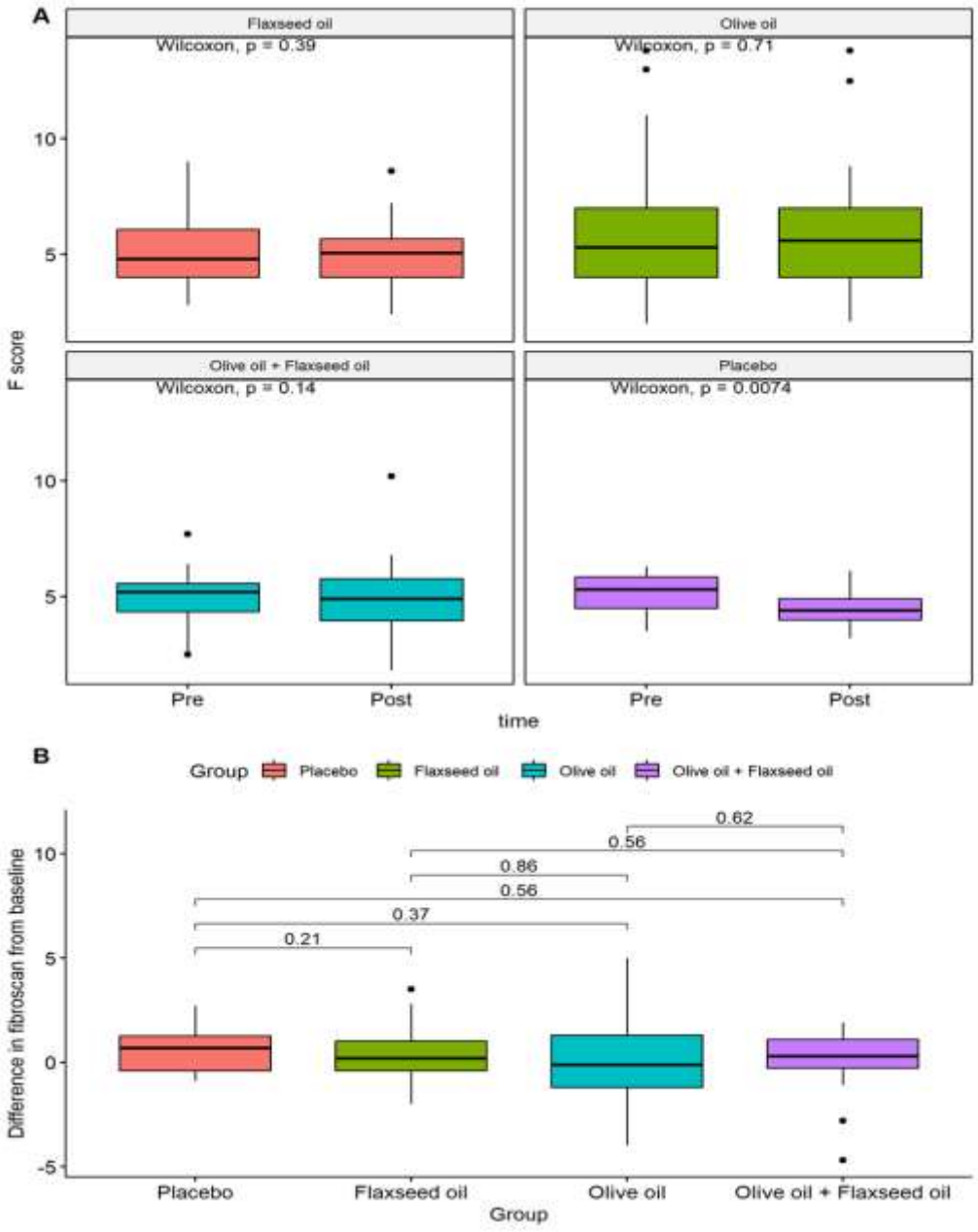

Figure 2: Comparing fibroscan among the study groups. A. Boxplots demonstrating the fibroscan among the study groups before and after treatment. B. Boxplots demonstrating the differences in fibroscan over follow-up time among the study groups with pairwise comparison comparing each group with the others. The calculated p-values for each pairwise comparison were corrected using Bonferroni correction.

