JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2024, 75, 3, 291-302
www.jpp.krakow.pl | DOI: 10.26402/jpp.2024.3.06

[2]A. KOPERSKA[1,2], M. MOSZAK[1], A. SERASZEK-JAROS[3], P. BOGDANSKI[3], M. SZULINSKA[3]

DOES BERBERINE IMPACT ANTHROPOMETRIC, HEPATIC, AND METABOLIC PARAMETERS IN PATIENTS WITH METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE? RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

[1]Department of Treatment of Obesity, Metabolic Disorders and Clinical Dietetics, Poznan University of Medical Sciences, Poznan, Poland; [2]Doctoral School, Poznan University od Medical Sciences, Poznan, Poland;
[3]Department of Bioinformatics and Computational Biology, Poznan University of Medical Sciences, Poznan, Poland

Globally, the metabolic dysfunction-associated fatty liver disease (MAFLD) holds the position as the most widespread chronic liver condition. Berberine (BBR) shows promise as a natural compound for managing obesity, hepatic steatosis, and metabolic disorders. The study aimed to investigate the effectiveness of BBR in addressing factors linked to MAFLD. This is a randomized, double-blind, and placebo-controlled clinical trial. Seventy individuals with MAFLD were enrolled in this study and randomly assigned in a 1:1 ratio to two groups. BBR (1500 mg/day) or placebo was administrated orally for 12 weeks. Selected anthropometric, hepatic, and metabolic parameters were assessed. After a 12-week intervention, the BBR group demonstrated a statistically significant decrease in alanine transaminase (ALT) p=0.0105, and de Ritis ratio p=0.0011 compared to the control group. In both groups we observed a decrease in trunk fat (kg) - BBR group p=0.0185, and placebo group p=0.0323. After three months, a significant divergence between the BBR and placebo groups was evident in the alteration of Δ total cholesterol (TC) p=0.0009, favoring the BBR group. Nevertheless, there were no significant differences detected in other lipid and glucose parameters. In the BBR group, we found significant correlations between changes and amelioration of certain variables: Δ body mass index (BMI) correlated with ΔALT (r=0.47; p=0.0089) and Δ aspartate aminotransferase (AST) (r=0.47; p=0.0081) levels; Δ trunk fat with Δ fatty liver index (FLI) (r=0.55; p=0.0337), Δ homeostasis model assessment for insulin resistant index (HOMA-IR) (r=0.37; p=0.0020), and AST (r=0.42; p=0.0202); Δ the de Ritis ratio correlated with Δ fibrosis-4 index (FIB-4) levels (r=0.59; p=0.0011); and ΔFLI correlated with ΔHOMA-IR (r=0.37; p=0.0409) and Δ visceral adiposity index (VAI) (r=0.54; p=0.0019), while no significant differences were observed in the Placebo group. The results show that BBR appears to be a bioactive compound that positively impacts MAFLD, however, additional research with extended intervention durations is required to fully assess its efficacy and potential clinical use.

Key words: *berberine, metabolic dysfunction-associated fatty liver disease, metabolic parameters, body mass index, homeostasis model assessment for insulin resistant index, visceral adiposity, liver fibrosis*

INTRODUCTION

The incidence of nonalcoholic fatty liver disease (NAFLD) has been steadily increasing over the past decade, particularly in the context of the global obesity epidemic. Notably, a recent proposal introduces a new definition for NAFLD, referred to metabolic dysfunction-associated fatty liver disease (MAFLD) (1, 2). This new terminology emphasizes the strong association between obesity, particularly abdominal obesity, and the increased risk of MAFLD. Experts have reached a consensus that metabolic dysfunction-associated fatty liver disease (MAFLD) is a more suitable and comprehensive term for describing the condition (2-4). The estimated global prevalence of MAFLD among obese adults is approximately 50%, with a relatively higher incidence observed in men (5). Considering that MAFLD can be viewed as a hepatic manifestation of systemic insulin resistance (IR), renaming NAFLD as MAFLD could facilitate more accurate identification and understanding of fatty liver disease (3, 6). The diagnostic criteria for MAFLD include the presence of hepatic steatosis, coupled with any one or more of the following: overweight/obesity, type 2 diabetes mellitus (T2DM), and indications of metabolic dysregulation (3). In recent times, berberine (BBR) has garnered significant attention for its promising potential in addressing metabolic disorders. BBR's therapeutic advantages are derived from its capacity to control a range of metabolic factors and pathological processes. It can enhance insulin secretion, improve IR, inhibit lipogenesis, alleviate adipose tissue fibrosis, decrease hepatic steatosis, and rectify gut microbiota disorders. It is important to note that much of the research involving BBR has been carried out on individuals with impaired carbohydrate metabolism, such as those with diabetes or impaired glucose tolerance. This emphasis has allowed for a deeper understanding of

BBR's effects on metabolic pathways in conditions characterized by dysregulated carbohydrate metabolism. Taken together, BBR stands out as a promising and potentially impactful drug candidate for treating metabolic diseases connected with IR and abdominal obesity (7). The purpose of this study was to assess the therapeutic impact of BBR on liver function and metabolic profiles in individuals diagnosed with MAFLD, where the main metabolic disorder is overweight or obesity, without accompanying carbohydrate metabolism disorders. In recent times, metabolic diseases, with obesity at the forefront, have attracted widespread attention, emerging as a significant global public health challenge. BBR is an isoquinoline alkaloid present in the root, rhizome, stem, or stem bark of various widely used medicinal plants, including *Coptis chinensis*, *Berberis aristata DC*, and *Berberis vulgaris* L. Numerous studies have documented the evident hypoglycemic, hypolipidemic, anti-obesity, hepatoprotective, and anti-inflammatory properties of BBR (7). It has a historical application in traditional Chinese medicine for the treatment of gastrointestinal infections (8). BBR is a yellow crystalline powder characterized by an intensely bitter taste (7). Clinical investigations and studies involving animals have generated compelling evidence, revealing the effectiveness of BBR in regulating glucose and lipid metabolism, and concurrently alleviating IR (8). BBR alleviates the accumulation of lipids in the liver, enhances hepatic steatosis, inhibits hepatic inflammation and oxidative stress, and regulates gut microbiota (7). It has been demonstrated that BBR can hinder the progression of hepatic steatosis to steatohepatitis and fibrosis. This is achieved through the regulation of lipid metabolism and the inhibition of lipogenesis (7). Several studies have indicated that BBR has the potential to prevent obesity by downregulating the expression of genes associated with the proliferation and differentiation of adipocytes (8, 9). According to the results of the meta-analysis, BBR exhibits positive impacts on the lipid profile, blood glucose levels, liver function, IR, and the condition of fatty liver among individuals diagnosed with NAFLD (10). Presently, due to the limited number and quality of incorporated trials, there is a call for additional clinical randomized controlled trials of elevated quality to further substantiate the efficacy of BBR in the treatment of NAFLD and MAFLD patients.

Our study, characterized by a rigorous protocol and the administration of a high dosage of BBR, underscores the evaluation of BBR's effectiveness in the context of MAFLD. The majority of earlier studies in NAFLD concentrated on the use of BBR at dosages that were one-half or one-third of the dosage employed in our research. It is noteworthy that the dosage 1500 mg/day it not authorized for use as dietary supplement.

It is important to emphasize that there is a lack of research dedicated to comprehensively investigating the varied effects of BBR on MAFLD and its metabolic characteristics. According to our current knowledge, this research marks the first attempt to evaluate the impact of BBR on hepatic steatosis in individuals diagnosed with MAFLD but without diabetes or any carbohydrate metabolism dysfunction.

The study aimed to explore whether the supplementation of BBR could have favorable effects on the health of overweight and class I obese patients with MAFLD by impacting anthropometric, hepatic, and metabolic parameters.

