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RESEARCH ARTICLE

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The effects of co-administration of artichoke leaf extract supplementation with metformin and vitamin E in patients with nonalcoholic fatty liver disease: A randomized clinical trial

Mohammad Bagher Majnooni[1] | Mari Ataee[2] | Gholamreza Bahrami[3] | Fatemeh Heydarpour[4] | Ina Yosifova Aneva[5] | Mohammad Hosein Farzaei[3] | Touraj Ahmadi-Juoibari[2]

[1]Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran

[2]Clinical Research Development Center, Imam Khomeini and Dr Mohammad Kermanshahi and Farabi Hospitals, Kermanshah University of Medical Sciences, Kermanshah, Iran

[3]Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

[4]Social Development and Health Promotion Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

[5]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

Correspondence
Mohammad Hosein Farzaei, Pharmaceutical Sciences Research Center, Kermanshah University of Medical Sciences, Kermanshah 6734667149, Iran.
Email: mh.farzaei@gmail.com

Touraj Ahmadi-Juoibari, Clinical Research Development Center, Imam Khomeini and Dr Mohammad Kermanshahi and Farabi Hospitals, Kermanshah University of Medical Sciences, Kermanshah 6715847141, Iran.
Email: dr.ahmadi-jouybari@yahoo.com

Medicinal plants are widely used as a complementary therapy to treat complex diseases, such as nonalcoholic fatty liver disease (NAFLD). Therefore, this study was done to investigate the effect of co-administration of artichoke leaf extract supplement (ALES) with conventional medicines on patients with NAFLD. The clinical trial was based on patients randomly divided into three groups involving metformin-vitamin E (ME), metformin-ALES (MA), and vitamin E-ALES (EA). The effectiveness of treatment in the treated groups was evaluated using liver ultrasonography and biochemical markers. After 12 weeks of treatment, the results showed that the rate of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was significantly reduced within all the study groups ($p < .05$). Liver ultrasonographic findings revealed that the rate of fat accumulation in liver of patients was decreased significantly within all the study groups and it was increased in the subjects with grade 0 fatty liver (without fat accumulation) in the MA and EA groups by 23.3 and 17.2%, respectively. In summary, the results of the present study showed that the concomitant use of ALES with metformin and vitamin E can have beneficial effects on amelioration of complications in patients with NAFLD. However, larger-scale clinical trial studies are required in this regard.

KEYWORDS
artichoke, clinical trial, complementary treatment, metformin, nonalcoholic fatty liver, vitamin E

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent metabolic diseases worldwide, manifested with accumulation of lipids, especially triglycerides, in liver cells' cytoplasm. This disease is the early stage of steatohepatitis, eventually leading to cirrhosis and hepatocellular carcinoma. Besides, NAFLD is strongly associated with other conditions, such as coronary artery disease and various cancers (Byrne & Targher, 2015; Lu et al., 2020). Therefore, finding effective treatment with minimal side effects is essential. Medicinal plants and their constituents have multiple protective and therapeutic effects, so they are widely used to treat diseases and their complex complications, including metabolic diseases such as NAFLD (Francini-Pesenti, Spinella, & Calò, 2019; Panyod & Sheen, 2020). However, medicinal plants and natural compounds as complementary and alternative therapeutics and conventional restorative therapy reduce the dose of chemical drugs with high side effects and increase their effectiveness (Čabarkapa et al., 2016; Rafieian-Kopaei, 2012). For example, co-administration of resveratrol, a polyphenol widely found in grapefruits, with meloxicam has been shown to relieve pain in patients with

osteoarthritis (Hussain, Marouf, Ali, & Ahmmad, 2018). The other studies have also shown that combinational therapy of ursodeoxycholic acid with curcumin and extracts of *Brassica rapa* leaves with metformin ameliorated complications of NAFLD and diabetes in rats, respectively (Gheibi, Ghaleh, Motlagh, & Azarbayjani, 2019; Hassanzadeh-Taheri et al., 2018). On the other hand, artichoke (*Cynara scolymus*, Asteraceae), as a herb widely used in traditional medicine, has shown therapeutic and protective effects in several diseases, especially metabolic disorders, such as diabetes, obesity-induced hypercholesterolemia, and hypertension in animal and clinical studies (Miraj & Kiani, 2016; Roghani-Dehkordi & Kamkhah, 2009; Villiger, Sala, Suter, & Butterweck, 2015). Besides, artichoke contains polyphenols, such as luteolin glycosides, chlorogenic acid, caffeic acid and its derivatives, cynarin, and apigenin with prominent hepatoprotective and antioxidant effects (Ben Salem et al., 2019; Zhu, Zhang, & Lo, 2004). Other researchers (Panahi et al., 2018) have demonstrated that the use of artichoke leaf extract supplement (ALES) (200 mg, three times a day) markedly decreased level of liver enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were also decreased. Besides, beneficial changes in liver ultrasonography were observed in patients with NAFLD. In another study, the ALES showed antioxidant activities and markedly reduced the level of oxidized-LDL in serum of the patients with metabolic syndrome (Rezazadeh, Aliashrafi, Asghari-Jafarabadi, & Ebrahimi-Mameghani, 2018). Also, other beneficial effects of the ALES include reduction of levels of LDL and total cholesterol and increasing level of high-density lipoprotein cholesterol (HDL-C) in patients with hypercholesterolemia (Rondanelli et al., 2013). Based on proper guidelines published for treatment of NAFLD, metformin and antioxidant supplements, such as vitamin E are among treatment protocols for this disease. However, there is insufficient clinical evidence on their effectiveness and these treatments, unlike treatment with the ALES, do not have multiple targets in treatment of NAFLD (Haukeland et al., 2009; Leoni et al., 2018). Accordingly, this randomized, single-blind clinical trial study was designed to investigate the effects of adding ALES to the treatment protocol of NAFLD containing metformin and vitamin E.

