EFFECT OF EMPAGLIFLOZIN VS PIOGLITAZONE VS METFORMIN WITH URSODEOXYCHOLIC ACID AND VITAMIN E IN NON-ALCOHOLIC FATTY LIVER DISEASE AND NON-ALCOHOLIC STEATOHEPATITIS IN EGYPTIAN PATIENTS WITHOUT DM

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming the most common cause of chronic liver disease and is now among the top causes of cirrhosis, hepatocellular carcinoma (HCC). No pharmacotherapies are approved for NAFLD in non-diabetic patients. **Aim:** We aimed to compare empagliflozin to pioglitazone and a combination of metformin, Ursodeoxycholic acid, and vitamin E (MET+UDCA+VitE) in nondiabetic patients with NAFLD, and to assess the impact of these therapies on autotaxin and cytokeratin 18. **Methods:** In this 12-month randomized, parallel, placebo-controlled trial, 200 non-diabetic adults with NAFLD were allocated to empagliflozin 10 mg daily (n=50), pioglitazone 15 mg daily (n=50), MET+UDCA+VitE (n=50), or placebo (n=50). The primary outcome was the change in steatosis grade on transient elastography. **Results:** Empagliflozin significantly reduced BMI ($p<0.001$), steatosis grade $p<0.001$), liver stiffness ($p= 0.008$), total cholesterol ($p<0.001$), LDL cholesterol ($-p<0.001$), white blood cells ($p=0.029$), cytokeratin-18 ($p<0.001$), and autotaxin ($-p<0.001$) versus compared to other treatment arms. Triglycerides decreased with pioglitazone ($p<0.001$) and MET+UDCA+VitE ($p<0.001$) only. No changes in aminotransferases were demonstrated with any treatment arm. **Conclusions:** In non-diabetic NAFLD patients, 12 months of empagliflozin treatment improved metabolic, inflammatory, and fibrotic parameters versus pioglitazone or MET+UDCA+VitE. The pleiotropic effects indicate empagliflozin may target pathological pathways beyond glucose control.
Running title: Empagliflozin vs. Pioglitazone for NAFLD.

Keywords: NAFLD; empagliflozin; pioglitazone; metformin; fibrosis.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become a major public health issue with a global prevalence estimated at 25%, though rates vary geographically (***Younossi et al.***, ***2016***). While the highest prevalence is reported in Middle Eastern and South American countries, African nations show lower estimates (***Younossi et al.***, ***2016***). Even early-stage NAFLD confers a significant cardiovascular risk and metabolic abnormalities, progressing to cirrhosis and liver failure in some patients (***S. Y. Tang et al.***, ***2023***). With lifestyle interventions alone limited by poor long-term adherence (***Schuppan & Schattenberg***, ***2013***), there is an unmet need for pharmacotherapy options.

For non-diabetic NAFLD, vitamin E is the first-line therapy based on its ability to prevent decompensation and the need for transplantation in advanced fibrosis (***Vilar-Gomez et al.***, ***2020***). However, vitamin E monotherapy does not improve histological outcomes in diabetes associated NAFLD. A combination of vitamin E and pioglitazone performs better than a placebo for diabetic NAFLD (***Bril et al.***, ***2019***). Alternatively, metformin remains first-line pharmacotherapy for diabetes despite no NAFLD benefit (***Mantovani et al.***, ***2020***). Thus pioglitazone is the only established drug for diabetes-related NASH (***Mantovani et al.***, ***2020***).

Beyond diabetes medications, sodium-glucose cotransporter 2 (SGLT2) inhibitors show pleiotropic effects in animal models and clinical trials, improving glycemic control, body weight, macrovascular events, and liver dysfunction (***Cusi et al.***, ***2019***). Though liver biopsy remains the gold standard NAFLD diagnostic (***Darweesh et al.***, ***2019***), circulating cytokeratin-18 (CK18) and autotaxin are emerging non-invasive biomarker for

fibrosis (***Cusi et al.***, ***2014***). Better understanding cytokeratin-18 and other novel markers could enhance non-invasive diagnosis, staging, and treatment monitoring in NAFLD. Given the sparse evidence of the efficacy of these medications in management of NAFLD in non-diabetic adult population, **the aim of the current study** is to compare empagliflozin against pioglitazone and a triple regimen of metformin, Ursodeoxycholic acid, and vitamin E (Metformin+UDCA+vitE) in this patient population, and to investigate the treatment effects on novel biomarkers including autotaxin and cytokeratin 18, which could further inform the development of personalize non-invasive diagnostic and prognostic tools for NAFLD management.

PATIENTS AND METHODS

Study Design

This was a 12-month, randomized, placebo-controlled, parallel-group trial conducted at Theodor Bilharz Research Institute and Al-Azhar University Hospitals in Egypt. The study design followed CONSORT guidelines for randomized controlled trials. The trial consisted of a 2-week screening period and a 12-month treatment period. Participants attended study visits at screening, baseline (week 0), and after 12 months of treatment (week 52). The study was approved by the Research Ethics Committee of Al-Azhar University and Theodor Bilharz Research Institute.

Participants

Adults aged 20-70 years diagnosed with NAFLD on prior abdominal ultrasound were enrolled in the period between April 2021 to April 2023. Inclusion criteria were age 20-70 years and ultrasonographic evidence of hepatic steatosis. Exclusion criteria were age <18 years, pregnancy or breastfeeding, positive serology for hepatitis B or C, known diagnosis of diabetes mellitus (HbA1c ≥6.5% or fasting glucose ≥126 mg/dL), liver cirrhosis, stage 4-5 chronic kidney disease (estimated glomerular filtration rate <30 mL/min/1.73m2), and BMI >45 kg/m^2.

Eligible participants provided written informed consent prior to participation. Demographic information, medical history, medication use, vital signs, and anthropometric measurements were collected at the screening visit.

Treatment

Patients were randomly assigned in a 1:1:1:1 ratio to receive empagliflozin, pioglitazone, combination therapy, or placebo. The empagliflozin group included 50 patients who received oral empagliflozin 10 mg once daily for 12 months. The pioglitazone group included 50 patients who received oral pioglitazone 15 mg once daily for 12 months. The combination therapy group included 50 patients who received oral metformin 500 mg twice daily, oral ursodeoxycholic acid 250 mg twice daily, and oral vitamin E 400 IU once daily for 12 months. The placebo group included 50 patients who received identical oral placebo pills once daily for 12 months. All patients were advised to maintain their usual diet and physical activity during the study period. Compliance was assessed through pill counts at follow-up visits. Randomization was performed by computer-generated permuted block sizes of 4.

Study Procedures and data collection

At the screening visit, informed consent was taken, and the data including demographic information, medical history, and medication use were collected. Physical examination included measurement of height, weight, BMI, and blood pressure. Blood samples were collected for complete blood count, liver enzymes, lipid profile, fasting glucose, HbA1c, hepatitis B and C serology, and renal function tests. Abdominal ultrasound and transient elastography were performed to confirm hepatic steatosis and rule out cirrhosis. The randomization schedule was prepared by an independent statistician using computer-generated block randomization. Sequentially numbered, sealed opaque envelopes concealed the allocation sequence until interventions were assigned.

Outcomes:

The primary outcome was change in steatosis grade on CAP. Secondary outcomes included change in BMI, liver stiffness on elastography, ALT, AST, GGT, ALP, total cholesterol, LDL, HDL, triglycerides, hemoglobin, white blood cells, platelets, C-reactive protein, cytokeratin-18 (CK 18), and autotaxin.

Assays

CK-18 was quantified by enzyme-linked immunosorbent assay (ELISA) using the CK-18 ELISA kit (EIAab Science, China). Absorbance was measured at 450 nm. CK-18 concentration was determined by comparing sample optical density to a standard curve. Results were reported as U/L.