METHODS

Study design

The study was a randomized, double-blind, placebo-controlled clinical trial. Ethical approval was obtained from the Bioethical Committee of Poznan University of Medical Sciences. The protocol was registered at the US National Institute of Health (ClinicalTrails.gov Identifier: NCT05523024). The participants were enlisted at the outpatient department of the University Hospital, Poznan, Poland. All participants provided informed written consent to participate in the research study.

Clinical parameters and medical data

During the initial visit, an evaluation of socio-demographic and medical information was carried out (socio-demographic data questionnaire, medical history interview, medical documents). Participants were provided with a medication journal to monitor their adherence to preparation intake and to document any potential side effects or missed doses. At the baseline and the endpoint liver steatosis and fibrosis scores: fibrosis-4 index (FIB-4), fatty liver index (FLI); de Ritis ratio, and visceral adiposity index (VAI) were calculated based on laboratory parameters. Laboratory tests included: alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), lipid metabolism parameters: total cholesterol (TC), high-density cholesterol (HDL), non-HDL, low-density lipoprotein (LDL), triglycerides (TG), carbohydrate metabolism parameters: fasting blood glucose (FBG), fasting blood insulin (FBI) concentration, homeostasis model assessment for insulin resistant index (HOMA-IR), inflammation parameters: high-sensitivity CRP (hs-CRP) and uric acid were performed for each group. Blood samples were obtained after an overnight period of fasting and rest and measurements were according to requirements specified by International Organization for Standardization (ISO).

During half of the study duration, remote consultations were conducted to evaluate adherence to study protocol and potential adverse effect occurrence.

Calculated parameters - formulas

a) FIB-4

The FIB-4 index is calculated using the formula:

FIB-4=Age(years)×AST(U/L)/[PLT(109/L)×ALT1/2(U/L)] (11);

b) FLI

FLI = (e0.953*loge (TG)+0.139*BMI+0.718*loge (GGT)+ 0.053*WC–15.745)/(1+e0.953*loge (TG)+0.139*BMI+ 0.718*loge (GGT)+0.053*WC–15.745)×100 (12);

c) VAI

Men:

VAI=[WC/39.68+(1.88×BMI)]×[TG/1.03]×[1.31/HDL];

Women:

VAI=[WC/36.58+(1.89×BMI)]×(TG/0.81)×(1.52/HDL) (13);

d) HOMA-IR

HOMA-IR = fasting insulin (microU/L) × fasting glucose (nmol/L)/22.5 (14);

e) De Ritis ratio

Ratio between the serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) (15).

Berberine supplement and allocation

Eligible patients (n=70) were enrolled into one of two groups if they met inclusion criteria and presented no exclusion criteria. An impartial allocation of participants to groups was ensured through the oversight of randomization by an independent statistician. The flowchart of the study design is presented in *Fig. 1*. Group 1 received a placebo (n=35), and group 2 (n=35) received berberine (Berberine hydrochloride 97% extract of *Berberis aristata*) 1500 mg per day in 3 dosages.

BBR was orally administered for 12 weeks - three times per day (3×2 capsules) before breakfast (7:00 to 9:00 a.m.), before dinner (1:00 to 2:00 p.m.), and before supper (6:00 to 7:00 p.m.).

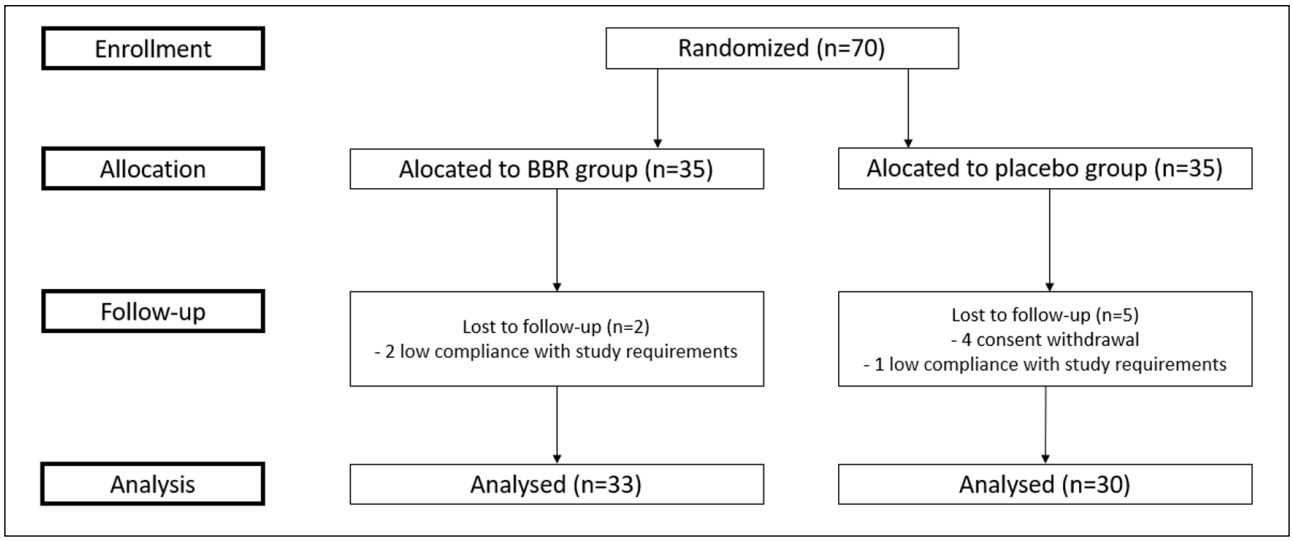

Fig. 1. A flowchart of the study design.

The placebo was designed to be distinguishable from the tested substance, varying in color, taste, smell, method of administration, and dosage. The placebo, comprising only excipients (potato starch), was also orally administered for 12 weeks. Both groups received an equal number of capsules (six) per day. During the first visit, each participant received a twelve-week supply of the assigned preparations. At the last visit, they were asked to hand back empty packages and medication journal.

Safety data

Below, we present the safety data. Both the berberine supplement and the placebo were safe for the participants and met all required standards.

Lead not more than 3ppm (µg/g); arsenic not more than 1ppm (µg/g); cadmium not more than 1ppm (µg/g); mercury not more than 0.1ppm (µg/g); residual pesticides not applicable; poly aromatic hydrocarbons (PAH): benzo(a)pyrene not more than 10ppb (µg/kg), PAH4 (sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) not more than 50ppb (µg/kg).

Microbiological profile: Total aerobic microbial count not more than 5000cfu/g; total yeasts and molds count not more than 100cfu/g; Escherichia coli Negative/10g; Salmonella Negative/25 g; Staphylococcus aureus Negative/10 g; Pseudomonas aeruginosa Negative/10 g; Bile tolerant Gram negative bacteria Negative/10 g; Coliforms Less than 10cfu/g.

Additional information: Genetic Modification Status: GMO free; BSE/TSE status: BSE/TSE free; gluten free; lactose free; for vegan.

BBR: manufactured by Sami Labs Limited - Peenya 19/1, 19/2, I Main II Phase, Peenya Industrial Area, Bangalore. 560 058, Karnataka, India. Supplier: Sabinsa Poland Sp z o. o.

Placebo: Standard Sp z o. o. Olszewskiego 10, 20-481 Lublin, Poland.

Diet and physical activity assessment

At the baseline (enrollment and allocation) and the endpoint anthropometric and body composition measurements were conducted, along with assessments of diet and physical activity: questionnaires FFQ6 (Food Frequency Questionnaire with 6 Answers) (16) and nutritional interview (24-hour record), physical activity interview (IPAQ, International Physical Activity Questionnaire) (17). FFQ6 and IPAQ are validated tools adapted to the Polish version. To minimize the potential bias in outcomes arising from modifications in diet and physical activity, participants were instructed to uphold their current practices in terms of physical activity and dietary habits. Verification of adherence to these instructions was ensured through nutritional and physical activity assessments conducted before the intervention, during remote control visits, and upon completion of the study.