2 | MATERIALS AND METHODS

2.1 | Patients

The study was carried out on male and female adult patients (aged between 18 and 70 years old) referred to the gastroenterology clinic (Kermanshah Province, Iran). Inclusion criteria were patients with fatty liver (grades 1–3) diagnosed by abdominal ultrasonography. Pregnancy or breastfeeding, alcohol consumption or alcoholic fatty liver disease, smoking, cardiovascular diseases, biliary diseases, chronic liver disease and hepatitis, renal failure, all types of cancer, thyroid diseases, hypoglycemia, hypolipidemia, the presence of gastrointestinal ulcers, consuming any herbal supplements and vitamins, as well as any medications that could influence liver function during the study, and any allergies to the drugs prescribed in the present study were among exclusion criteria. Clinical evidence and biochemical tests were used to confirm exclusion criteria. Also, titration measurement of anti-hepatitis B and C antibodies was used to diagnose viral hepatitis in all subjects. The patients were randomly divided into three groups: (a) oral administration of 1,000 mg/day of metformin in two divided doses (every 12 hr) plus vitamin E (400 UI/day); (b) oral administration of 1,000 mg/day of metformin in two divided doses (every 12 hr) plus 800 mg/day of ALES in two divided doses (every 12 hr), and (c) oral administration of 800 mg/day of ALES in two divided doses (every 12 hr) plus vitamin E (400 UI/day). Each capsule of ALES (400 mg) was standardized based on 20 mg of chlorogenic acid (Tishoke, Goldaru Company, Isfahan, Iran). Also, aerobic exercise (30 min, three times a week) and reduction of consuming fatty and high-calorie foods were recommended to all patients. There were no co-interventions with the studied drug treatments (Zelber-Sagi, Ratziu, & Oren, 2011). The patient's compliance (through telephone follow-up and verbal questions during the treatment period) and number of the returned drugs were assessed at the second visit. This study was approved by the Ethics Committee of the Kermanshah University of Medical Sciences (KUMS.rec.1395.367) and was registered in the Iranian registry of clinical trials (IRCT) ID: IRCT2017040429278N1.

2.2 | Sample size

The sample size was estimated as 21 people in each group using previous similar studies and taking into account Type I error of $\alpha = .05$, Type II error of $\beta = .1$, and the minimum acceptable difference in mean ALT and AST rate of 4 between the two groups of MA and ME by the following formula, and assuming the probability of sample loss, 30 people in each group were considered.

$$\frac{2 * \left(Z1 - \frac{\alpha}{2} + Z1 - \beta\right)^2 * \delta^2}{d^2}$$

2.3 | Anthropometric indices

Body weight and height were measured to the nearest 0.1 kg and 1 mm using an automated scale and a wall-mounted stadiometer, respectively. The following formula of weight (kg)/height^2 (m^2) was used to calculate body mass index (BMI). All measurements were carried out in standing position with no shoes.

2.4 | Biochemical measurements

Fasting (12 hr overnight) blood samples were collected before and after 12 weeks of the treatment period in ethylenediaminetetraacetic acid (EDTA) tubes and were stored at −80 ° C. The blood samples

were centrifuged at 3,000 rpm for 10 min to separate sera. Fasting blood sugar (FBS), plasma total cholesterol (Total-Chol), TG, LDL-C, HDL-C, ALT, AST, alkaline phosphatase (ALP), and insulin were analyzed enzymatically on a Hitachi 717 auto biochemical analyzer using Pars-Azmoon kits (Tehran, Iran). All laboratory equipment were calibrated, and blinded duplicate samples were utilized.

2.5 | Liver ultrasonography

Liver ultrasonography was carried out at the beginning and after 12 weeks of the experimental protocol by a Mindray DC-8 diagnostic ultrasound system (convex 3.5–5.0 MHz). Ultrasound assessments were done by the same ultrasound system and radiologist that were blinded to the group assignment. Hepatic steatosis was categorized via sonographic echogenicity of the liver as grade 0 (lack of fat accumulation), grade 1 (a mild increase in echogenicity), grade 2 (the moderate increase in echogenicity with slightly impaired visualization of echogenic walls of portal vein branches and without obscuration of the diaphragm), and grade 3 (severe increase in echogenicity with significantly impaired visualization of echogenic walls of portal vein branches and obscuration of the diaphragm). All lobes and surfaces of the liver (right, left, upper, and lower) were assayed.

2.6 | Identification of artichoke leaves' active compounds by liquid chromatography with tandem mass spectrometry (LC–MS/MS)

Separation of ALES was performed isocratically on MZ PerfectSil target C18 column (25.0 mm × 4.0 mm ID., 5 m) using a C18 guard column (MZ-Anaysentechnik GmbH, Germany). The column temperature was maintained at 38°C, and a mixture of methanol and 0.1% formic acid in water (70:30, v/v) was eluted as mobile phase at a flow rate of 0.5 ml/min. Quantitative analysis was done using an Agilent 1200 series LC system consisting of a quaternary delivery pump, a thermostatted column compartment, a degasser (Agilent Technologies, Germany), and a Rheodyne 7725i manual injector valve with a 20-L sample loop (Cotati, CA, USA). Mass analysis was conducted by an Agilent 6410 Triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) run by Agilent Mass Hunter Workstation B.01.03. Ionization was obtained using electrospray ionization (ESI) with a capillary voltage of 4,000 V. Nitrogen was utilized as nebulizer gas with nebulizer pressure of 40 psi and source temperature of 100°C. Drying gas (nitrogen) was heated to 300°C and was delivered at a flow rate of 10 L/min. Fragmentor voltage and collision energy for all analytes were equal to 5 V and 35, respectively, and dwell time was equal to 200 ms. The mass spectra results were analyzed by determining m/z of the parent ions and their correspondence to m/z of the obtained fragments (their daughter ions). Compared to fragmentation patterns of similar compounds, a comparison was also done with other phytochemical studies on *C. scolymus* (Ben Salem et al., 2019; Ncube et al., 2014).