Serum autotaxin was measured using an ELISA kit (R&D Systems, USA). The optical density was measured at 450 nm. Autotaxin concentration was calculated by comparison to a standard curve. Results were reported in ng/mL.

High-sensitivity C-reactive protein (hsCRP) was measured by latex-enhanced immunoturbidimetric assay on a Roche Cobas 6000 analyzer (Roche Diagnostics, Switzerland). Results were reported in mg/L, with a normal range 0-5 mg/L.

Sample Size Calculation

The sample size was determined using R software (Version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria) based on the primary outcome of change in steatosis grade on CAP. Assuming a 20% difference between groups, power of 80%, and alpha error of 0.05, a sample size of 50 participants per group (200 total) was required.

Statistical Analysis

Continuous variables were summarized as mean ± standard deviation for normally distributed data and median (interquartile range) for non-normal data. Categorical variables were presented as counts and percentages.

Normality was assessed using the Shapiro-Wilk test and visual inspection of histograms. One-way ANOVA was used to compare normally distributed continuous variables between groups at baseline, while the Kruskal-Wallis test was applied for non-normal variables. Categorical variables were compared using the Chi-square test or Fisher's exact test.

The within-group change from baseline was evaluated using paired t-test for normally distributed data or Wilcoxon signed-rank test for non-normal data. Between-group differences were assessed by one-way ANOVA with post hoc Tukey test or Kruskal-Wallis test followed by Wilcoxon test. Missing data was handled using the last observation carried forward imputation. All analyses were performed using SPSS (version 26.0, IBM, NY, USA) on an intention-to-treat basis. Data visualization was adopted using R software (Version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study Participants

A total of 205 patients were screened for eligibility. Five patients were excluded for not meeting inclusion criteria. The remaining 200 eligible patients were randomized into four groups: empagliflozin (n=50), pioglitazone (n=50), metformin + ursodeoxycholic acid + vitamin E (n=50), and placebo (n=50). All patients completed the 12-month study period with no loss to follow-up. Baseline characteristics were balanced across treatment arms (Table 1), with exception to BMI, hemoglobin levels, and platelet count. The mean BMI was 31.7 kg/m2, ranging from 29.2 kg/m^2 in the empagliflozin group to 32.4 kg/m^2 in the placebo group ($p<0.001$). Liver enzymes, fibrosis markers, lipid profiles, and hematologic parameters did not significantly differ between groups at baseline, except for higher triglycerides in the placebo group ($p=0.016$), lower hemoglobin in Metformin + UDCA + Vitamin E group ($p=0.004$), and lower platelets count in empagliflozin group ($p=0.005$).

Metabolic Parameters

Compared to baseline, BMI significantly decreased with only empagliflozin (-1.7±1.7 kg/m2, $p<0.001$), while MET+UDCA+VitE, pioglitazone, or placebo did not result in a significant change in BMI at the end of treatment (Table 2, Figure 1).

Triglycerides significantly decreased from baseline in the placebo, MET+UDCA+VitE, and pioglitazone groups ($p<0.001$ for all) but not with empagliflozin. However, triglyceride changes did not significantly differ between pioglitazone and MET+UDCA+VitE groups ($p=0.38$) (Table 2, Figure 2).

Total cholesterol significantly declined from baseline with empagliflozin ($p<0.001$), MET+UDCA+VitE ($p=0.031$), and pioglitazone (=0.022) but not placebo. The reduction in total cholesterol differed between groups ($p<0.001$), with the greatest decrease seen with empagliflozin compared to MET+UDCA+VitE ($p=0.002$) and pioglitazone ($p=0.014$). The reduction in total cholesterol was comparable between Met+ UDC +Vit E and pioglitazone ($p=0.95$) (Table 2, Figure 3).

LDL cholesterol significantly decreased from baseline in all active treatment groups (p=0.023, 0.031, <0.001, for pioglitazone, Met+ UDC +Vit E, empagliflozin, respectively) but not placebo (Table 2, Figure 4). Pairwise comparisons suggested that empagliflozin, pioglitazone, and Met+ UDC +Vit E demonstrated a comparable reduction in LDL compared to each other ($p>0.5$). HDL cholesterol did not significantly change from baseline in any group, with no between-group differences ($p=0.7$) (Table 2).

Liver Parameters

Liver enzymes did not significantly change from baseline in any group, with no between-group differences in ALT, AST, ALP, or GGT (Table 2).

Imaging Markers

Fibroscan significantly improved from baseline only with empagliflozin ($p=0.008$) (Table 2). Between-group differences were significant ($p=0.001$), with greater improvement in the empagliflozin group compared to MET+UDCA+VitE and pioglitazone (p= 0.003, <0.001, respectively) [Table 2, Figure 5]. Both pioglitazone and Met+ UDC +Vit E demonstrated a comparable improvement in fibroscan compared to each other ($p=0.72$).

Hepatic steatosis grade declined significantly from baseline with empagliflozin ($p<0.001$) and placebo ($p=0.01$) but not with other groups. Between-group differences were significant ($p<0.001$), with greater reduction in the empagliflozin group compared to MET+UDCA+VitE and pioglitazone (both $p<0.001$). Both pioglitazone and Met+ UDC +Vit E demonstrated a comparable non-significant reduction in steatosis compared to each other ($p=0.13$) [Table 2, Figure 6].

Hematologic Parameters

Hemoglobin modestly declined from baseline only with empagliflozin (-0.4±0.6 g/dL, $p=0.017$). The reduction differed significantly between empagliflozin and other groups ($p=0.003$ for MET+UDCA+VitE, $p=0.014$ for pioglitazone) (Table 2). White blood cells (WBCs) were only reduced significantly with empagliflozin treatment

(p=0.029). Other hematologic parameters did not significantly change.

Biomarkers

Cytokeratin-18 significantly declined only with empagliflozin (-74.6±81.1 U/L, p<0.001). The reduction differed significantly between empagliflozin and other groups (p=0.005 for MET+UDCA+VitE, p=0.04 for pioglitazone) (Table 2, Figure 7). Similarly, Autotaxin significantly declined only with empagliflozin (-115.6±87.0 mg/dL, p<0.001). The reduction was significantly greater with empagliflozin compared to all other groups (all p<0.001) (Table 2, Figure 8).

Table 1. Comparing baseline demographic, laboratory, and clinical characteristics among the study groups (N=200).

Characteristic	Placebo (n=50)	Empagliflozin (n=50)	Metformin + UDCA + Vitamin E (n=50)	Pioglitazone (n=50)	p[1]
BMI (kg/m^2)	32.4 ± 4.0	29.2 ± 2.6	31.8 ± 4.0	32.3 ± 3.6	**<0.001**
ALT (U/L)	30.0 ± 11.0	31.4 ± 11.2	26.7 ± 9.8	31.6 ± 11.7	0.12
AST (U/L)	27.3 ± 9.8	29.8 ± 9.9	25.7 ± 9.2	29.5 ± 10.1	0.2
ALP (U/L)	80.1 ± 30.9	69.9 ± 21.2	71.3 ± 23.3	75.2 ± 26.1	0.3
GGT (U/L)	28.9 ± 9.2	25.6 ± 9.3	26.6 ± 8.3	27.1 ± 10.6	0.5
Fibroscan (kPa)	6.3 ± 1.8	7.2 ± 2.3	6.2 ± 1.6	6.3 ± 2.0	0.11
Total cholesterol (mg/dL)	295.7 ± 47.6	306.4 ± 48.1	292.2 ± 48.0	303.6 ± 48.9	0.5
Triglycerides (mg/dL)	185.3 ± 35.0	165.7 ± 44.9	165.1 ± 26.6	176.2 ± 30.3	**0.016**
LDL (mg/dL)	193.9 ± 42.0	193.3 ± 50.6	206.8 ± 60.0	205.9 ± 50.7	0.12
HDL (mg/dL)	41.0 ± 9.0	40.8 ± 8.5	38.6 ± 9.0	41.4 ± 8.1	0.3
Steatosis grade	317.4 ± 34.7	321.8 ± 35.0	311.9 ± 36.4	312.6 ± 29.9	0.5
Hemoglobin (g/dL)	12.0 ± 1.6	12.8 ± 1.4	11.8 ± 1.4	11.9 ± 1.3	**0.004**
Platelets (x109/L)	250.5 ± 44.8	224.0 ± 39.4	250.5 ± 52.4	250.4 ± 42.0	**0.005**
WBC count (x109/L)	6.4 ± 1.8	6.5 ± 1.8	6.7 ± 1.6	6.1 ± 2.0	0.5
CK18 (U/L)	236.8 ± 93.0	222.2 ± 90.0	276.9 ± 241.2	347.9 ± 387.2	0.4
ATX (mg/L)	321.7 ± 105.5	325.7 ± 120.9	326.1 ± 119.7	359.1 ± 121.3	0.4