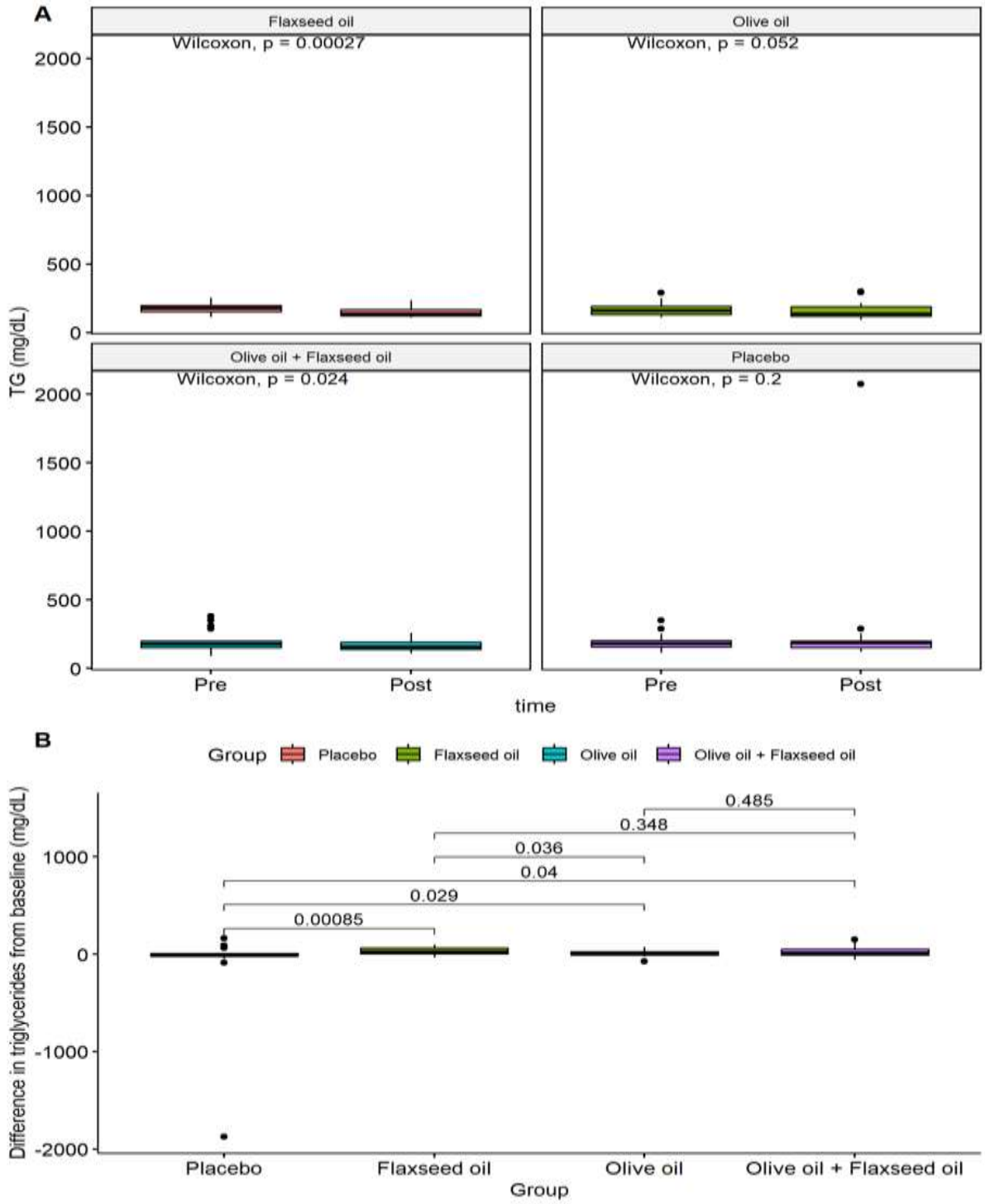

Figure 3: Comparing triglyceride levels among the study groups. A. Boxplots demonstrating the triglyceride levels among the study groups before and after treatment. B. Boxplots demonstrating the differences in triglyceride levels over follow-up time among the study groups with pairwise comparison comparing each group with the others. The calculated p-values for each pairwise comparison were corrected using Bonferroni correction.