2.7 | Outcomes

Reduction of hepatic steatosis was a primary outcome. Besides, the secondary outcomes included improvement of anthropometric measures and serum biochemical factors, especially ALT and AST, as well as total-Chol, TG, LDL-C, HDL-C, ALP, and insulin resistance.

2.8 | Statistical analysis

Data were analyzed using SPSS software version 11.5 (SPSS Inc., Chicago, Illinois, USA), and the results were expressed as mean ± standard deviation. The intention-to-treat (ITT) analysis was performed on all the randomized patients who received at least one dose of the study medications. The Kolmogorov–Smirnov test was used for determining normality of distribution of variables. Within-group comparisons were conducted using the paired-samples *t*-test (for normally distributed variables) or Wilcoxon signed-rank test (for non-normally distributed variables). Between-group comparisons were performed using one-way analysis of variance (ANOVA) and Chi-squared test (for normally distributed variables) or Kruskal–Wallis test (for non-normally distributed variables), and multinomial logistic regression analysis was used to adjust the effect of weight (STATA software, V 14.2). A p value of less than .05 was considered as statistically significant in all statistical analyses.

3 | RESULTS

3.1 | Baseline characteristics of patients under study

Ninety patients participated in the study, and all of them continued the survey until the end without any side effects (Figure 1). Weight was significantly higher in the MA group (96.07 ± 14.37 kg) than EA (85.60 ± 18.66 kg) and ME (80.83 ± 12.12 kg) groups (Tukey's post hoc test). Due to the fact that, in univariate analysis, there was a statistically significant difference between the two groups of ME and MA and also between groups of EA and MA in terms of weight, so multinomial logistic regression analysis was used to adjust the effect of weight. The results of this analysis showed that a significant difference in weight between the groups had no significant effect on the main variables of the study, such as liver enzymes (AST and ALT) and fatty liver grades (0–4). Besides, BMI, a more reliable indicator than weight in NAFLD, did not differ significantly between them (Fan, Wang, & Du, 2018; Zheng, Chen, Chen, Lu, & Chen, 2012). Other baseline variables like gender, age, and fatty liver grades were not significantly different between the groups (Table 1).

3.2 | Serum levels of liver enzymes

ALT, AST, and ALP are liver enzymes assessed in the present study. Our results showed that serum levels of these enzymes were decreased within the groups after the intervention, with a significant decrease for

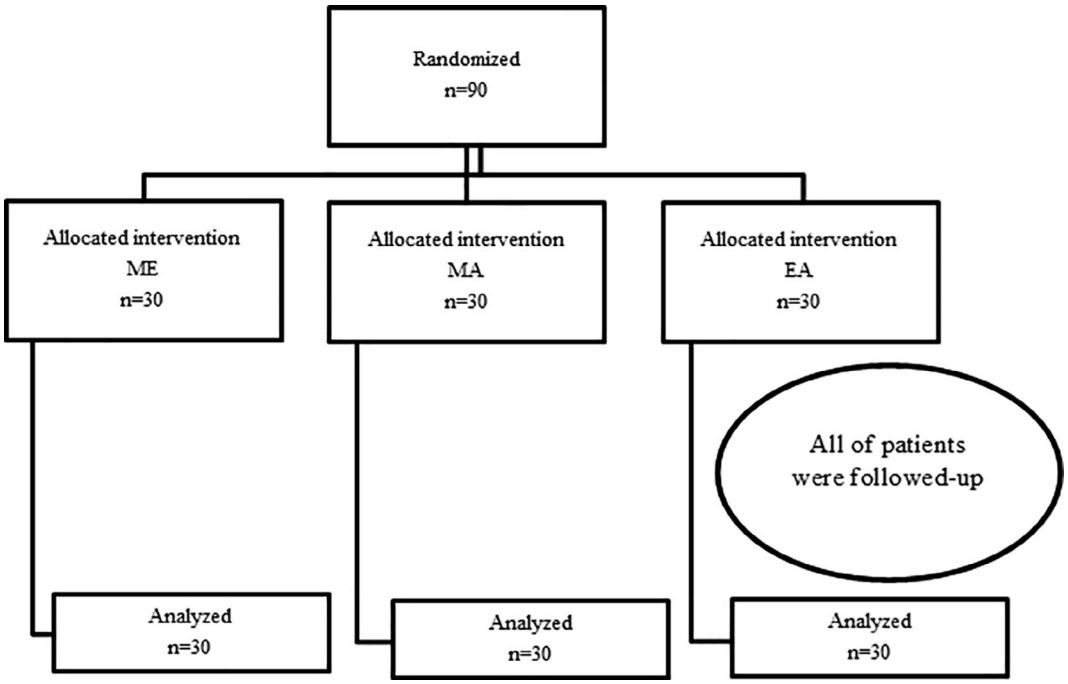


FIGURE 1 Flow diagram of the study. EA, Vitamin E-artichoke leaf extract supplement; MA, metformin-artichoke leaf extract supplement; ME, metformin-vitamin E

TABLE 1 Comparison of baseline characteristics of the patients between the three groups studied

Parameters	ME ($n = 30$)	MA ($n = 30$)	EA ($n = 30$)	p value
Gender				
Male	17 (56.7%)	20 (66.7%)	18 (58.1%)	$p = .73$
Female	13 (43.3%)	10 (33.3%)	13 (41.9%)	
Age (year)	38.28 ± 8.42[a]	36.93 ± 9.67	42.17 ± 11.09	$p = .11$
Weight (kg)	80.83 ± 12.12	96.07 ± 14.37	85.60 ± 18.66	$p = .001^{**}$
Grade of fatty liver				
Grade 1	6 (20.00%)	3 (10.00%)	7 (23.30%)	$p = .72$
Grade 2	15 (50.00%)	16 (53.30%)	14 (46.70%)	
Grade 3	9 (30.00%)	11 (36.70%)	9 (30.00%)	

Abbreviations: EA, vitamin E-artichoke; MA, metformin-artichoke; ME, Metformin-vitamin E.
[a]Data are presented as mean (±*SD*).
*Significant difference between groups (Chi-square test, $p < .05$).
**Significant difference between groups (one-way ANOVA, $p < .05$).