Anthropometric parameters: waist circumference (WC) - measured between the iliac crest and the lower rib after a normal exhale. WC was measured twice using stretch-resistant medical tape with a precision of 0.5 cm; weight and height - measured with an accuracy of 0.1 kg, and height was estimated to the nearest 0.5 cm; body mass index (BMI): calculated by dividing the weight by the square of the height (kg/m^2) (18). Fat mas %, trunk fat (kg) assessed by bioimpedance analysis (BIA).

Obesity is defined as the excessive accumulation of fat tissue in the body, exceeding the optimal values necessary for maintaining good health. BMI is a commonly used measure to assess body weight in relation to height. Another method used to identify obese patients and measure body fat content is BIA (33). Analysis of body mass composition was performed using the electrical bioimpedance method with InBody 370. This analysis adhered to the guidelines set by the European Society for Clinical Nutrition and Metabolism (ESPEN) (19).

Individuals

A total of 70 MAFLD patients after the screening process were initially invited to participate in the study.

The inclusion criteria were:

1) MAFLD (according to the definition proposed in 2020). MAFLD is diagnosed in patients when they have both hepatic steatosis and any of the following three metabolic conditions: overweight/obesity, diabetes mellitus, or evidence of metabolic dysregulation (MD) in lean individuals (2). Hepatic steatosis was identified based on an ultrasound scan.

2) body mass index (BMI): 27.0 kg/m^2 to 34.9 kg/m^2

3) abdominal obesity-related waist circumference >80 cm (women) and >94 cm (men) - in accordance to International Diabetes Federation (20);

4) age 40 to 60 years;

5) women ≥1 year since last menstruation;

6) stable body weight in the 3 months prior to the trial (permissible deviation is ± 3 kg).

The exclusion criteria:

1) history of following alternative diets within 3 months before the study;

2) history of use of any dietary supplements in the 3 months before the study;

3) history of intake of antibiotics, probiotics, prebiotics within 3 months before the study;

4) secondary form of obesity, pharmacological treatment for obesity (in the 3 months before the study), history of bariatric surgery;

5) another liver diseases: high risk of NASH (assessed on the FIB-4 (score >2.67), autoimmune hepatitis, hepatitis B and C, toxic hepatitis, cirrhosis, Wilson's disease, hemochromatosis;

6) other gastrointestinal disorders, especially: inflammatory bowel disease (IBD), celiac disease, gastritis and duodenitis, pancreatic disorders, gastrointestinal symptoms suggestive of IBS;

7) clinically significant acute inflammatory process (elevated hsCRP);

8) abnormal kidney function (GFR <60mL/min/1,73m^2;

9) T2DM;

10) dyslipidemia or hypertension - requiring the introduction and/or change of pharmacological treatment in the 6 months before the trial or during intervention. Acceptable first-grade hypertension, with the use of a single medication to manage and control blood pressure; dyslipidemia was managed using mono pharmacotherapy, excluding the use of statins;

11) another chronic pharmacotherapy: nonsteroidal anti-inflammatory drugs, proton pump inhibitors, anticoagulants, drugs causing metabolic alteration, *e.g.*, SFAs (second-generation antipsychotics);

12) diseases requiring nutritional requirement and chronic supplementation;

13) alcohol (>30g/d for men and >20g/d for women), nicotine or drug abuse;

14) mental disorders, including eating disorders;

15) cancer, autoimmune diseases;

16) any other condition which may influence on final results of the study or pose a risk for subjects health.

No serious adverse events (SAE) were reported by the participants who consumed the BBR supplement throughout the study. The occurrence of mild gastrointestinal events, such as flatulence or bitter aftertaste, did not necessitate any adjustments to the supplementation regimen. Additionally, there was no need for any additional medical treatment among the patients during the study period. Consent withdrawal was attributed to personal matters. The flowchart (*Fig. 1*) provides details on the number of patients for whom follow-up was not possible, along with the reasons for this occurrence.

Statistical analysis

All calculations and statistical analyses were performed using TIBCO Software Inc. (2017). Statistica (data analysis software system), version 13. Quantitative variables were presented using the mean value and standard deviation, median, minimum, and maximum values. Using the Shapiro-Wilk test, the compliance of their distributions with the Gaussian curve was checked. Most of the variables did not comply with normal distribution, therefore comparative analyses were performed using non-parametric tests. The Mann-Whitney test was used to compare results between groups and Wilcoxon test was used to compare results before and after the intervention. For correlation the Spearman rang test was used. For analyses with statistically significant results, the power of the test was calculated, ranging from 79% to 98%. Only in the case of HOMA-IR is it 30%. For all tests, results were considered significant if the p-value was less than 0.05.

RESULTS

Baseline and postintervention outcomes

Variables assessed in BBR and control group at the baseline are present in *Table 1*. At the baseline, significant differences between groups existed in age (p=0.0175), HDL (p=0.0352), and FIB-4 (p=0.0189). The age criteria were met in both groups and HDL was ranged within the norm. Postintervention outcomes in BBR and control groups are presented in *Table 2*. There was no significant change in the fibrosis score (*e.g.*, FIB-4) or other studied parameters between the BBR and placebo groups after 12 weeks. Results indicate the effectiveness of BBR in reducing trunk fat (kg) (p=0.0185), ALT (p=0.0105), and de Ritis ratio (p=0.0011) after the 12-week intervention (*Table 3*). Significant outcomes are presented in *Fig. 2* and *Fig. 3*. However, no significant changes were observed in other lipid or glucose parameters, as well as liver-related indicators.

Changes in Δ variables in the berberine and placebo groups after 3 months

Change between the BBR and placebo groups after 3 months (*Table 4*) was observed in Δ TC (p=0.0009). We noted a significant correlation in the change of selected variables in the BBR group following the intervention (*Table 5*, *Figs. 4-8*). Specifically, changes in delta BMI correlated with ALT (r=0.47; p=0.0089) and AST levels (r=0.47; p=0.0081), while changes in trunk fat were correlated with FLI (r=0.55; p=0.0337), HOMA-IR (r=0.37; p=0.0020), and AST (0.42; p=0.0202). Additionally, the de Ritis ratio was correlated with FIB-4 levels (r=0.59; p=0.0011), while FLI was correlated with HOMA-IR (r=0.37; p=0.0409) and VAI (r=0.54; p=0.0019). While no significant changes were observed in these parameters within the placebo group. The FIB4 index, serving as a liver fibrosis biomarker, presents itself as a potential alternative to liver biopsy for diagnosing and managing liver disease.

Adverse effects, safety and compliance

It is noteworthy that BBR treatment in a few cases induced mild and transient gastrointestinal reactions during the initial days/weeks. During the 12-week intervention, no SAE was reported. Flatulence or a bitter aftertaste were the most commonly reported symptoms. Participants did not require any medical intervention or experience health deterioration due to the supplementation of BBR. The tolerability of BBR supplementation in 1500 mg/day dosage was assessed as acceptable. In the berberine group, two individuals were excluded from the final analysis due to poor adherence to the study protocol. Meanwhile, in the placebo group, four participants discontinued their involvement by withdrawing consent, and one individual was excluded due to poor compliance.

DISCUSSION

The diagnostic criterion for MAFLD encompasses the presence of hepatic steatosis along with any one or more of the following: overweight/obesity, T2DM, or signs of metabolic dysregulation (3). Building upon the promising potential of BBR

Table 1. Berberine (BBR) and control group baseline characteristics.