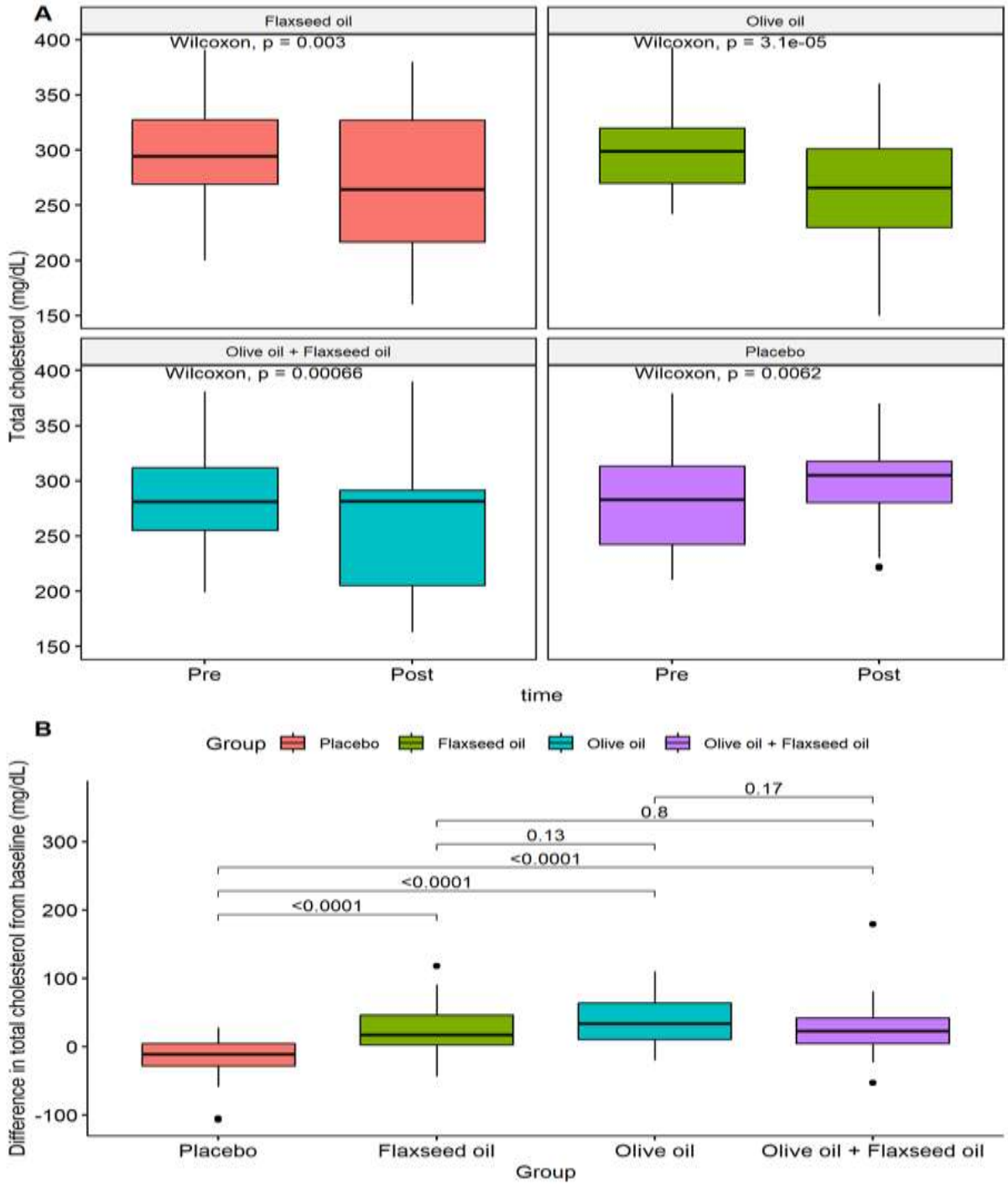

Figure 4: Comparing total cholesterol levels among the study groups. A. Boxplots demonstrating the total cholesterol levels among the study groups before and after treatment. B. Boxplots demonstrating the differences in total cholesterol levels over follow-up time among the study groups with pairwise comparison comparing each group with the others. The calculated p-values for each pairwise comparison were corrected using Bonferroni correction.