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; ATX; autotaxin.

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

Outcome	Placebo			Empagliflozin			Metformin + UDCA + Vitamin E			Pioglitazone			p[2]
	Pre	Post	p[1]	Pre	Post	p[1]	Pre	Post	p[1]	Pre	Post	p[1]	
BMI (kg/m^2)	32.4±4.0	31.7±4.3	0.42	29.2±2.6	27.5±2.1	**<0.001**	31.8±4.0	30.6±4.2	0.16	32.3±3.6	32.2±3.8	>0.9	**<0.001**
Triglycerides (mg/dL)	185.3±35.0	149.9±36.3	**<0.001**	165.7±44.9	160.6±53.9	0.2	165.1±26.6	135.3±27.5	**<0.001**	176.2±30.3	143.1±33.5	**<0.001**	**<0.001**
Total cholesterol (mg/dL)	295.7±47.6	282.3±49.3	0.42	306.4±48.1	258.2±57.5	**<0.001**	292.2±48.0	265.1±52.0	**0.031**	303.6±48.9	275.7±62.3	**0.022**	**<0.001**
LDL (mg/dL)	193.9±42.0	185.6±46.1	0.1	193.3±50.6	163.7±60.4	**<0.001**	206.8±60.0	187.4±55.5	**0.016**	205.9±50.7	184.5±50.9	**0.023**	**<0.001**
HDL (mg/dL)	41.0±9.0	42.7±9.0	0.24	40.8±8.5	43.6±8.7	0.14	38.6±9.0	40.9±9.4	0.25	41.4±8.1	43.1±8.0	0.41	0.7
ALT (U/L)	30.0±11.0	29.2±9.7	0.75	31.4±11.2	30.6±9.6	0.55	26.7±9.8	26.2±9.4	0.93	31.6±11.7	30.5±9.8	0.72	>0.9
AST (U/L)	27.3±9.8	27.2±9.6	0.98	29.8±9.9	29.1±8.5	0.81	25.7±9.2	25.0±8.7	0.83	29.5±10.1	29.2±9.8	0.98	0.9
ALP (U/L)	80.1±30.9	79.9±29.7	0.94	69.9±21.2	70.4±20.5	0.31	71.3±23.3	70.3±21.9	0.87	75.2±26.1	75.7±24.9	0.96	0.9
GGT (U/L)	28.9±9.2	28.9±9.4	0.86	25.6±9.3	24.8±8.6	0.26	26.6±8.3	26.6±9.0	0.94	27.1±10.6	27.8±10.1	0.75	0.3
Fibroscan (kPa)	6.3±1.8	5.9±1.7	0.23	7.2±2.3	5.9±1.3	**0.008**	6.2±1.6	5.8±1.6	0.15	6.3±2.0	6.0±1.8	0.49	**0.001**
Steatosis grade	317.4±34.7	291.5±38.1	**0.01**	321.8±35.0	272.5±28.4	**<0.001**	311.9±36.4	293.2±45.9	0.06	312.6±29.9	301.6±39.9	0.24	**<0.001**
Hemoglobin (g/dL)	12.0±1.6	11.8±1.2	0.56	12.8±1.4	12.4±1.2	0.06	11.8±1.4	11.8±1.0	0.74	11.9±1.3	12.0±0.9	0.79	**0.017**
Platelets (x109/L)	250.5±44.8	251.5±35.2	0.58	224.0±39.4	234.4±42.3	0.2	250.5±52.4	257.0±42.7	0.36	250.4±42.0	263.3±37.2	**0.04**	0.3
WBC count (x109/L)	6.4±1.8	6.4±1.9	0.81	6.5±1.8	5.7±1.3	**0.029**	6.7±1.6	6.7±1.4	0.91	6.1±2.0	6.6±1.9	0.23	0.13
CK18 (U/L)	236.8±93.0	218.3±72.2	0.56	222.2±90.0	147.6±38.5	**<0.001**	276.9±241.2	280.0±383.3	0.77	347.9±387.2	247.2±181.5	0.24	**<0.001**
ATX (mg/L)	321.7±105.5	303.0±133.4	0.45	325.7±120.9	210.0±136.7	**<0.001**	326.1±119.7	285.0±130.3	0.07	359.1±121.3	325.5±144.2	0.24	**<0.001**

[1] p-value calculated using Wilcoxon signed rank test for paired data.
[2] p-value calculated using the 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; CRP, C-reactive protein; ATX; autotaxin.