ALT and AST enzymes ($p < .05$, Table 2). On the other hand, there was no significant difference in serum levels of ALT, AST, and ALP between the groups after the intervention. Besides, comparison of mean differences (MD) in liver enzymes' serum level revealed that the levels of AST and ALT had the greatest decrease in the EA group, and the level of ALP was significantly reduced in the MA group.

3.3 | Serum levels of cholesterols, TG, insulin, and FBS

Serum levels of LDL-C, FBS, TG, and insulin were decreased within the groups after the intervention, although the difference was not statistically significant ($p > .05$). Serum levels of TG were decreased in the EA group more than the other two groups, which was significant compared with the ME group. Serum levels of HDL-C were significantly higher in the MA group than this before the treatment ($p < .05$). In addition, serum levels of total cholesterol (MD = 11) in the MA group had the highest decrease compared with other groups.

3.4 | Liver ultrasonography and BMI

The liver ultrasonography results (Table 3) showed a sizeable improvement within all three groups of MA, EA, and ME. The amount of fat accumulation in the liver was significantly reduced ($p = .001$) so that

TABLE 2 Biochemical characteristics of patients with NAFLD at baseline and after the 12-week intervention

Parameters	ME	MA	EA
FBS (mg/dl)			
Before	92.33 ± 10.10	96.67 ± 11.01	95.68 ± 9.88
After	90.43 ± 9.56	94.90 ± 8.24	92.39 ± 9.51
MD (95% CI) within groups	1.9 (0.948 to 4.748)	1.77 (−30.38 to 12.24)	3.29 (−0.36 to 6.94)
Insulin (μU/ml)			
Before	14.49 ± 8.61	19.59 ± 11.41	15.82 ± 9.10
After	12.89 ± 5.26	17.55 ± 11.14	14.25 ± 6.92
MD (95% CI) within groups	1.59 (−0.75 to 3.94)	2.03 (−0.09 to 4.17)	1.57 (−0.67 to 3.82)
TG (mg/dl)			
Before	211.40 ± 96.26	185.07 ± 106.19	169.10 ± 58.87
After	209.93 ± 98.89	176.43 ± 72.56	152.26 ± 66.28[a]
MD (95% CI) within groups	1.46 (−19.50 to 22.43)	8.63 (−15.83 to 33.09)	16.83 (−6.91 to 40.59)
Total-Chol (mg/dl)			
Before	180.50 ± 25.72	206.50 ± 36.72[b]	193 ± 43.7
After	182.10 ± 28.49	195.50 ± 40.38	194.48 ± 53.56
MD (95% CI) within groups	−1.6 (−10.42 to 7.22)	11(−3.42 to 25.42)	−1.48 (−18.78 to 15.81)
LDL-C (mg/dl)			
Before	104.07 ± 25.53	127.80 ± 59.60	110.45 ± 30.76
After	103.83 ± 22.36	119.83 ± 30.37	114 ± 34.80
MD (95% CI) within groups	0.23 (−7.46 to 7.93)	7.96 (−15.90 to 31.03)	−2.51 (−15.47 to 10.43)
HDL-C (mg/dl)			
Before	46.16 ± 21.44	42.34 ± 9.65	45.97 ± 13.48
After	45 ± 12.42	49.69 ± 13.59	46.44 ± 14.40
MD (95% CI) within groups	1.15 (−5.14 to 7.45)	−7.34 (−12.40 to −2.28)*	−0.04 (−6.18 to5.23)
ALT (U/L)			
Before	58 ± 23.64	63.87 ± 21.58	65.06 ± 23.52
After	40.92 ± 15.54	41.23 ± 12.54	38.29 ± 23.05
MD (95% CI) within groups	17.08 (9.55 to 24.60)*	23.63 (14.52 to 30.73)**	26.77 (18.72 to 34.82)**
AST (U/L)			
Before	39.07 ± 38.50	35.80 ± 12.75	43.68 ± 18.04[c]
After	32.37 ± 23.62	29.83 ± 9.87	27.87 ± 10.79
MD (95% CI) within groups	6.70 (−0.16 to −13.56)**	5.96 (1.71 to 10.21)*	15.8 (10.07 to 21.54)**
ALP (U/L)			
Before	186.60 ± 56.90	197.79 ± 67.39	185.21 ± 45.98
After	184.67 ± 41.17	182.32 ± 51.24	180.64 ± 59.90
MD (95% CI) within groups	1.93 (−14.16 to 18.02)	15.46 (−1.22 to 32.15)	4.57 (−9.08 to 18.22)
BMI (kg/m^2)			
Before	28.09 ± 3.17	31.71 ± 3.77	28.90 ± 3.92
After	27.39 ± 2.97	30.50 ± 3.48	28.53 ± 3.98
MD (95% CI) within groups	0.69 (0.45 to 0.93)*	1.21 (0.71 to 1.71)*	0.36 (0.17 to 0.57)*

Abbreviation: ALP, Alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Chol, cholesterol; CI, confidence interval; EA, vitamin E-artichoke leaf extract supplement; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MA, metformin-artichoke leaf extract supplement; MD, mean difference; ME, metformin-vitamin E; TG, triglycerides.

[a]Significant difference between the MA and EA groups after the intervention (Post hoc Tukey, $p < .05$).

[b]Significant difference between the MA and ME groups before intervention (Post hoc Tukey, $p < .05$).

[c]Significant difference between the MA and EA groups before intervention (Mann–Whitney *U* test, $p < .05$).

*Significant difference within the group (paired sample T-test, $p < .05$).