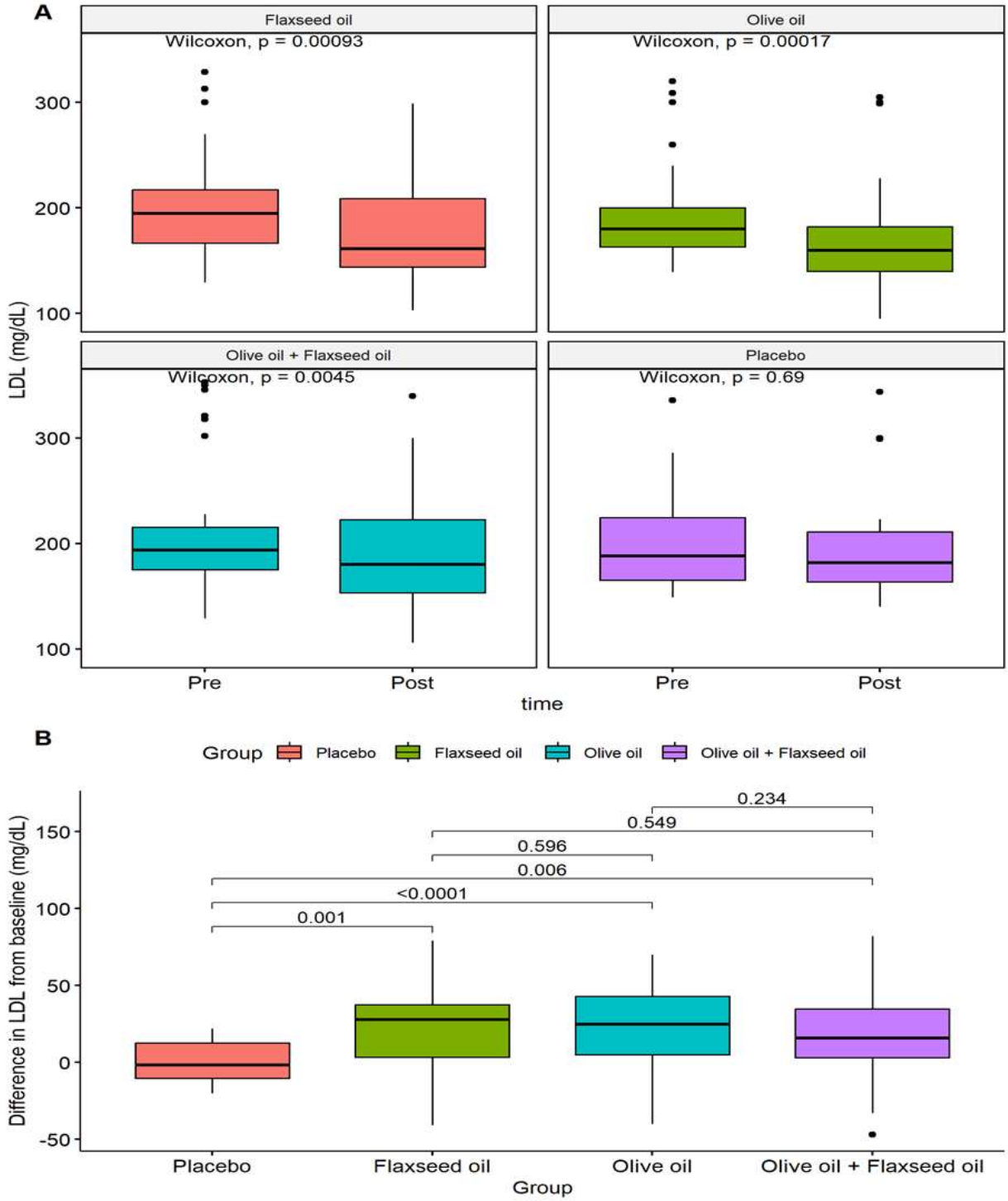

Figure 5: Comparing LDL levels among the study groups. A. Boxplots demonstrating the LDL levels among the study groups before and after treatment. B. Boxplots demonstrating the differences in LDL levels over follow-up time among the study groups with pairwise comparison comparing each group with the others. The calculated p-values for each pairwise comparison were corrected using Bonferroni correction.