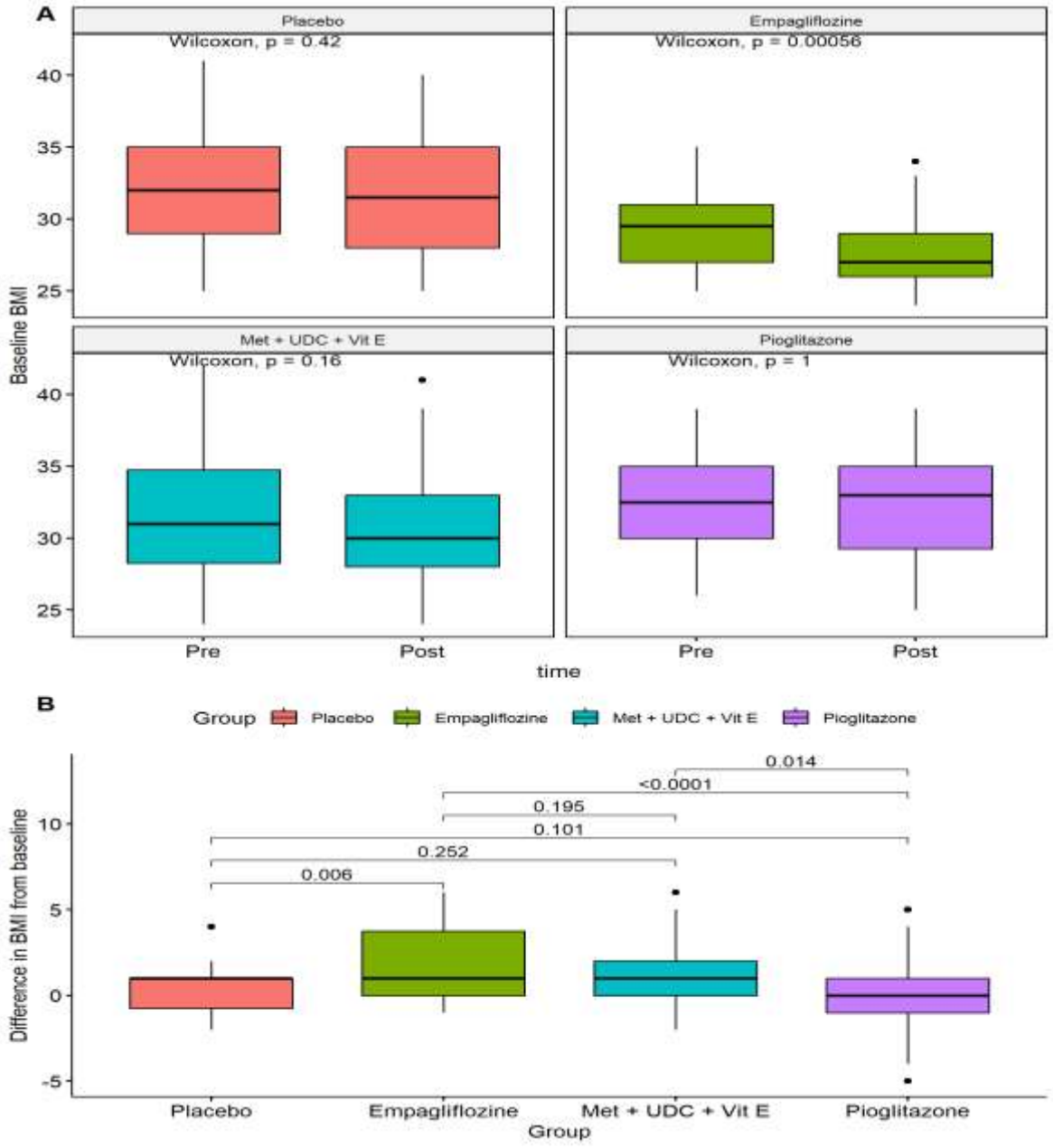

Figure 1: Comparing body mass index among the study groups. A. Boxplots demonstrating the body mass index among the study groups before and after treatment. B. Boxplots demonstrate the differences in body mass index over follow-up time among the study groups with pairwise comparison comparing each group.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

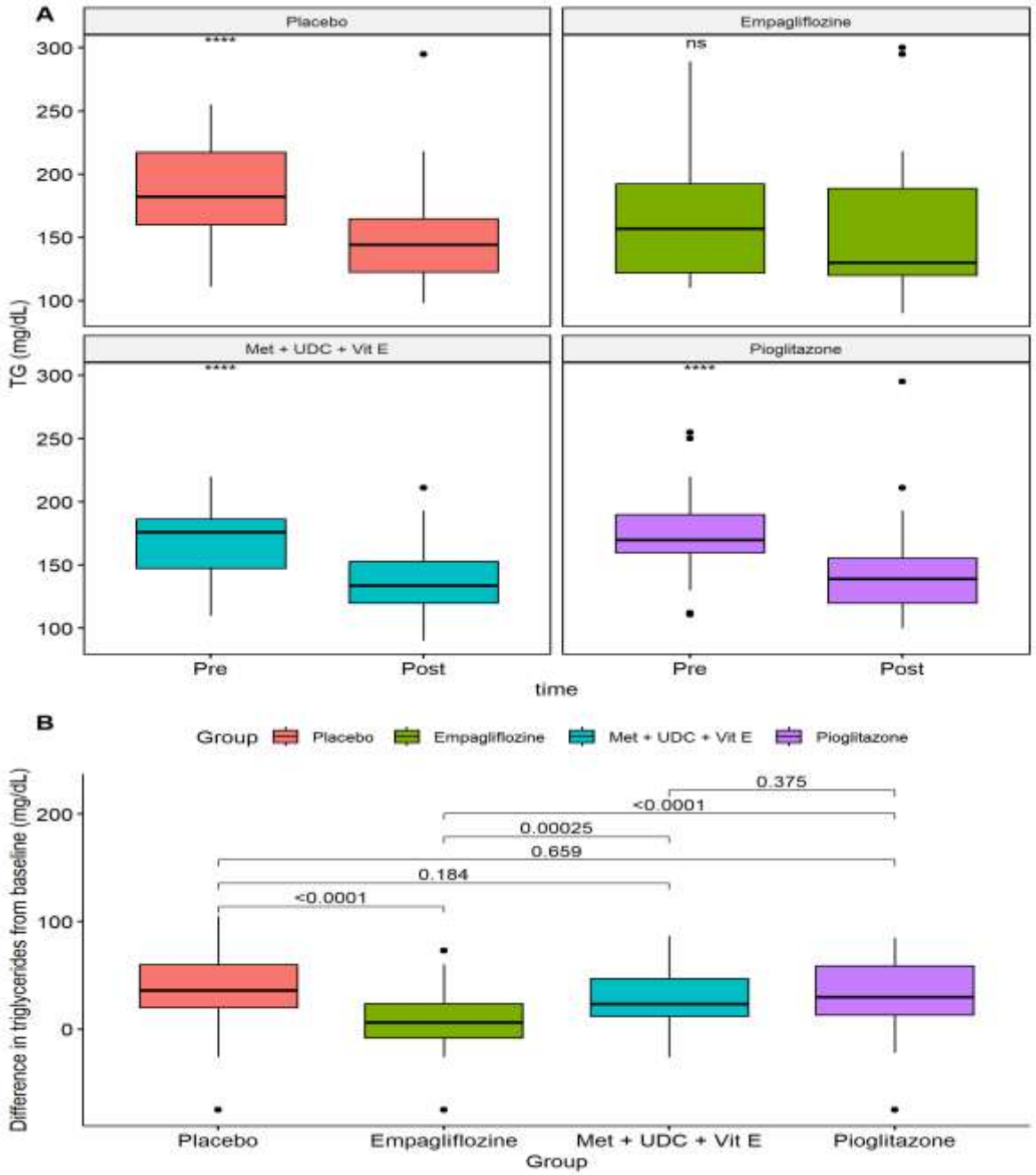

Figure 2: Comparing triglyceride levels among the study groups. A. Boxplots demonstrating the triglyceride levels among the study groups before and after treatment. B. Boxplots demonstrate the differences in triglyceride levels over follow-up time among the study groups with pairwise comparison comparing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