**Significant difference within the group (Wilcoxon rank-sum test, $p < .05$).

TABLE 3 Comparison of the results of fatty liver grades of patients with NAFLD at baseline and after the 12-week intervention (*N*, %)

	ME	MA	EA	
Before				
Grade 1	6 (20.00%)	3 (10.00%)	7 (23.30%)	$p = .72$
Grade 2	15 (50.00%)	16 (53.30%)	14 (46.70%)	
Grade 3	9 (30.00%)	11(36.70%)	9 (30.00%)	
After				
Grade 0	3 (10.00%)	7 (23.30%)	5 (17.20%)	$p = .17$
Grade 1	12 (40.00%)	4 (13.30%)	13 (44.80%)	
Grade 2	9 (30.00%)	12 (40.00%)	6 (20.70%)	
Grade 3	6 (20.00%)	7 (23.23%)	5 (17.20%)	
	$p = .001$[a]	$p = .001$[a]	$p = .001$[a]	

[a]Significant difference within the group (Wilcoxon rank-sum test, $p < .05$).

the number of people with no fat accumulation in liver (grade 0) was increased by 23.30, 17.20, and 10% in MA, EA, and ME groups, respectively, after the intervention. Also, BMI was another variable measured in this study. Our results showed that the BMI was decreased significantly within all groups ($p < .05$), and this decrease was more in MA (MD = 1.2) group than the others.

3.5 | Identification of artichoke leaves' active compounds

Although the present study was performed on a standardized commercial product, LC–MS/MS was used for further analyses. High-resolution mass spectra for the LC–MS/MS analysis were obtained in negative ion mode. According to the highest intensity in total ion chromatogram (TIC) results, molecular ion at 516 m/z and its obtained fragments at 353 and 335 m/z corresponded to caffeoylquinic acid glucoside (Figure 2a). The molecular ion at 447 m/z, which produced a fragment at 286 m/z, was attributed to luteolin glycoside (Figure 2b). Also, the molecular ion at 354 m/z and its daughter ions at 191, 179, and 135 m/z corresponded to chlorogenic acid (Figure 2c), and the molecular ion at 341 m/z and its two fragment ions at 179 and161 m/z were identified as caffeoyl-glucoside (Figure 2d). The molecular ion at 179 m/z and its fragment ion at 135 m/z (Figure 2e) were identified as caffeic acid (Ben Salem et al., 2019; Ncube et al., 2014).

4 | DISCUSSION

NAFLD is one of the main common causes of chronic liver diseases, which is highly prevalent throughout the world. In addition to its complications, the studies have shown that it could be correlated with other conditions, including diabetes, cardiovascular disease, various types of cancer, some endocrine diseases, and chronic kidney disease (Kim et al., 2018; Musso et al., 2014; Sinn et al., 2020). The authentic guidelines have recommended lifestyle modification, daily exercise, reducing consumption of food rich in calories in the form of fats and fructose to prevent this disease. In addition, various drug protocols, such as the use of thiazolidinediones (e.g., pioglitazone and rosiglitazone), polyunsaturated fatty acids (e.g., eicosapentaenoic acid), and statins (e.g., atorvastatin, simvastatin, rosuvastatin, fluvastatin, and lovastatin) have been proposed for the treatment of NAFLD (Leoni et al., 2018; Zelber-Sagi et al., 2011). Metformin as a drug increasing insulin sensitivity, and vitamin E as an antioxidant supplement are other medicaments used in the treatment of NAFLD (Bugianesi et al., 2005). However, various studies have not definitely confirmed these drugs' effectiveness, and there is controversy on their function. Besides, long-term use of these drugs can be associated with multiple side effects (Bugianesi et al., Haukeland et al., 2009). However, the use of medicinal plants and their constituents due to their fewer side effects and multi-target effectiveness has always been considered by researchers to treat complex and chronic diseases, such as NAFLD (Yao et al., 2016). Artichoke is one of the plants whose protective and therapeutic effects on NAFLD have been reported in experimental studies and folk medicine (El Sayed et al., 2018; Gebhardt & Fausel, 1997). Our results showed that the concomitant use of ALES with vitamin E and metformin caused beneficial changes in serum levels of lipid, TG, LDL-C, and HDL-C, as well as fatty liver grades in patients with NAFLD. On the other hand, the concomitant use of ALES with metformin significantly increased the serum level of HDL-C compared with the baseline in MA group ($p < .05$). Rondanelli et al. (2013) showed that taking 250 mg of ALES twice a day for 8 weeks significantly reduced the levels of total cholesterol and LDL-C and also increased the level of HDL-C ($p < .001$) in patients with moderate hypercholesterolemia. The results of a meta-analysis study confirmed that the artichoke extract significantly decreased the levels of total cholesterol and LDL-C (Sahebkar et al., 2018). It should be mentioned that, here, there was no significant difference in reduction of serum lipid levels in patients between ALES and ME groups. Therefore, it would be concluded that ALES can be a promising candidate to improve change in pattern of serum lipids and reduce serum levels of lipids in patients with NAFLD.

Elevated serum levels of liver enzymes of AST and ALT occur in most patients with NAFLD, and their changes are used as markers to control disease progression and treatment in these patients (Bayard, Holt, & Boroughs, 2006). Our results showed that serum levels of AST