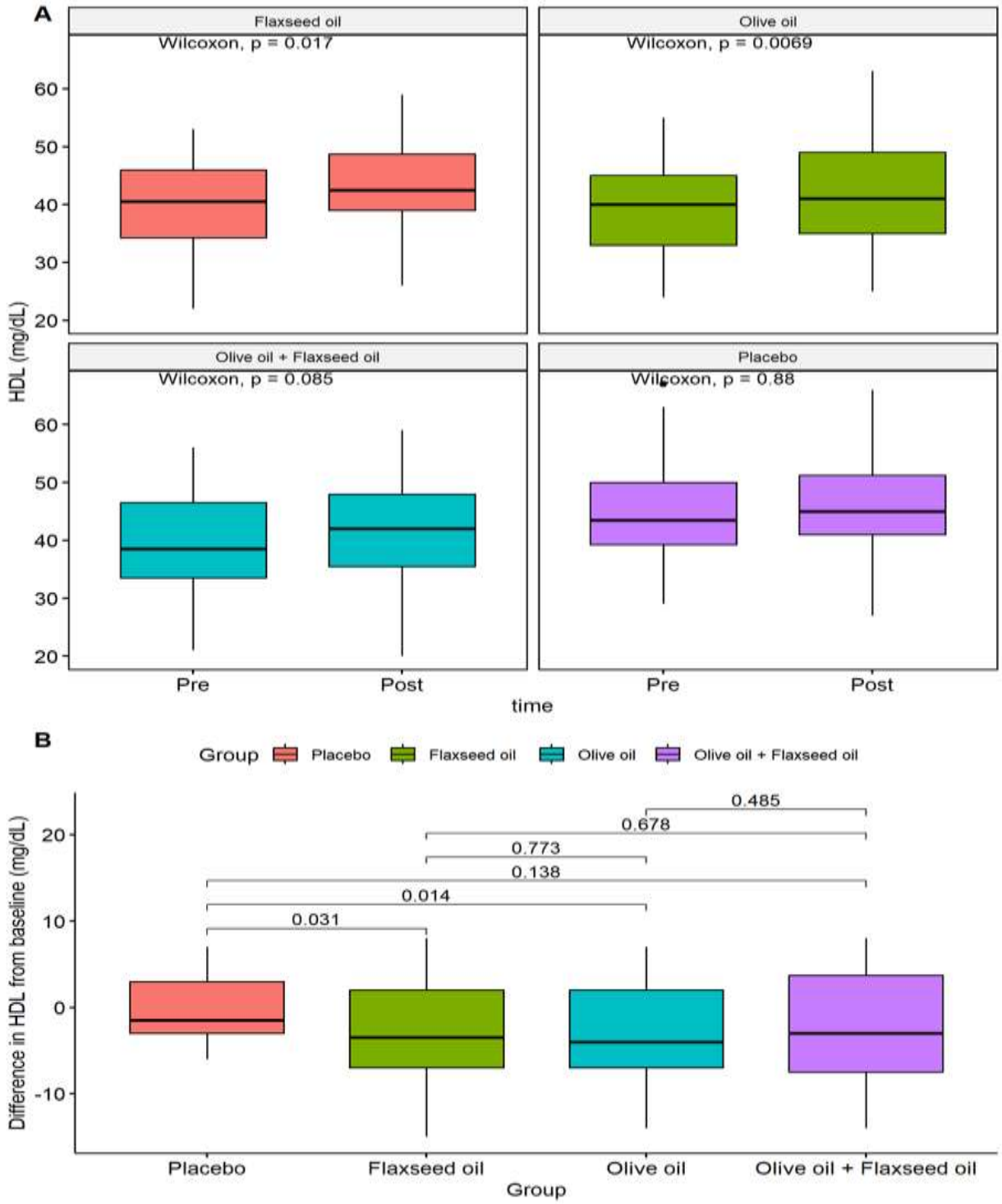

Figure 6: Comparing HDL levels among the study groups. A. Boxplots demonstrating the HDL levels among the study groups before and after treatment. B. Boxplots demonstrating the differences in HDL levels over follow-up time among the study groups with pairwise comparison comparing each group with the others. The calculated p-values for each pairwise comparison were corrected using Bonferroni correction.