Variables	**BBR group** (n=33)				**Control group** (n=30)				**Differences between groups**
	Mean±SD	**Median**	**Min.**	**Max.**	**Mean±SD**	**Median**	**Min.**	**Max.**	
Age	49.09±5.00	50.0	40.0	59.0	53±6.25	52.5	42.0	63.0	**0.0175***
BMI [kg/m^2]	32.22±3.46	31.7	25.8	39.9	30.9±3.55	29.8	25.8	39.8	0.0975
WC [cm]	103.93±12.81	104.0	82.0	133.9	99.47±12.37	98.4	73.5	127.5	0.1901
FM [%]	39.76±6.23	40.4	24.2	50.9	40.5±4.96	41.0	31.2	50.8	0.6969
Trunk fat [kg]	18.95±3.75	18.1	13.8	26.9	19.19±6.18	18.55	11.1	43.00	0.7070
TC [mg/dl]	215.58±37.93	215.0	128.0	298.0	222.87±42.94	221.0	150.0	287.0	0.6082
HDL [mg/dl]	52.73±11.83	51.0	34.0	83.0	59.17±12.57	60.0	33.0	88.0	**0.0352***
TG [mg/dl]	128.73±61.07	111.0	58.0	320.0	111.83±41.01	105.0	52.0	208.0	0.4155
LDL [mg/dl]	137.06±33.55	137.00	65.00	221.00	141.47±43.12	133.00	75.00	225.00	0.8967
HOMA-IR	1.69±0.83	1.6	0.6	4.3	1.73±1.16	1.4	0.6	5.5	0.6041
FLI	68.49±25.58	75.9	16.2	99.3	58.18±25.24	67.1	17.2	100.0	0.0948
FIB-4	0.97±0.41	0.87	0.50	2.01	0.73±0.27	0.69	0.27	1.34	**0.0189***
VAI	2.04±1.38	1.7	0.7	7.5	1.69±0.79	1.4	0.7	3.6	0.4395
UA [mg/dl]	5.45±1.44	5.6	2.7	9.0	5.45±1.08	5.5	3.7	9.3	0.7689
ALT [U/l]	33.94±23.99	24.0	14.0	120.0	30.50±16.38	26.0	10.0	91.0	0.9292
AST [U/l]	22.73±10.55	20.0	10.0	60.0	24.1±7.38	23.0	14.0	48.0	0.1188
GGTP [U/l]	46.55±85.79	27.0	11.0	466.0	37.14±61.51	25.0	11.0	352.0	0.8776
de Ritis ratio	0.76±0.24	0.76	0.32	1.48	0.90±0.37	0.82	0.48	2.40	0.1809
non-HDL	162.85±40.16	156.0	77.0	260.0	163.77±42.29	157.5	105.0	252.0	0.7481
FBG [mg/dL]	89.73±8.58	90.0	76.0	125.0	90.43±12.08	88.5	75.0	130.0	0.5428
FBI [uU/mL]	7.60±3.55	7.6	2.8	19.4	7.49±4.13	6.3	2.6	21.3	0.7367

BMI, body mass index; WC, waist circumference; FM, fat mass; TC, total cholesterol; HDL, high-density cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistant index; FLI, fatty liver index; FIB-4, fibrosis-4 index, VAI, visceral adiposity index; UA, uric acid; ALT, alanine transaminase; AST, aspartate aminotransferase; GGTP, gamma-glutamyltransferase; FBG, fasting blood glucose; FBI, fasting blood insulin; * p-value <0.05.

Table 2. Postintervention outcomes in berberine (BBR) and control groups.

Variables	**BBR group** (n=33)				**Control group** (n=30)				**Differences between groups**
	Mean±SD	**Median**	**Min.**	**Max.**	**Mean±SD**	**Median**	**Min.**	**Max**	**p-value**
BMI [kg/m^2]	32.16±3.64	32.50	25.70	39.40	30.57±3.63	29.95	23.30	39.20	0.0701
WC [cm]	103.55±10.99	105.00	75.50	123.25	99.70±10.52	98.10	77.25	123.50	0.1412
FM [%]	39.33±6.35	41.20	24.30	50.00	40.24±4.62	40.15	29.00	49.20	0.8691
Trunk fat [kg]	18.82±3.77	18.20	12.30	25.4	18.06±4.16	17.80	10	26.4	0.4713
TC [mg/dl]	211.81±30.83	208.00	126.00	263.00	220.60±40.63	220.50	152.00	295.00	0.5704
HDL [mg/dl]	52.03±11.83	50.00	35.00	83.00	58.37±13.16	59.00	34.00	90.00	0.0545
TG [mg/dl]	136.79±91.35	108.00	44.00	435.00	119.90±54.80	101.00	52.00	267.00	0.6082
LDL [mg/dl]	133.97±26.14	134.00	69.00	181.00	141.77±41.91	142.50	57.00	112.00	0.3658
HOMA-IR	1.68±0.89	1.45	0.29	5.05	1.89±1.63	1.49	0.00	7.61	0.9945
FLI	67.4±25.68	78.10	14.20	98.60	61.70±25.61	67.10	17.21	100.00	0.3682
FIB-4	0.79±0.33	0.77	0.18	1.82	0.96±0.34	0.93	0.38	1.83	0.0500
VAI	2.14±1.99	1.65	0.00	8.67	1.98±1.36	1.55	0.58	7.01	0.9238
UA [mg/dl]	5.54±1.47	5.42	2.48	8.10	5.28±0.94	5.15	4.00	8.30	0.3531
ALT [U/l]	28.06±16.92	22.50	11.00	75.00	26.9±13.68	24.00	9.00	72.00	0.7039
AST [U/l]	22.56±10.09	20.00	7.00	55.00	39.67±96.05	21.00	12.00	547.00	0.7579
GGTP [U/l]	36.13±54.13	24.50	12.00	320.00	46.24±60.46	27.00	10.00	293.00	0.4936
de Ritis ratio	0.89±0.28	0.87	0.35	1.54	0.91±0.29	0.86	0.51	2.11	0.9267
non-HDL	159.82±31.38	154.00	87.00	224.00	162.17±38.69	163.00	93.00	247.00	0.9836
FBG [mg/dL]	88.91±8.65	87.00	77.00	121.00	94.30±21.07	90.50	73.00	171.00	0.3075
FBI [uU/mL]	7.56±3.45	6.70	1.50	16.90	8.79±6.89	7.10	2.90	39.50	0.8238

BMI, body mass index; WC, waist circumference; FM, fat mass; TC, total cholesterol; HDL, high-density cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistant index; FLI, fatty liver index; FIB-4, fibrosis-4 index, VAI, visceral adiposity index; UA, uric acid; ALT, alanine transaminase; AST, aspartate aminotransferase; GGTP, gamma-glutamyltransferase; FBG, fasting blood glucose; FBI, fasting blood insulin.

Table 3. Pre-intervention and postintervention differences in variables in the berberine (BBR) and placebo groups.

Variables	BBR group			Placebo group		
	Mean±SD		p-value	Mean±SD		p-value
	before	After 12 weeks		before	after 12weeks	
BMI [kg/m^2]	32.22±3.46	32.16±3.64	0.9835	30.9±3.55	30.57±3.63	0.0619
WC [cm]	103.93±12.81	103.55±10.99	0.5748	99.47±12.37	99.70±10.52	0.7241
FM [%]	39.76±6.23	38.68±7.07	0.0752	40.5±4.96	40.24±4.62	0.4299
Trunk fat [kg]	18.95±3.75	18.32±4.26	**0.0185***	19.19±6.18	18.06±4.16	**0.0323***
TC [mg/dl]	215.58±37.93	211.81±30.83	0.2278	222.87±42.94	220.60±40.63	0.8119
HDL [mg/dl]	52.73±11.83	52.03±11.83	0.2207	59.17±12.57	58.37±13.16	0.6204
TG [mg/dl]	128.73±61.07	136.79±91.35	1	111.83±41.01	119.90±54.80	0.2137
LDL [mg/dl]	137.06±33.55	133.97±26.14	0.6509	141.47±43.12	141.77±41.91	0.7189
HOMA-IR	1.69±0.83	1.68±0.89	0.5249	1.73±1.16	1.89±1.63	**0.0461***
FLI	68.49±25.58	67.4±25.68	0.9765	58.18±25.24	61.70±25.61	0.6766
FIB-4	0.73±0.27	0.79±0.33	0.2242	0.73±0.27	0.79±0.33	0.5905
VAI	2.04±1.38	2.20±1.99	0.9238	1.69±0.79	1.98±1.36	0.1718
UA [mg/dl]	5.45±1.44	5.54±1.47	0.8079	5.45±1.08	5.28±0.94	0.1494
ALT [U/l]	33.94±23.99	28.06±16.92	**0.0105***	30.50±16.38	83.5±310.32	0.1577
AST [U/l]	22.73±10.55	22.56±10.09	0.8693	24.1±7.38	39.67±96.05	0.3246
GGTP [U/l]	46.55±85.79	36.13±54.13	0.7648	37.14±61.51	46.24±60.46	0.7089
de Ritis ratio	0.89±0.24	0.74±0.28	**0.0011***	0.90±0.37	0.91±0.29	0.4123
non-HDL	162.85±40.16	159.82±31.38	0.2278	163.77±42.29	162.17±38.69	0.8936
FBG [mg/dL]	89.73±8.58	88.91±8.65	0.3674	90.43±12.08	94.30±21.07	0.2369
FBI [uU/mL]	7.60±3.55	7.56±3.45	0.7613	7.49±4.13	8.79±6.89	0.0949