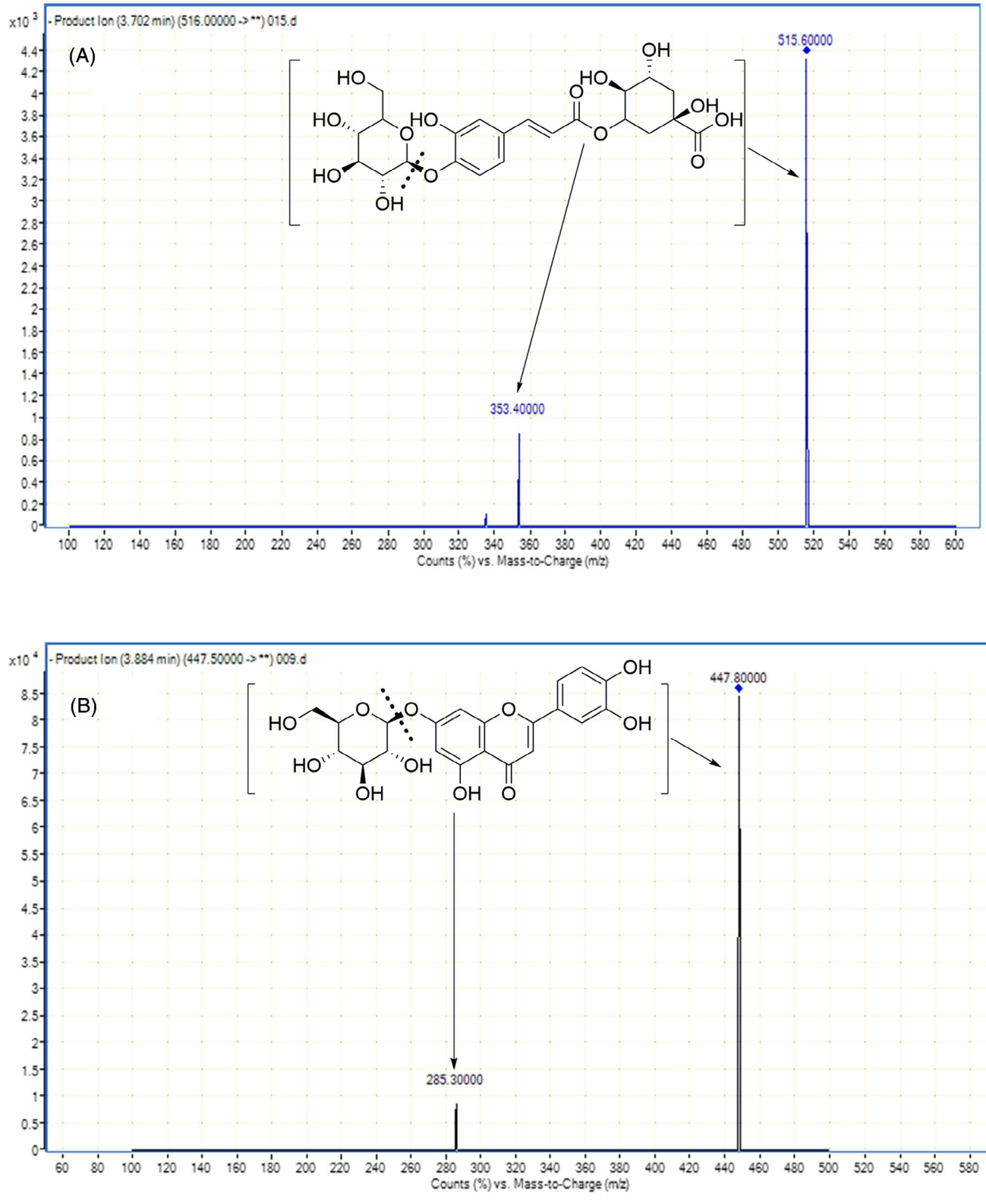
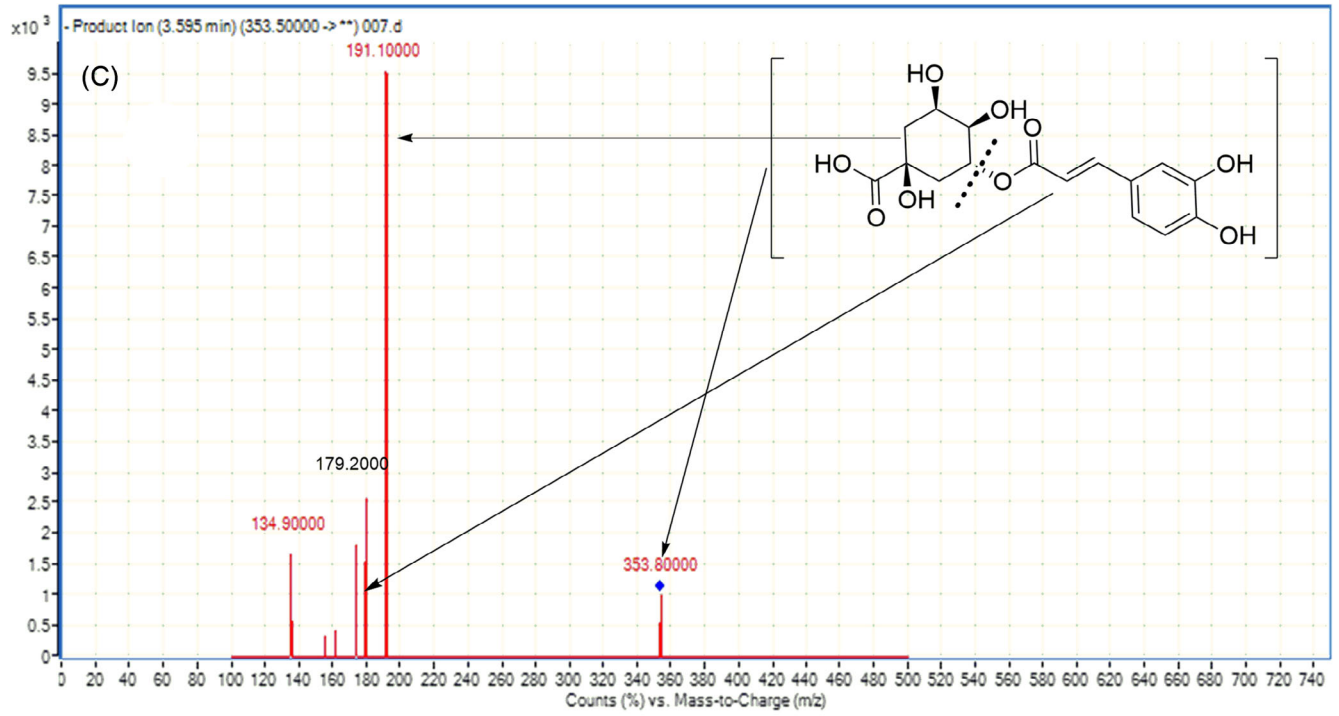