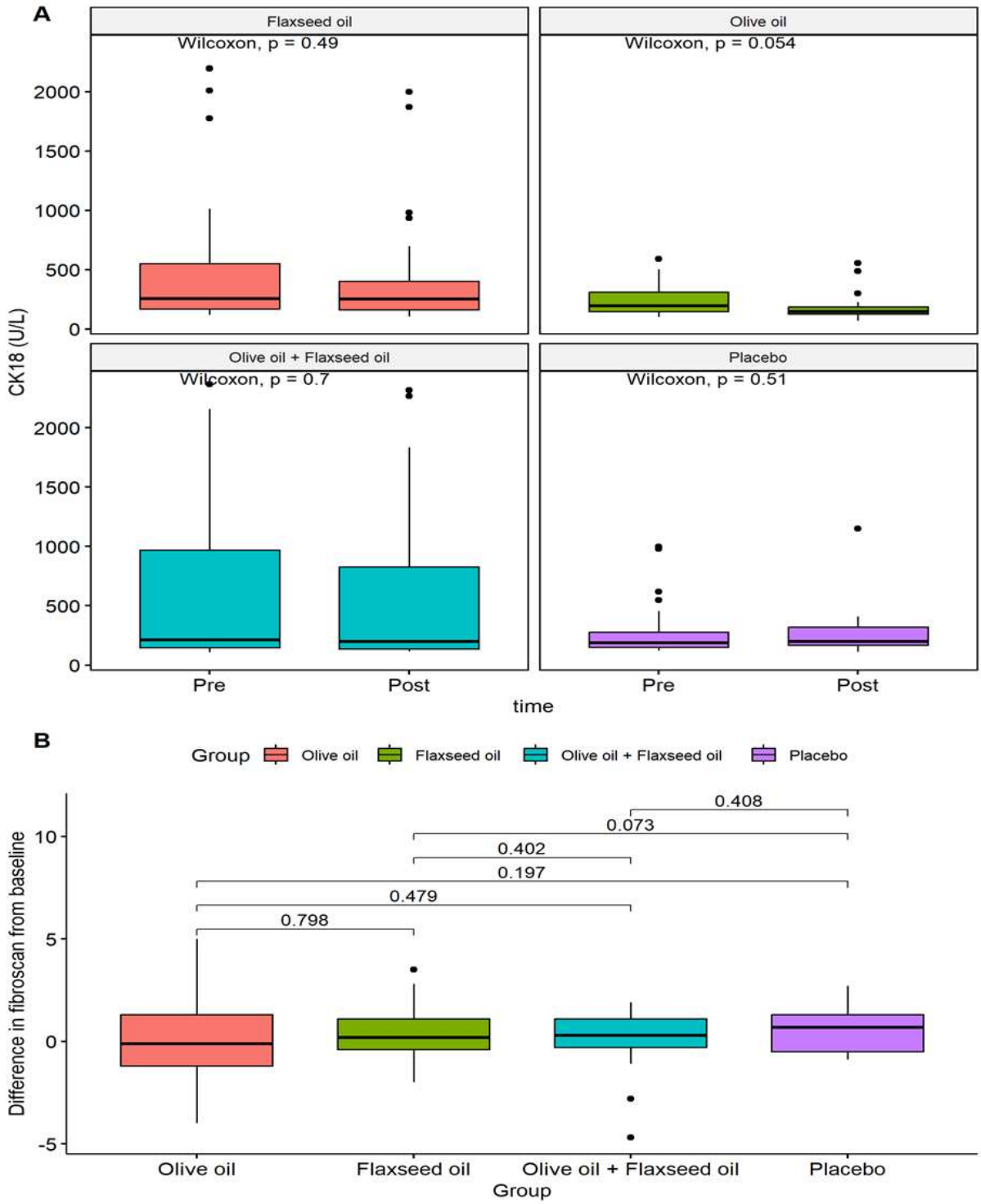

Figure 7: Comparing CK18 levels among the study groups. A. Boxplots demonstrating the CK18 levels among the study groups before and after treatment. B. Boxplots demonstrating the differences in CK18 levels over follow-up time among the study groups with pairwise comparison comparing each group with the others. The calculated p-values for each pairwise comparison were corrected using Bonferroni correction.

DISCUSSION

The present study was a randomized controlled clinical trial that aimed to investigate the effects of flaxseed oil, olive oil, and a combination of flaxseed oil and olive oil on lipid profiles in patients with NAFLD. A total of 119 patients with NAFLD were randomly assigned to receive flaxseed oil (45 mL/day), olive oil (22 mL/day), or a combination of flaxseed oil and olive oil for 12 weeks.

The main findings of the current study were the significant effect of flaxseed oil, and olive oil, and

their combinations on the improvement of lipid profiles of NAFLD patients, including low-density lipoproteins (LDL) and total cholesterol (TC).

The effect of flaxseed oil was significantly higher among the study groups in reducing TG, while olive oil demonstrated a slightly greater increase in HDL. The combined treatment with both oils offered no additional benefit over individual oils, rather, it was associated with a reduced HDL compared to individual oils.