BMI, body mass index; WC, waist circumference; FM, fat mass; TC, total cholesterol; HDL, high-density cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistant index; FLI, fatty liver index; FIB-4, fibrosis-4 index, VAI, visceral adiposity index; UA, uric acid; ALT, alanine transaminase; AST, aspartate aminotransferase; GGTP, gamma-glutamyltransferase; FBG, fasting blood glucose; FBI, fasting blood insulin; *p-value <0.05.

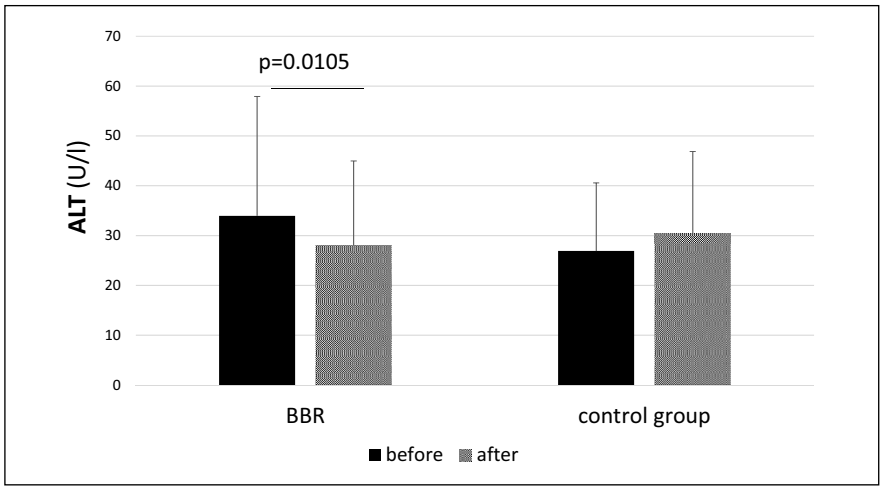

Fig. 2. Differences in ALT [U/I] in the berberine (BBR) and control group before and after a 12-week intervention.

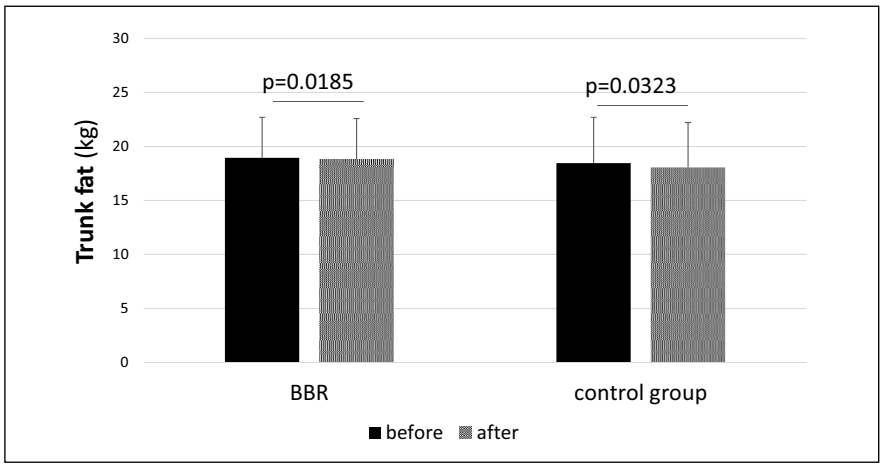

Fig. 3. Differences in Trunk fat (kg) in the berberine (BBR) and control group before and after a 12-week intervention.

Table 4. Changes in anthropometric and biochemical variables in the berberine (BBR) and placebo groups after 3 months.

Variable	Group	Mean±SD	p-value
Δ BMI [kg/m^2]	BBR Placebo	0.07±1.03 0.33±0.96	0.2719
Δ WC [cm]	BBR Placebo	–1.90±6.14 –0.30±5.31	0.4608
Δ FM [%]	BBR Placebo	–0.57±1.56 –0.26±2.45	0.8467
Δ Trunk fat [kg]	BBR Placebo	0.45±1.15 0.41±1.29	0.8028
Δ TC [mg/dl]	BBR Placebo	–163.82±32.01 –198.63±53.30	**0.0009***
Δ HDL [mg/dl]	BBR Placebo	0.70±5.82 0.80±7.02	0.6081
Δ TG [mg/dl]	BBR Placebo	–8.06±50.19 –8.07±38.73	0.2821
Δ LDL [mg/dl]	BBR placebo	–0.20±21.35 –0.30±27.44	0.9707
Δ HOMA-IR	BBR Placebo	0.01±0.48 –0.39±0.91	0.0673
Δ FLI	BBR Placebo	–0.46±12.54 –1.76±11.11	0.9478
Δ FIB-4	BBR Placebo	–0.05±0.21 0.06±0.25	0.2156
Δ VAI	BBR Placebo	2.14±1.99 –0.24±0.93	0.6745
Δ UA [mg/dl]	BBR Placebo	–0.08±0.87 0.18±0.68	0.2459
Δ ALT [U/l]	BBR Placebo	6.50±19.62 2.59±16.30	0.3321
Δ AST [U/l]	BBR Placebo	0.47±8.21 1.76±6.64	0.6005
Δ GGTP [U/l]	BBR Placebo	10.94±48.12 7.11±48.3	0.8503
Δ de Ritis ratio	BBR Placebo	–0.13±0.20 0.00 ±0.28	0.0898
Δ non-HDL [mg/dl]	BBR Placebo	3.03±23.58 1.60±21.60	0.4897
Δ FBG [mg/dL]	BBR Placebo	0.82±5.75 –3.87±12.14	0.1474
Δ FBI [uU/mL]	BBR Placebo	0.04±2.05 –1.53±4.31	0.1504

BMI, body mass index; WC, waist circumference; FM, fat mass; TC total cholesterol; HDL, high-density cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistant index; FLI, fatty liver index; FIB-4, fibrosis-4 index, VAI, visceral adiposity index; UA, uric acid; ALT alanine transaminase; AST, aspartate aminotransferase; GGTP, gamma-glutamyltransferase; FBG, fasting blood glucose; FBI, fasting blood insulin; p-value <0.05.