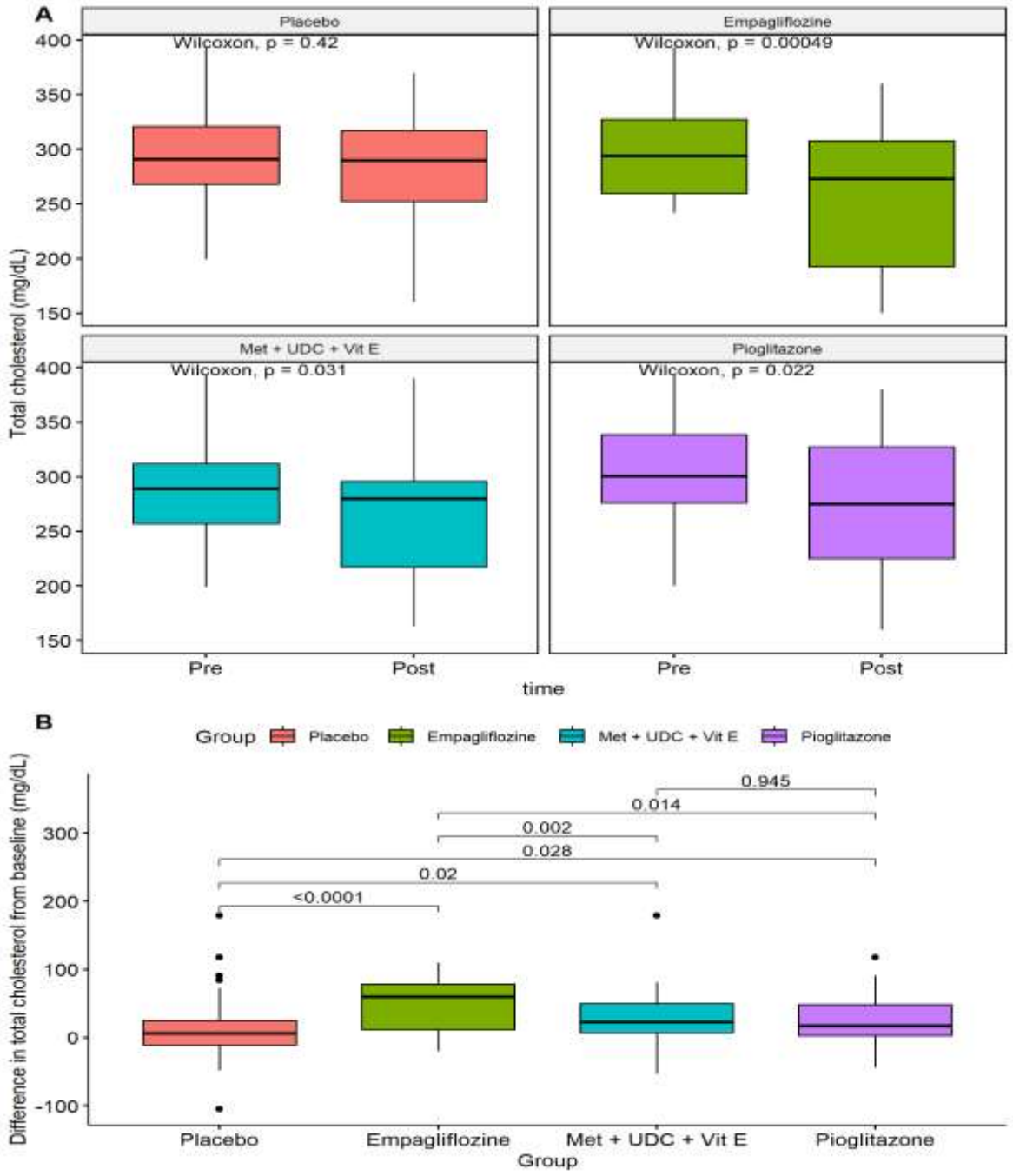

Figure 3: Comparing total cholesterol levels among the study groups. A. Boxplots demonstrating the total cholesterol levels among the study groups before and after treatment. B. Boxplots demonstrate the differences in total cholesterol levels over follow-up time among the study groups with pairwise comparison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

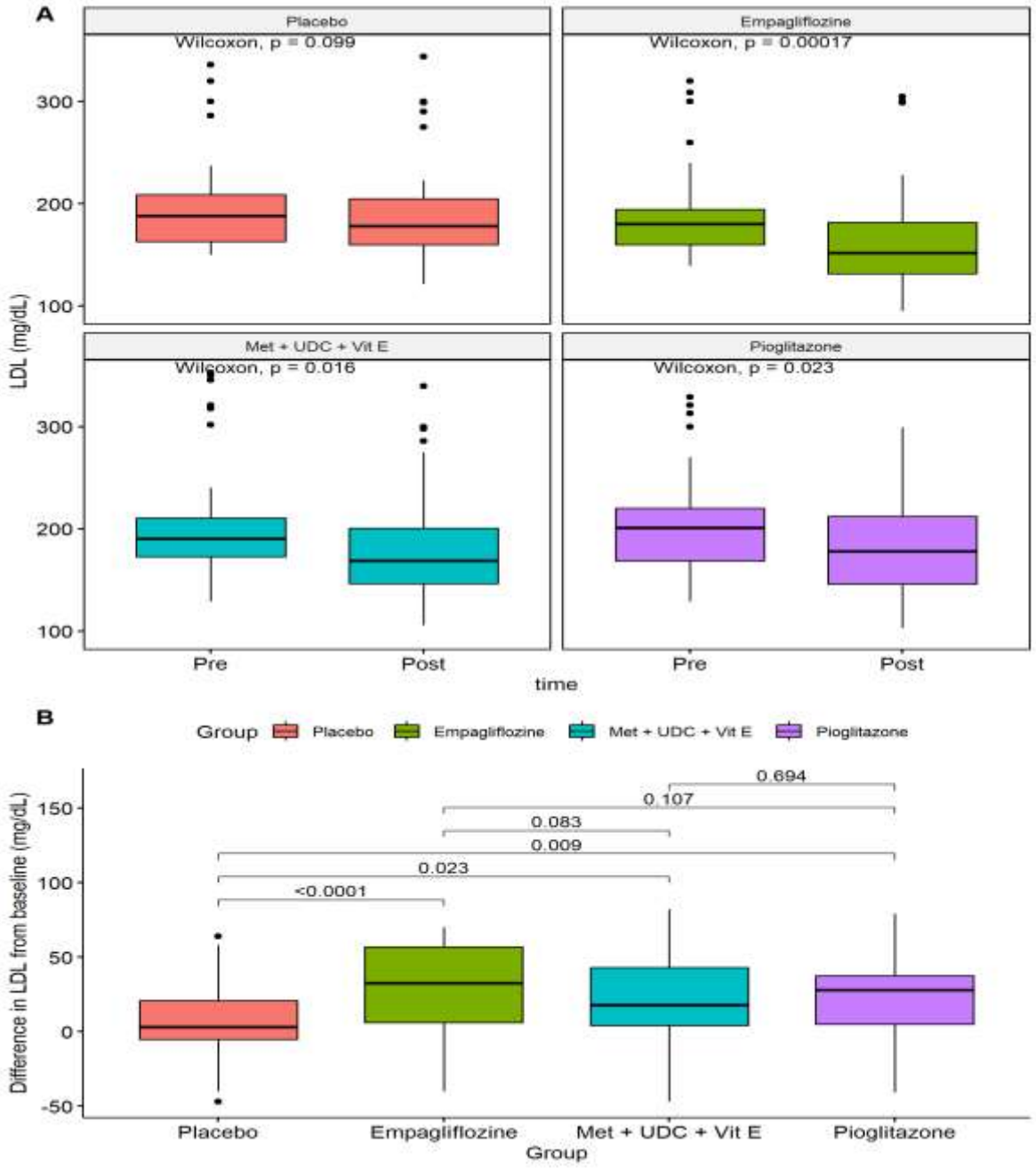

Figure 4: Comparing LDL levels among the study groups. A. Boxplots demonstrating the LDL levels among the study groups before and after treatment. B. Boxplots demonstrate the differences in LDL levels over follow-up time among the study groups with pairwise comparison comparing each group. The calculated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