The effects on liver functions, steatosis, fibrosis, and CK 18 were insignificant, except for the olive oil, which demonstrated a significant reduction in the steatosis score at the end of treatment.

There were non-significant differences in the effects on the markers of inflammation, suggesting limited or absent anti-inflammatory effects of the studied oils.

Olive oil demonstrated a significant reduction of steatosis score (p=0.014), with non-significant effects on liver functions and fibrosis in the current study. In line with the current finding, Pinto et al. (PREDIMED study) conducted a randomized parallel group clinical trial, including 100 subjects at high cardiovascular risk who were randomly assigned to a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, or a control diet. After a median follow-up of 3 years, the prevalence of hepatic steatosis was significantly lower in the group following the Mediterranean diet supplemented with extra-virgin olive oil compared to the other two groups [9]

Unexpectedly, we found a significant reduction in F scores within the placebo group (p=0.0074) after treatment. This might be attributed to several factors. First, the regression to the mean phenomenon which is a statistical discrepancy that occurs when extreme initial measurements tend to be closer to the mean in subsequent measurements [10]. Therefore, patients with high baseline F scores might show improvement by chance, while patients with low baseline F scores might show worsening, leading to a reduction in F scores in the placebo group. Second, the Hawthorne effect, where patients who participate in clinical trials might change their behavior due to the awareness of being observed or receiving medical attention [11]. They might become more adherent to healthier lifestyle choices, such as better diet and exercise habits, during the trial. These changes could lead to improvements in liver health and reduced F scores in the placebo group. Third, the natural course of some liver diseases can involve periods of improvement followed by deterioration. The observed reduction in F scores in the placebo group could be the result of the natural fluctuation of the fibrosis rather than the effect of the placebo itself [12]. Fourth, the observed reduction may be attributed to the effect of concomitant treatments, where patients in the placebo group might be receiving other medications or treatments for their liver disease or related conditions during the trial. These additional interventions could influence the F scores independent of the placebo effect.

It is worth mentioning that to the best of our knowledge, the non-significant improvement of fibrosis with both oils despite the significant improvement of steatosis with olive oil treatment may be related to the short duration of the current study. In other terms, Liver fibrosis, in particular, is a slow process that develops over time and reversing or halting its progression may require a longer intervention period [13].

The studied oils demonstrated a significant impact on the lipid profiles of the included patients at the end of treatment period. The current results suggested that flaxseed oil significantly reduced triglycerides (by 31.9 mg/dL), total cholesterol (by 24.5 mg/dL) and LDL (by 20.6 mg/dL) compared to baseline values. Alternatively, Olive oil did not significantly reduce triglycerides compared to baseline, but it did significantly reduce total cholesterol (by 39.7 mg/dL) and LDL (by 24.8 mg/dL). The flaxseed oil + olive oil combination significantly reduced triglycerides (by 25.9 mg/dL), total cholesterol (by 27.7 mg/dL) and LDL (by 16.5 mg/dL) compared to baseline levels. HDL was significantly increased by both olive oil (by 3.5 mg/dL) and flaxseed oil (by 3 mg/dL). In line with the current findings, Nigam et al. demonstrated a significant increase in HDL level and a non-significant decrease in triglyceride levels in the olive oil group of NAFLD patients [14].

Our findings are also consistent with those of Ghobadi et al. who conducted a meta-analysis of randomized controlled trials to investigate the effects of olive oil consumption compared to other plant oils on blood lipids. The study included 27 randomized controlled trials with a total of 1,059 participants. The authors found that olive oil consumption had a significant effect on reducing total cholesterol ($p<0.001$), LDL cholesterol ($p<0.001$), and triglycerides ($p<0.001$) compared to other plant oils [15]. Despite the coincident findings, Ghobadi et al. inconsistently reported a significant reduction of TGs with olive oil. This could be attributed to the inclusion of diverse studies, populations, doses, and duration of treatment in their meta-analysis. Similarly, Al Jamal & Ibrahim showed that four weeks of consumption of olive oil significantly lowered the levels of TGs, total cholesterol, and LDL in dyslipidemic diabetic patients [16]. Khan et al. demonstrated that extra virgin olive oil showed 14-25% reduction in plasma lipids with 8-12% increase in HDL-cholesterol level after six weeks of treatment of adult diabetic patients with dyslipidemia [17]. The significant reduction of TGs in these two studies

with olive oil treatment cannot be directly compared to the current study since they exclusively included a different population (diabetic patients), who were underrepresented in the current study (only 18% of the included patients in the current study were diabetic). The reduction of TG posed by olive oil administration was also shown by Rezaei et al., who reported a significant reduction of serum TG after the ingestion of olive oil. However, this reduction in TG levels cannot be attributed confidently to olive oil only; since they applied a hypocaloric diet in their study, which may be also a confounding reason for reducing hypertriglyceridemia [18].