in addressing NAFLD, we initiated a double-blind, placebo-controlled clinical trial to investigate its effects on anthropometric, metabolic, and hepatic outcomes in patients diagnosed with MAFLD. Most of the studies present outcomes in NAFLD patients predating the change in nomenclature to MAFLD. Currently, there is a lack of effective pharmacological treatments for NAFLD and MAFLD. The available therapeutic choices are primarily confined to lifestyle modification and symptom management, along with addressing the comorbidities or complications associated with the condition (21). Reports indicate that BBR effectively hinders the accumulation of lipids and mitigates lipotoxicity by activating AMP-activated protein kinase (AMPK) and insulin signaling pathways. It also regulates inflammation, endoplasmic reticulum (ER) stress, oxidative stress, and mitochondrial function across various tissues and organs, as documented by Zhu *et al*. in 2016. This synergistic mechanism proves advantageous in addressing MAFLD and its associated complications (21). BBR demonstrates anti-inflammatory effects by reducing the expression of proinflammatory cytokines, including IL-1β, IL-6, IL-17, IL-18, and TNF-α (1, 7, 22). Abnormalities in various enzymes indicative of liver function, including ALT, AST, and GGT, are frequently observed in individuals with NAFLD or MAFLD. BBR has been demonstrated to lower the levels of these liver enzymes, thereby enhancing liver function in individuals with NAFLD (22). The results of pharmacokinetic studies indicate that, after oral administration, BBR metabolites are concentrated in the liver at levels 50–70 times higher than in the plasma. This specific localization in the liver is likely a contributing factor to the observed therapeutic effect of BBR on NAFLD (21). In our study in the BBR group post-intervention, we noted a significant reduction in trunk fat (p=0.0185), indicating the efficacy of BBR in decreasing fat mass and hepatic steatosis. While the difference in FIB-4 between the BBR and placebo groups was not statistically significant after 3 months of intervention (p=0.005), we noted a trend and observed that the mean of this parameter was lower in the BBR group (0.79±0.33) compared to the control group (0.96±0.34). In our research, noteworthy findings were the significant improvement in ALT levels (p=0.0105) and the de Ritis ratio (p=0.0011) after 12 weeks of BBR supplementation compared to the placebo group. Despite patients with normal ALT levels being eligible for the study, a de Ritis ratio below 1 suggests a proinflammatory etiology of MAFLD. BBR was shown to ameliorate NAFLD and related metabolic disorders by significantly reducing TG and TC levels and hepatic fat content (23). According to a meta-analysis, NAFLD patients who received BBR exhibited a more significant decrease in the level of TC compared to those undergoing other drug therapies or lifestyle interventions (10). In our study, significant differences in the change of ΔTC (p=0,0009) were noted between the BBR and placebo groups, even though individuals did not exhibit extreme dysfunction in their lipid profiles during randomization, which confirms the positive effect of BBR. Unfortunately, we did not observe an improvement in other lipid or liver parameters. Similar studies showed that over 12 weeks, obese individuals receiving BBR treatment (0.5 g three times a day) experienced a 12.2% reduction in total cholesterol (TC) and a 23% decrease in triglycerides (TG). Additionally, they exhibited mild weight loss and elevated calcitriol levels (7). BBR can cause 15% to 20% LDL-C reduction *via* an increase in LDL receptor activity (24). In our research, we did not notice any significant change in LD-C. BBR demonstrated effectiveness in reducing body weight, BMI, waist circumference, FBG, postprandial blood glucose (PBG), TC, TG, and HbA1c in obese patients with T2DM (7, 25). A phase 4 clinical trial for the treatment of NASH involving BBR has been initiated. Various mechanisms have been proposed to elucidate how BBR may alleviate NAFLD. One such mechanism involves BBR enhancing obesity management by activating thermogenesis in both brown adipose tissue (BAT) and white adipose tissue (WAT) (26, 27). In the dysfunction associated with obesity, WAT is unable to effectively store fat, leading to the release of excess free fatty acids (FFA) into the bloodstream. These accumulated FFAs deposit in non-adipose tissues such as the liver, causing detrimental effects on hepatocytes (28). BBR effectively lowers body weight and enhances insulin sensitivity in overweight individuals with NAFLD through the promotion and activation of BAT (29).

Table 5. The correlation of the change in selected variables in the berberine (BBR) and placebo groups after a 12-week intervention.

Correlation	BBR group		Placebo group	
	R	p	R	p
Δ BMI [kg/m^2] and **Δ ALT** [U/l]	0.47	**0.0089***	0.06	0.7643
Δ BMI [kg/m^2] and **Δ AST** [U/l]	0.47	**0.0081***	–0.12	0.5523
Δ Trunk fat [kg] and **Δ FLI**	0.55	**0.0337***	0.18	0.7903
Δ Trunk fat [kg] and **Δ HOMA-IR**	0.37	**0.0020***	–0.05	0.3984
Δ de Ritis ratio and **Δ FIB-4**	0.59	**0.0011***	0.61	**0.0003***
Δ HOMA-IR and **Δ FLI**	0.37	**0.0409***	0.05	0.8320
Δ VAI and **Δ FLI**	0.54	**0.0019***	0.53	**0.0068***
Δ ALT [U/l] and **Δ Trunk fat** [kg]	0.42	**0.0202***	0.004	0.9817
Δ AST [U/l] and **Δ Trunk fat** [kg]	0.23	0.2284	0.03	0.8601

BMI, body mass index; HOMA-IR, homeostasis model assessment for insulin resistant index; FLI, fatty liver index; FIB-4, fibrosis-4 index, VAI, visceral adiposity index; ALT alanine transaminase; AST, aspartate aminotransferase; *p-value <0.05.

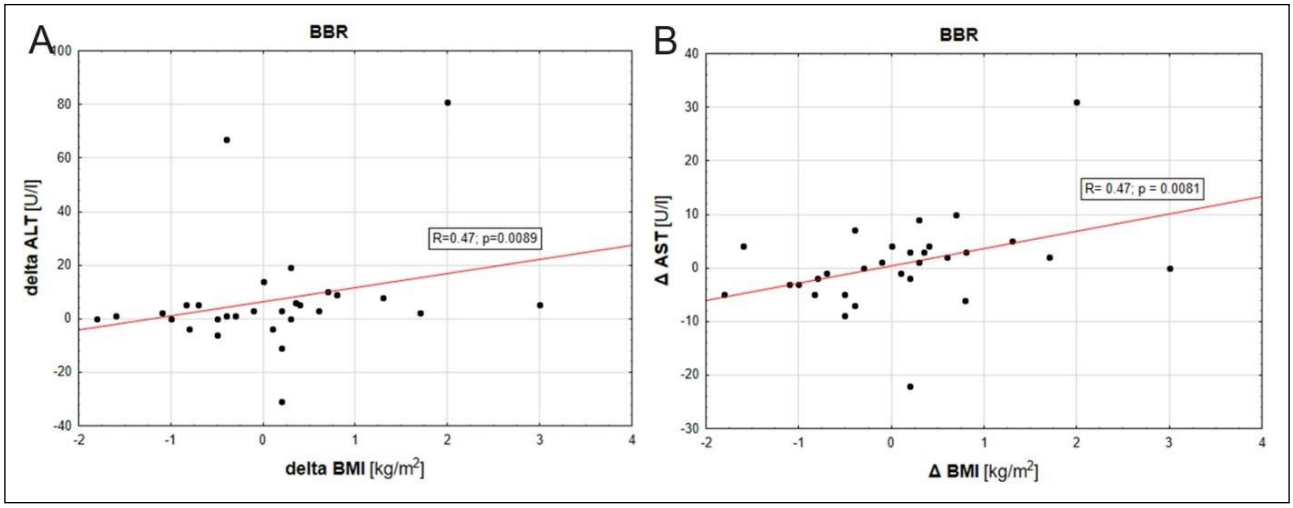

Fig. 4. The correlation between the change in ΔBMI and ΔALT, ΔBMI and ΔAST in the berberine (BBR) group.

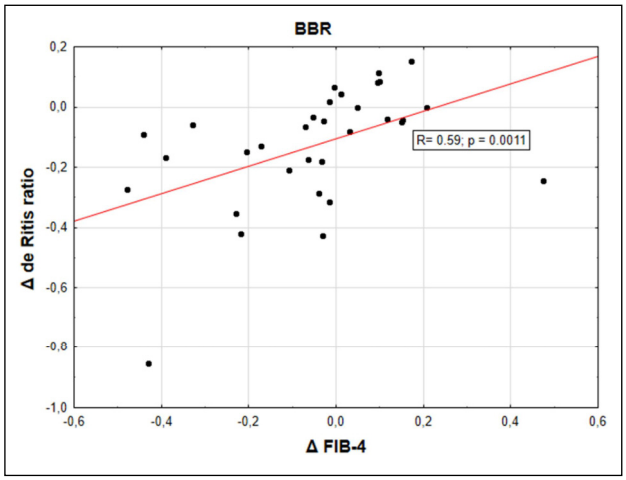

Fig. 5. The correlation between the change in ΔFIB-4 and Δde Ritis ratio in the berberine (BBR) group.