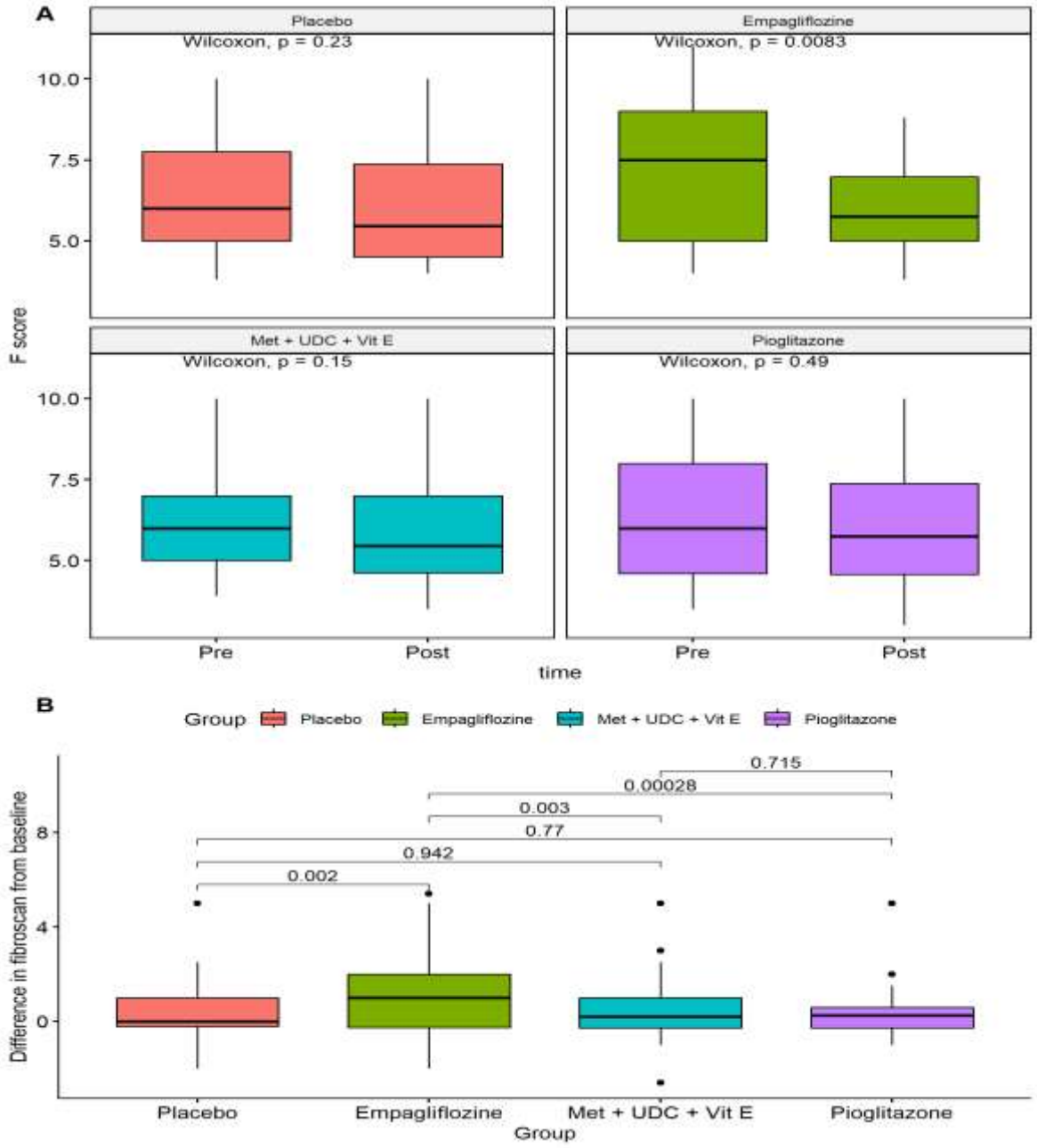

Figure 5: Comparing fibroscan among the study groups. A. Boxplots demonstrating the fibroscan among the study groups before and after treatment. B. Boxplots demonstrate the differences in fibroscan over follow-up time among the study groups with pairwise comparison comparing each group. The calculated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

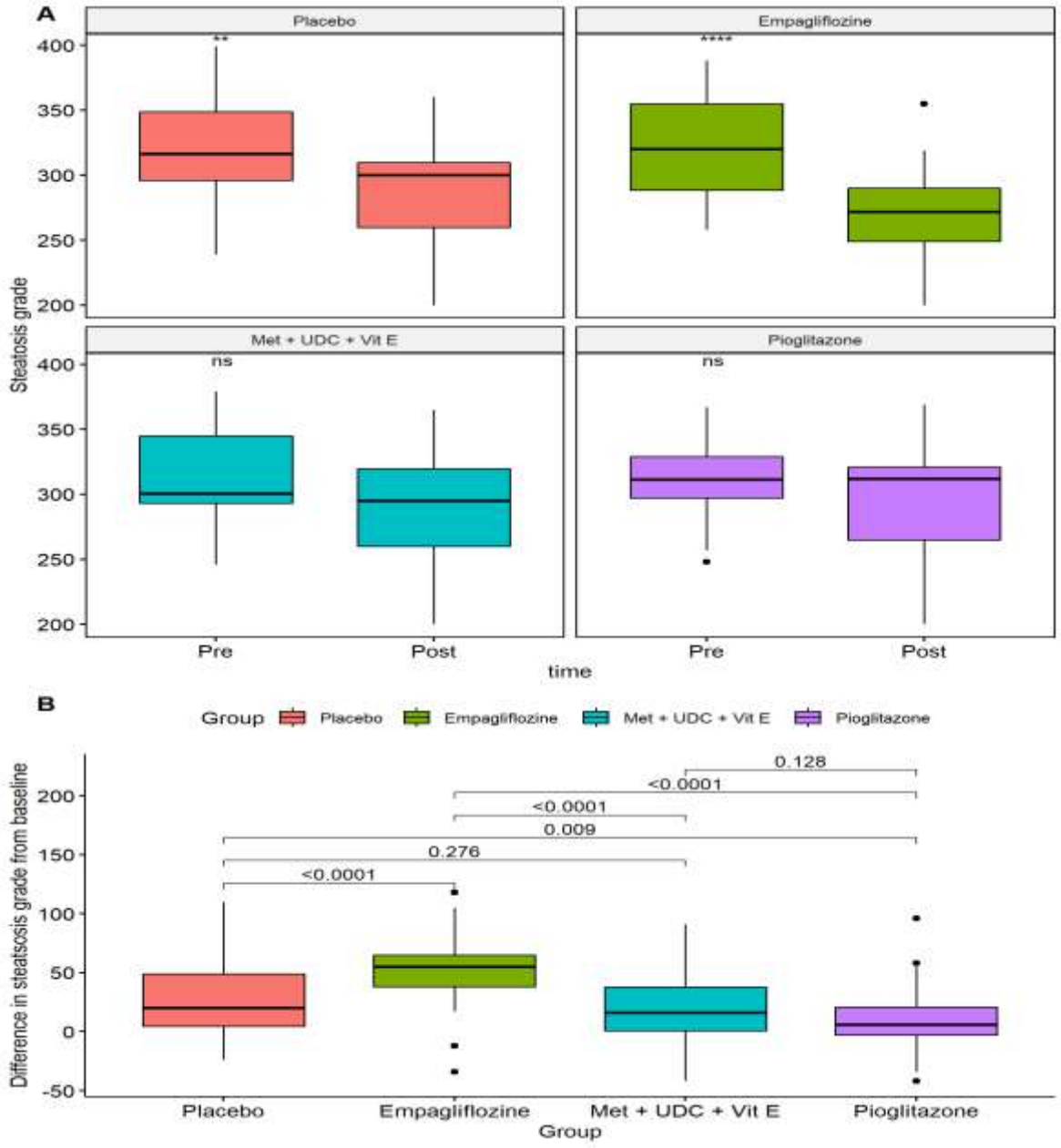

Figure 6: Comparing steatosis among the study groups. A. Boxplots demonstrating the steatosis among the study groups before and after treatment. B. Boxplots demonstrate the differences in steatosis over follow-up time among the study groups with pairwise comparison comparing each group. The calculated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