For flaxseed oil, in line with our findings, Saxena et al. evaluated 50 dyslipidemic subjects allocated between flaxseed oil and control group. Both groups were prescribed similar dietary guidelines. The experimental group received 30 g of roasted flaxseed powder for 3 months. A highly significant reduction in total cholesterol, triglycerides, LDL-C, and VLDL-C levels, with simultaneous elevation in HDL-C levels was observed within the flaxseed oil group [19]. Similarly, Pan et al. conducted a meta-analysis of randomized controlled trials to investigate the effects of flaxseed and its derivatives on blood lipid profiles. Their results showed that flaxseed interventions significantly reduced total and LDL cholesterol concentrations, particularly in females and individuals with high initial cholesterol concentrations. No significant changes were found in the concentrations of HDL cholesterol and triglycerides [20]. The inconsistent non-significant effect of flaxseed on HDL and TGs could be related to the difference in demographic characteristics and the use of different forms of flaxseed other than the oil in the included studies.

Surprisingly, the effect of combination oil therapy demonstrated a weaker effect compared to individual treatments. Despite that, both flaxseed oil and olive oil significantly increased HDL compared to baseline values. However, flaxseed plus olive oil did not significantly change HDL. Despite not being reported in the literature, several hypotheses could explain the observed attenuated response to the combination of flaxseed oil and olive oil, as opposed to their individual administration. The interaction of diverse bioactive compounds, each possessing distinct properties and modes of action, could potentially interfere with or neutralize their individual benefits when these oils are combined. Furthermore, the body's finite capacity to absorb and metabolize the active constituents of both oils may lead to competition for absorption, diminishing the overall efficacy of each oil. The ingredients of PUFA in flaxseed oil and MUFA in olive oil may compete on the metabolizing enzymes required for the production of the endogenously active forms, resulting in a diminished response compared to when each oil is administered independently. For instance, MUFAs need stearoyl-CoA desaturase-1 (SCD1) to produce oleic acid and palmitoleic acid, which is shown to be suppressed by PUFA intake [21]

The current study has many different implications on the clinical management of NAFLD. First, the selection of individual therapies should be tailored on a case-by-case basis. Based on the current study findings, flaxseed oil only should be indicated if the patient demonstrated elevated triglycerides without a significant associated hepatic steatosis. Alternatively, olive oil only should be administered if the patients demonstrated evidence of steatosis without hypertriglyceridemia. Combined oils accordingly should be indicated if the patient presents with both hypertriglyceridemia and hepatic steatosis. Second, the reduced effect of the combined oils suggests the need to separate the administration of each oil from the other by two hours to minimize the possible conflicting effects on the absorption of each other. In particular, patients should be educated about this tip prior to starting the administration of the oils combination. Third, the preferential effects of the studied oil concluded from the current study may provide evidence for a safer and more cost-effective alternative to statin therapy in the management of hypercholesterolemia, which is well-known to cause serious hepatotoxicity and myopathy as a common adverse effect [22].

However, there are certain limitations to consider. The short 12-week duration, though suitable for assessing metabolic changes, may be insufficient to evaluate improvements in fibrosis. The findings are restricted to surrogate metabolic markers, and longer-term studies with histological outcomes are needed. Nevertheless, this study provides good preliminary evidence on the lipid-lowering and anti-steatotic potential of these natural oils in NAFLD management. Future studies should investigate the effects of these oils in a longer duration of treatment with an adequate sample size to characterize the possible cardiovascular benefits and anti-fibrotic effects of their use.

CONCLUSION

In patients with NAFLD, olive oil improved liver fat, and flaxseed oil enhanced lipid profiles over 12 weeks. These readily available natural oils could complement lifestyle changes in NAFLD management. Olive oil may help in reducing steatosis and disease progression. Flaxseed oil offers cardiovascular benefits by improving triglycerides and HDL. These findings support incorporating olive oil and flaxseed oil into dietary counseling for patients with NAFLD and dyslipidemia.

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