Scientific investigations endorse the application of BBR in addressing NAFLD. Following a 16-week BBR intervention, a notable amelioration was detected in the concentrations of TG, TC, PBG, HOMA-IR, hepatic fat content, apolipoprotein B (APOB), ALT, and AST in individuals diagnosed with NAFLD (23). We noted a significant correlation in the change of selected variables in BBR compared to the placebo group following the intervention. Specifically, changes in delta BMI correlated with ALT (r=0.47; p=0.0089) and AST levels (r=0.47; p=0.0081), while changes in trunk fat were correlated with FLI (r=0.55; p=0.0337) and HOMA-IR (r=0.37; p=0.0020). Additionally, the de Ritis ratio was correlated with FIB-4 levels (r=0.59; p=0.0011), while FLI was correlated with HOMA-IR (r=0.37; p=0.0409) and VAI (r=0.54; p=0.0019). These interdependencies suggest that the positive impact of BBR on selected parameters is associated with favorable changes in others. It demonstrated the interplay between anthropometric, hepatic, and metabolic factors and their mutual influence. Scientific data underscore the importance of exercise in the treatment of NAFLD and MetS. According to Reljic *et al*., a highly time-efficient high-intensity interval training (HIIT) protocol, requiring only 28 minutes per week, led to significant improvements in NAFLD fibrosis scores (NFS) and several cardiometabolic health indices in obese MetS patients with elevated NFS grades (30). Physical activity may enhance the benefits of BBR, indicating a need for further studies in this area. Another interesting observation was provided by Kovar J. *et al* (31). Their study found that NAFLD subjects accumulated almost five times more fat in their livers than healthy subjects with normal hepatic fat content (HFC) (31). Additionally, the consumption of a high-fat load led to fat accumulation in the liver of NAFLD patients. This accumulation was diminished by glucose co-administration but not by fructose

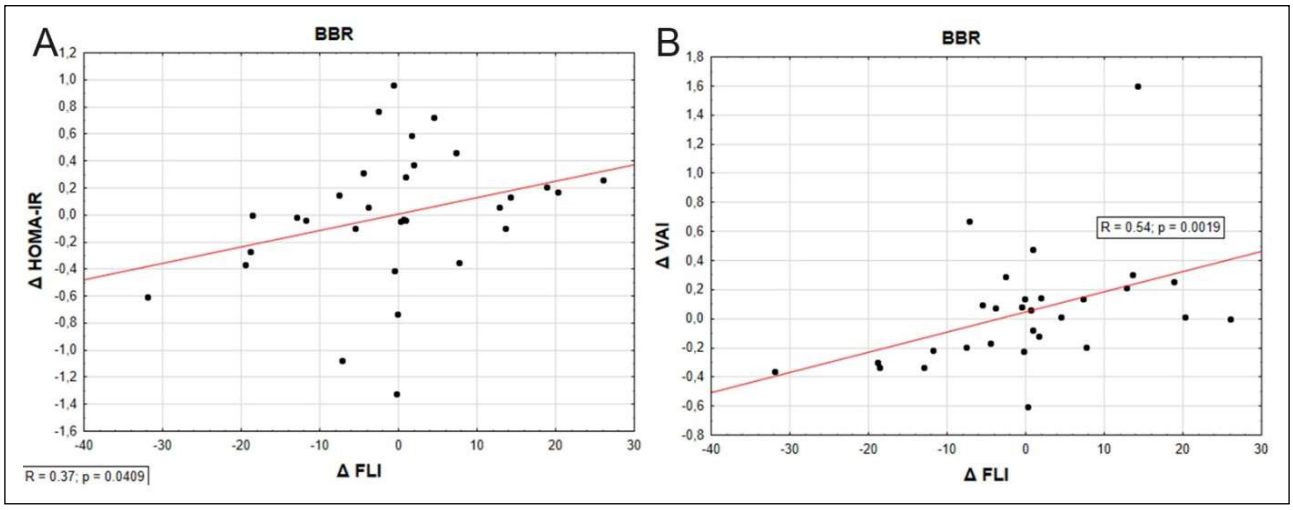

Fig. 6. The correlation between the change in ΔFLI and ΔHOMA-IR, ΔFLI and ΔVAI in the berberine (BBR) group.

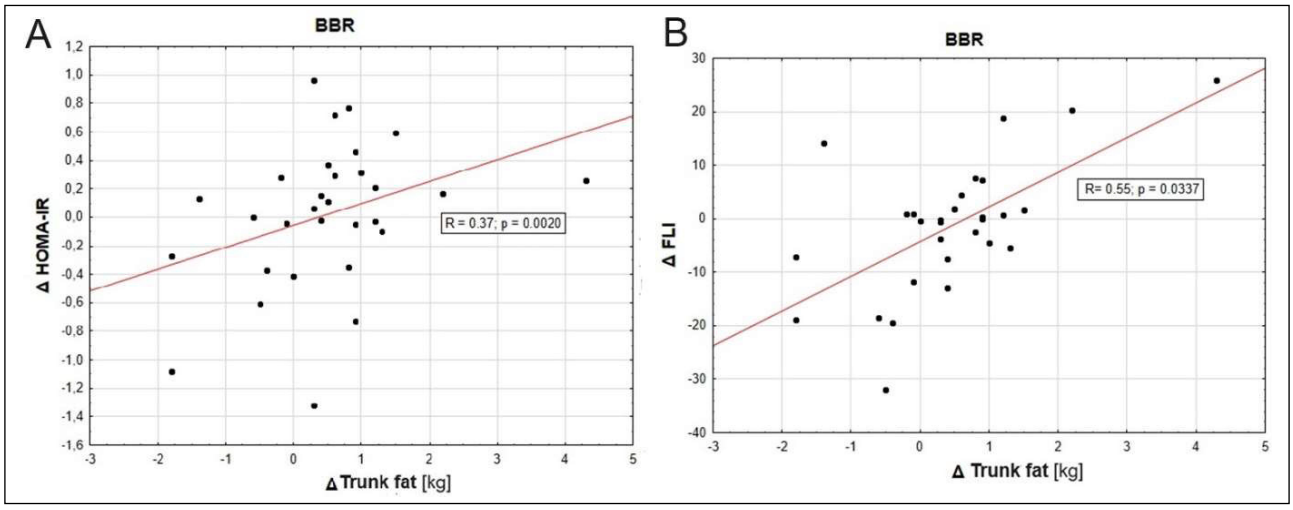

Fig. 7. The correlation between the change in ΔTrunk fat and ΔHOMA-IR, ΔTrunk fat and ΔFLI in the berberine (BBR) group.

co-administration (31). Interestingly, the NAFLD and IR are strongly associated with Peyronie's disease (PD) (32).