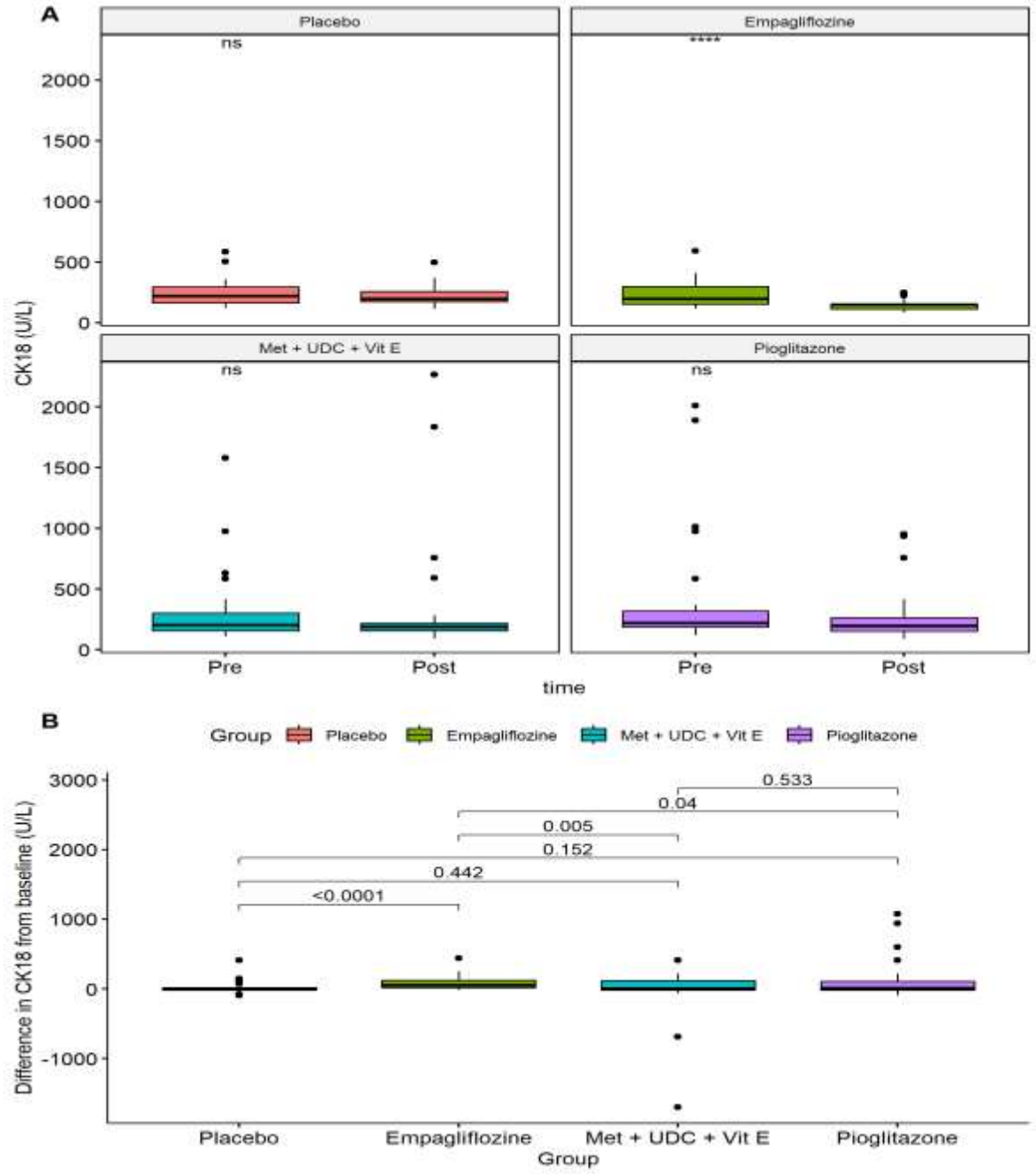

Figure 7: Comparing CK18 levels among the study groups. A. Boxplots demonstrating the CK18 levels among the study groups before and after treatment. B. Boxplots demonstrate the differences in CK18 levels over follow-up time among the study groups with pairwise comparison comparing each group. The calculated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

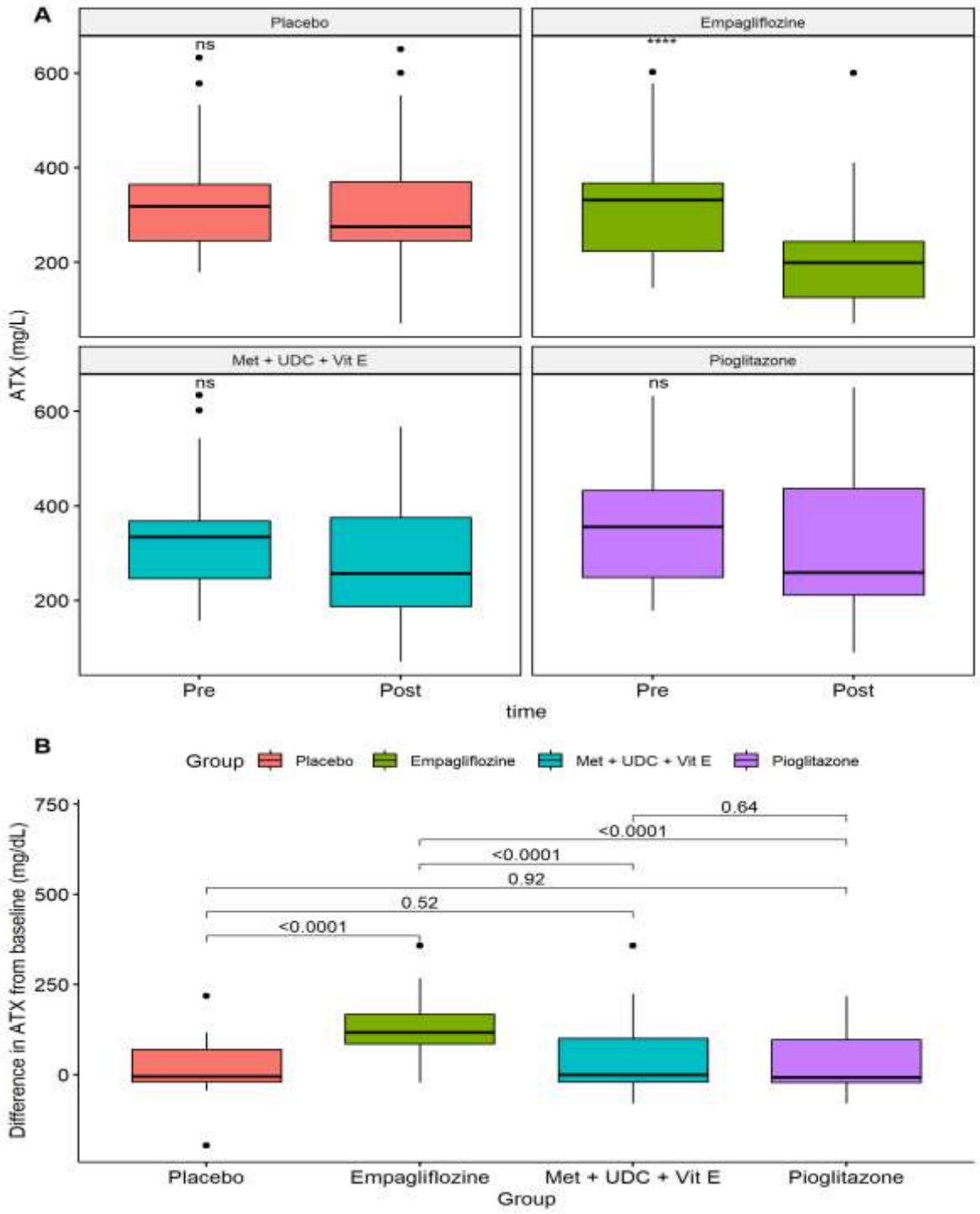

Figure 8: Comparing autotoxin levels among the study groups. A. Boxplots demonstrating the autotoxin levels among the study groups before and after treatment. B. Boxplots demonstrate the differences in autotoxin levels over follow-up time among the study groups with pairwise comparison comparing each group. The calculated p-values for each pairwise comparison were corrected using Bonferroni correction.

DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) encompasses a disease spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), which can progress to cirrhosis and hepatocellular carcinoma (***Buzzetti et al.***, ***2016***). Numerous agents have been investigated for NAFLD, but currently there are no consensus on the optimal management of non-diabetic NAFLD.

In this 12-month randomized controlled trial of 200 non-diabetic patients with NAFLD, we compared the efficacy of empagliflozin, pioglitazone, metformin plus ursodeoxycholic acid and vitamin E (MET+UDCA+VitE), and placebo on metabolic parameters, liver enzymes, imaging markers of steatosis and fibrosis, and novel biomarkers of fibrosis, including cytokeratin 18 and autotaxin. The key findings from the current study are that Empagliflozin, an SGLT2 inhibitor, showed superior improvements in BMI, steatosis grade, and Fibroscan-measured liver stiffness compared to other treatment modalities. Empagliflozin also reduced total cholesterol and LDL cholesterol to a greater extent than pioglitazone or MET+UDCA+VitE but did not lower triglycerides. In contrast, pioglitazone and MET+UDCA+VitE significantly decreased triglycerides without impacting hepatic steatosis or fibrosis. Serum biomarkers cytokeratin 18 and autotaxin, implicated in NAFLD pathogenesis, were markedly reduced only with empagliflozin.

At baseline, patients' characteristics were comparable among the study groups, except for BMI, hemoglobin, and platelet counts. Nevertheless, this was suggested to impose little impact on the current findings since we did not study the longer-term cardiovascular implications of these treatments, which could be potentially confounded by both different baseline levels of hemoglobin and platelets (***Işık & Soner***, ***2022***). BMI was one of the studied metabolic outcomes of this study, however, we analyzed the differences from baseline values among the different groups for all studied outcomes, including BMI, to alleviate the effects of baseline differences.

Our findings suggested that empagliflozin significantly decreased liver stiffness and steatosis grade, but not the liver enzymes. However, the significant hepatic improvement was also associated with a significant reduction of the studied biomarkers including CK 18 and autotaxin, which were exclusively reduced with empagliflozin therapy only. This partially aligns with the previous studies showing SGLT2 inhibitors improved controlled attenuation parameter, liver stiffness measurement, and aminotransferases (***Kahl et al.***, ***2022***; ***Kuchay et al.***, ***2018***). In particular, a post hoc analysis of EMPA-REG OUTCOME trial demonstrated that empagliflozin reduced liver fat fraction, ALT, and AST versus placebo over 20 weeks in diabetic NAFLD (***Kahl et al.***, ***2022***). However, we did not detect significant declines in ALT, AST, GGT or ALP with empagliflozin. In contrast, a recent meta-analysis demonstrated no significant improvements in controlled attenuation parameter (CAP) score, hepatic steatosis, liver stiffness measurement (LSM) score, ALT, AST, LDL, or triglycerides (TG) following empagliflozin treatment in a total of 212 patients with NAFLD (***X. Tang et al.***, ***2022***). However, the validity of the conclusion of this meta-analysis is questionable since they included only 3 RCTs with a total of 212 NAFLD patients only. The conflicting findings may be explained on the basis that these studies included diabetic NAFLD, who may respond differently to the studied therapies compared to our non-diabetic population. Interestingly, the current study demonstrated that Empagliflozin uniquely reduced BMI, likely reflecting caloric loss through urinary glucose excretion (***Ferrannini et al.***, ***2016***). It may also improve adipose insulin sensitivity and exert systemic anti-inflammatory effects based on the observed white blood cell decline through the current study, a result that agree with the current literature (***Xu et al.***, ***2019***).

For pioglitazone, we did not observe significant steatosis or fibrosis improvements in non-diabetics, concurring with the PIVENS study (***Chalasani et al.***, ***2009***). However, other trials demonstrated histological benefits with pioglitazone, especially in diabetes (***Cusi et al.***, ***2016***; ***Sharma et al.***, ***2012***). A recent meta-analysis contrastingly found that pioglitazone improved liver histopathology and enzymes in both diabetic and non-diabetic NAFLD (***Wang et al.***, ***2023***). Longer treatment and baseline glucose dysregulation likely explain the clinical advantages seen with pioglitazone in these studies compared to our study, which exclusively included non-diabetic patients. As expected, we did not detect BMI reduction with pioglitazone versus placebo in line with the current evidence (***Yang et al.***, ***2014***).

We did not observe significant hepatic improvements with metformin+UDCA+ vitamin E. Some studies suggest modest benefits on aminotransferases and imaging with vitamin E combined with silymarin (***Aller et al.***, ***2015***), or atorvastatin (***Foster et al.***, ***2011***). A recent meta-analysis indicated that adjuvant vitamin E therapy provides significant biochemical and histological improvements in adult patients with NAFLD (***Amanullah et al.***, ***2019***). The contrasting finding from these studies may be attributed to inclusion of different population (diabetic NAFLD), with different combinations, formulations, and treatment durations of vitamin E therapy. One meta-analysis did agree with our findings of LDL cholesterol reduction with vitamin E (***Amanullah et al.***, ***2019***). For UDCA, similar to our findings, two studies agreed it did not significantly improve steatosis or histology (***Lin et al.***, ***2022***; ***Zhang et al.***, ***2020***), while only higher doses improved aminotransferases and fibrosis markers in another trial (***Ratziu et al.***, ***2011***).

Our study unexpectedly revealed a reduction in steatosis scores and triglyceride levels in the placebo group post-treatment. This might be attributed to the Hawthorne Effect, where participants, aware of

being observed, might adopt healthier behaviors. Additionally, the unblinded nature of our study introduces potential observer bias, which could affect the steatosis score evaluations.

The findings from this randomized controlled trial have several implications on the clinical practice of NAFLD/NASH management. In patients with non-diabetic NAFLD, a treatment window of 12 months of empagliflozin treatment may confer greater benefits on steatosis, fibrosis, and metabolic parameters compared to pioglitazone or a combination of MET+UDCA+VitE. The potent effects of empagliflozin on reversing histological disease activity may support the use of SGLT2 inhibitors as first-line pharmacotherapy for this population. The lack of significant improvements with pioglitazone monotherapy implies it may not be an optimal choice for non-diabetic NAFLD compared to empagliflozin. Conversely, pioglitazone and MET+UDCA+VitE should be indicated if concomitant hypertriglyceridemia was presented.

This trial has several strengths. The inclusion of multiple treatment arms allowed for direct comparison of different pharmacological options for NAFLD management. The primary outcome of change in steatosis grade was assessed using transient elastography, which provides an objective and quantitative measure of hepatic fat content. Novel circulating biomarkers were examined to elucidate mechanisms of treatment response. A sample size of 200 participants provided adequate statistical power to detect differences between groups. There was no loss to follow-up, minimizing attrition bias.

However, some limitations should be noted. The open-label design was susceptible to observer bias during outcome assessments. The generalizability of findings is limited as the cohort comprised Egyptian adults without diabetes or cirrhosis. The interventions were not compared to or combined with lifestyle counseling, which represents the cornerstone of care.

Conclusion

In non-diabetic adults with NAFLD, empagliflozin treatment for 12 months resulted in superior improvements in BMI, liver stiffness, steatosis grade, cholesterol levels, and novel biomarkers compared to pioglitazone or metformin plus UDCA and vitamin E. Although transient elastography provides non-invasive quantification of hepatic steatosis and fibrosis, the distinctive metabolic and anti-inflammatory effects of empagliflozin observed in this study indicate it may target key pathways of NAFLD progression beyond glucose control. If corroborated by larger clinical trials, these findings suggest empagliflozin could emerge as a promising first-line pharmacotherapy for non-diabetic patients with NAFLD. However, long-term studies are still needed to confirm durability of treatment response and impact on clinical outcomes.

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