FIGURE 2 Mass spectra of the fragmentation patterns of caffeoylquinic acid glucoside (a), Luteolin 7-glucoside (b), Chlorogenic acid (c), Caffeoylglycoside (d), and Caffeic acid (e)

and ALT were decreased significantly ($p < .05$) in all three groups. Comparing MDs in the study groups indicated that the concomitant use of ALES with vitamin E and metformin further reduced serum levels of ALT and AST than the concomitant use of metformin with vitamin E. There are contradictory results on the effect of metformin on serum levels of AST and ALT in different studies. Bugianesi



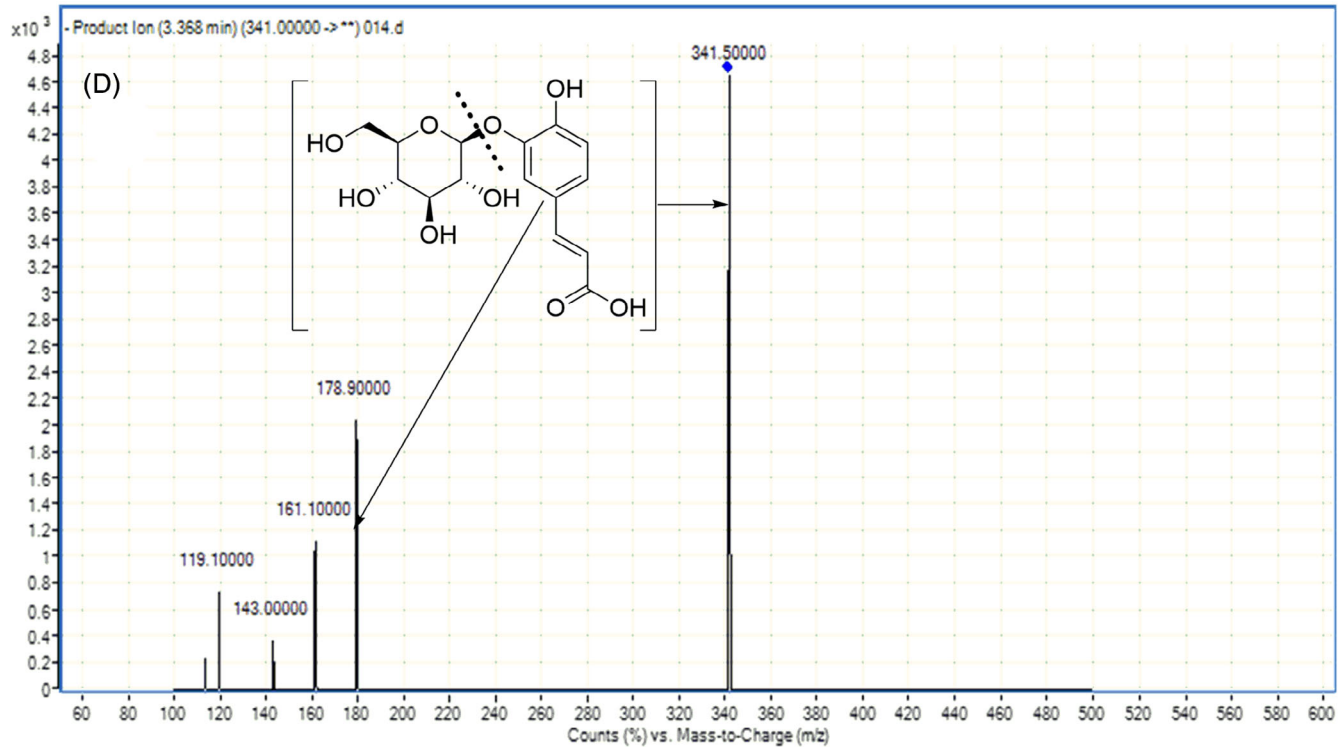


FIGURE 2 (Continued)

et al. (2005) reported that serum levels of AST and ALT were significantly reduced in patients with NAFLD ($p < .001$) after the treatment with metformin (2 g/day, 12 weeks), while Shields, Thompson, Grice, Harrison, and Coyle (2009) showed that treatment with sustained-release formulation of metformin (500 mg/day) for 48 weeks caused no significant difference in reduction of liver enzymes' level compared with placebo ($p < .05$). Bugianesi et al. also found that vitamin E (800 IU/day, 12 weeks) did not significantly reduce serum levels of AST and ALT compared with pre-treatment, while the results of the present study showed that application of ALES along with vitamin E

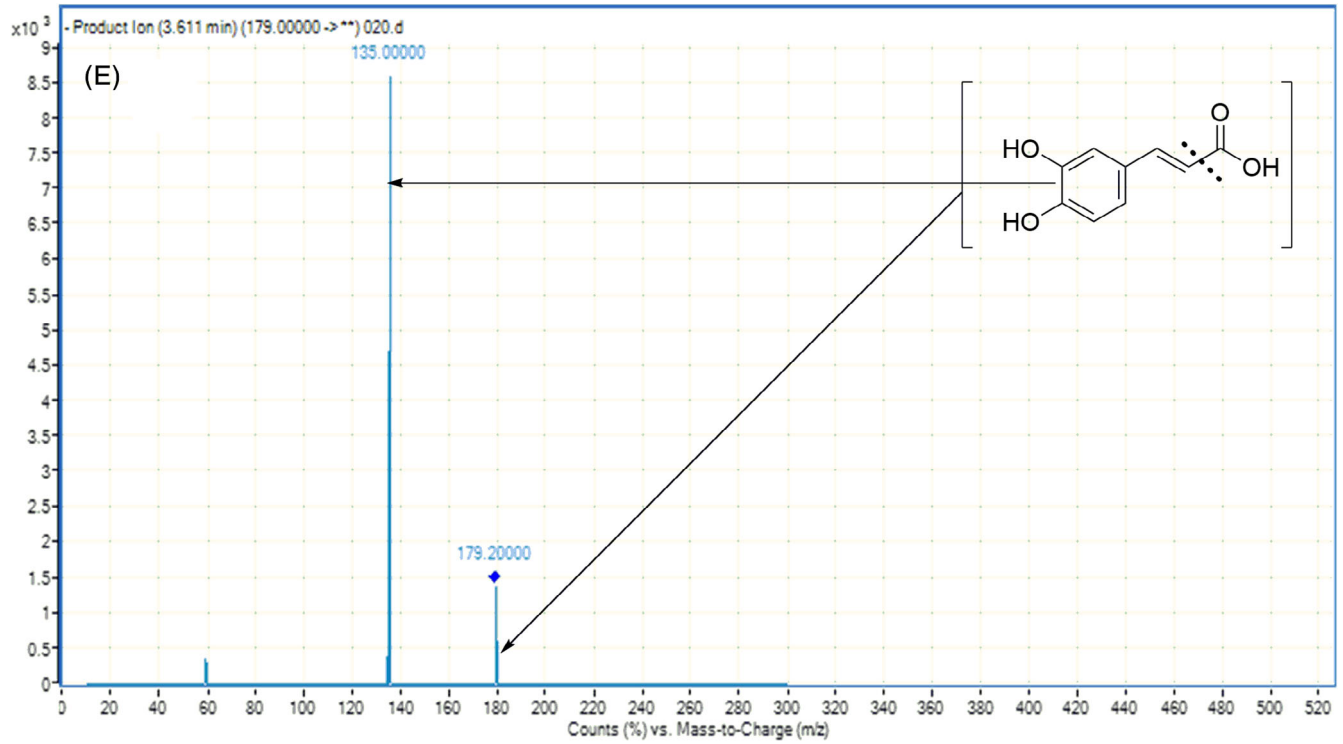


FIGURE 2 (Continued)

(400 UI/day) significantly reduced serum levels of AST and ALT. Besides, Panahi et al. (2018) reported that serum levels of AST and ALT were reduced considerably by administering 200 mg of ALES three times a day after for 8 weeks in patients with NAFLD. The results of this study are consistent with our research on the effect of ALES on reduction of serum levels of ALT and AST in patients with NAFLD. However, more comprehensive clinical studies are needed to recommend ALES alone in order to reduce these two enzymes' serum levels in patients (Panahi et al.). According to the liver ultrasonography results, as one of the standard diagnostic methods, fatty liver is divided into four types. Grade 0 represents no fatty liver and the increase in grades indicates severity of fatty liver. In the present study, the liver ultrasonography results showed beneficial significant changes in fatty liver grades in patients compared with baseline within all groups, especially in the MA group (Table 3). On the other hand, these improving changes can indicate beneficial effects of ALES in combination with conventional therapies of NAFLD. According to studies, this improving effect may be related to the presence of polyphenolic compounds in ALES. Ben Salem et al. (2019) performed an analysis on an ethanol solution extract of artichoke leaf through LC–MS/MS to find polyphenolic compounds with hepatoprotective effects in rats. They reported the presence of compounds, such as chlorogenic acid, caffeoylquinic acid, apigenin, luteolin, quercetin, caffeic acid, and kaempferol in the leaf extract, which may be responsible for the therapeutic and protective effects of artichoke to treat fatty liver in obese rats. The data obtained from the LC–MS/MS analysis confirmed the presence of similar polyphenolic compounds including luteolin, chlorogenic acid, caffeic acid, and its derivatives (Ben Salem et al.). Lowering levels of cholesterol and triglycerides (Cao, Wu, Tian, & Guo, 2019; Meguro, Hasumura, & Hase, 2013), increasing insulin sensitivity (Kwon, Jung, Park, Yun, & Choi, 2015; Lecoultre et al., 2014), inhibiting hydroxy-methyl-glutaryl coenzyme A (Sahebkar et al., 2018), increasing synthesis of bile acid (Park et al., 2020), and antioxidant activities (Choi et al., 2014; Li et al., 2018) are among basic mechanisms of polyphenols like luteolin, chlorogenic acid, caffeic acid and their derivatives for prevention and treatment of NAFLD. In addition, luteolin reduces absorption of cholesterol by inhibiting the Niemann–Pick C1-Like 1 (NPC1L1) protein, as a protein present in gastrointestinal tract wall and hepatocytes with a critical role in absorption of cholesterol showing the privileged effects in treatment of NAFLD (Kobayashi, 2019).

It is noteworthy that, in addition to the results of the present study, comprehensive studies are needed to confirm the use of an herbal supplement instead of conventional drug therapy. Therefore, until the effectiveness of these herbal supplements is proven by valid drug guidelines, they must be used as adjunctive therapy and approved drug protocols (Gheibi et al., 2019; Hassanzadeh-Taheri et al., 2018; Mosihuzzaman & Choudhary, 2008). There were some limitations in this study, for example, the lack of more complete diagnostic tests and a short treatment period. However, further research is recommended to investigate cellular signaling mechanisms in the studied groups in order to clarify their synergistic effects. Other recommendations include investigation of the effects of prescribing ALES along with other treatment protocols provided in authentic guidelines.

5 | CONCLUSION

Our findings briefly showed the critical clinical role of co-administration of the ALES with metformin and vitamin E for ameliorating complications of liver function in patients with NAFLD. However, large-scale clinical trials are needed to confirm these results.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

ORCID

Ina Yosifova Aneva https://orcid.org/0000-0002-6476-5438

Mohammad Hosein Farzaei https://orcid.org/0000-0001-7081-6521

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