While dual-energy X-ray absorptiometry (DXA) is established as a gold standard for body composition analysis, we opted for bioelectrical impedance analysis (BIA) due to its practicality in clinical settings and its ability to offer valuable insights (33). Anthropometric parameters and body composition in our research were assessed using BIA. Natural medicines are increasingly acknowledged on a global scale as valuable reservoirs for crafting innovative drugs, owing to their notable therapeutic effectiveness and minimal adverse effects (7). In Nejati *et al*., in a randomized controlled trial concluded that BBR had no significant impact on lipid levels, FBG, liver enzymes, and body weight in patients with NAFLD (34). Although BBR is widely employed in the treatment of NAFLD, it does not surpass the efficacy of lifestyle modifications, such as adopting a low-fat, low-calorie diet, and engaging in regular physical activity (34). In a study involving 184 patients with NAFLD across three medical centers, the inclusion of BBR alongside lifestyle intervention led to a significant improvement in NAFLD compared to those undergoing lifestyle intervention alone (23). BBR significantly reduced hepatic lipid accumulation by modulating fatty acid synthesis and metabolism (35). Chen *et al*. illustrated that administering BBR supplements (1.5 g/d) over 12 weeks resulted in a notable reduction of BMI and waist circumference in individuals with obesity and prediabetes (22, 36). A meta-analysis encompassing six randomized clinical trials with 501 participants revealed that the administration of BBR (1000 mg/day) led to significant improvements in lipid parameters, IR, liver markers, and the extent of hepatic steatosis (10, 37). Moreover, combining BBR with nutraceuticals has demonstrated effective regulation of plasma lipids, and plasma glucose in a cohort of 1,161 patients diagnosed with MetS (38). In our study, while we didn't observe significant improvement in HOMA-IR within the BBR group, it's noteworthy that the control group experienced a significant worsening of this parameter after 12 weeks. Observed changes in variables between the BBR and placebo groups over the three months suggest a positive trend in HOMA-IR and the de Ritis ratio ΔHOMA-IR (p=0.0673), Δde Ritis ratio (p=0.0898). Expanding the group size could potentially offer more conclusive evidence to support these findings. The study protocol was designed to avoid factors that could affect outcomes. The limitations of the study are the poor bioavailability of the BBR. Enhancing the bioavailability of BBR remains an unresolved issue that requires future attention and solutions (7). Certain parameters showed no significant difference before and after the intervention, as the recruited

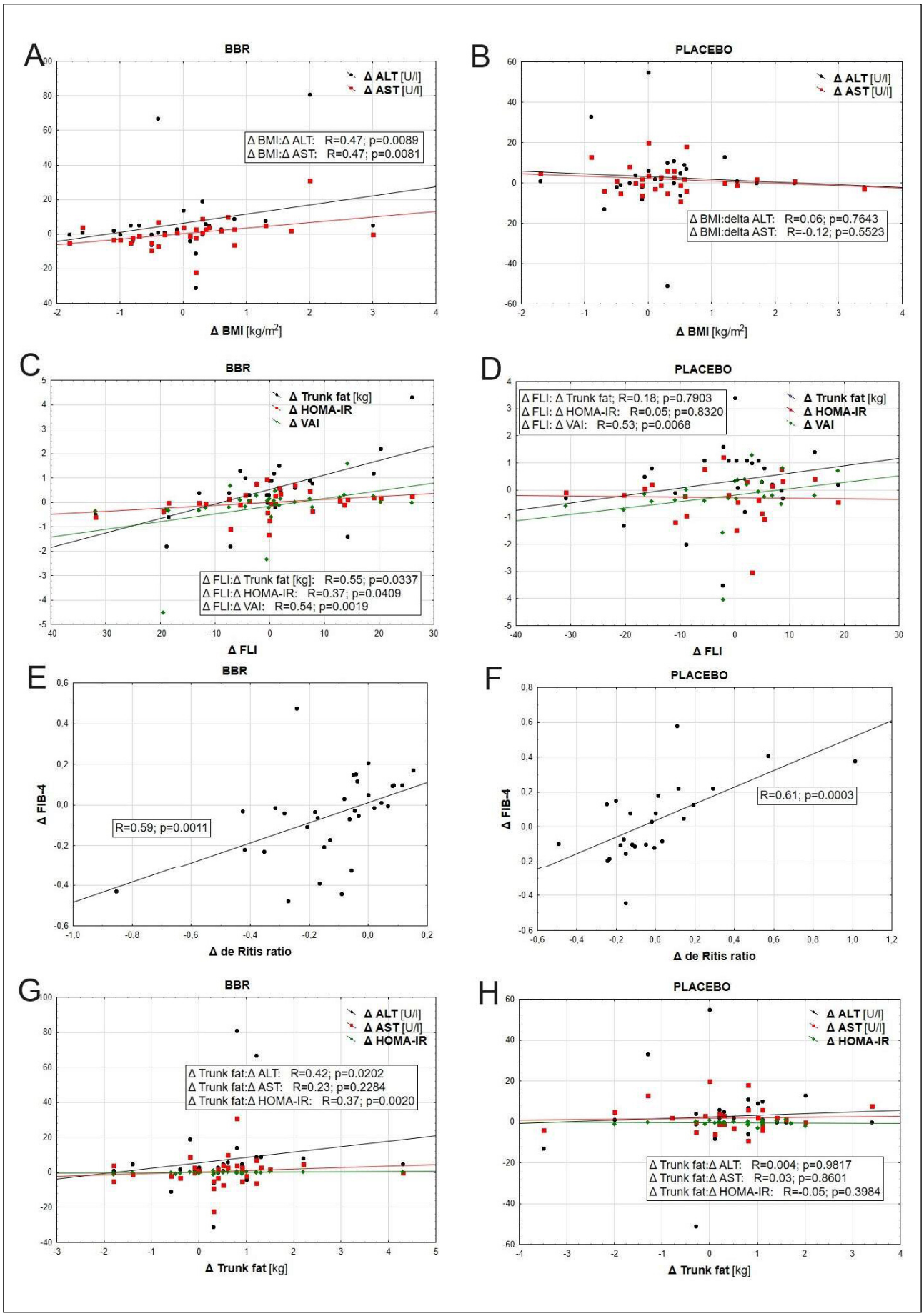

Fig. 8. The correlation of the change in selected variables in the berberine (BBR) and placebo groups after a 12-week intervention.

groups did not exhibit serious metabolic disorders such as diabetes, unstable dyslipidemia, and hypertension, or class II or III obesity with accompanying comorbidities. In this study Patient compliance was very good, but adherence to regimens and taking pills 3 times/per day might be challenging for many. We propose extending the intervention duration to thoroughly assess outcomes. It is possible that larger sample sizes or longer intervention periods could further elucidate the trends we observed and provide clearer and more significant outcomes. It would also be worthwhile to assess changes in hepatic steatosis using ultrasound scans after three months. Conducting studies with higher BBR dosages may be essential to elucidate the precise clinical effects of BBR in patients with MAFLD. The constraints include the incapacity to analyze the relationship between the dosage and its effects, and in the case of extended interventions, the correlation between time and effectiveness.

Achieving and maintaining long-term compliance with lifestyle changes is a persistent challenge. Consequently, a substantial unmet need exists for a new drug to effectively treat MAFLD. Our study findings provide valuable insights into the potential of BBR for managing MAFLD. These discoveries present a potential new option for preventing and treating metabolic diseases. BBR stands out as a promising natural solution for addressing obesity and MAFLD. Conducting extensive, prolonged, and multi-center clinical trials is imperative to assess the effectiveness of BBR in the treatment of MAFLD.

Authors' contribution: A.K., M.S., M.M., P.B. conceptualization; A.K., M.S planned and drafted the manuscript; A.K., M.S., P.B, M.M contributed to the full manuscript and provided critical reviewing and suggestions throughout the different versions of this manuscript; M.S., P.B. supervision; M.S writing - review and editing; A.K., M.M., M.S, P.B. project management, data curation; A.S.J. statistical analysis. All authors have read and agreed to the published version of the manuscript.

Funding: The research was financed from a large research grant from statutory funding for young researchers - doctoral students for 2022 (502-14-21191730-45058).

Acknowledgment: This project was supported by FORMEDS. FORMEDS was responsible for providing the drugs; did not participate in the design of the trial; collection, management, analysis, and interpretation of the data and writing of the report or the decision to submit the report for publication; and did not receive funding for this project.

All authors have read and agreed to the published version of the manuscript.

Conflict of interests: None declared.

REFERENCES

1. Orlandi P, Solini A, Banchi M, *et al*. Antiangiogenic drugs in NASH: evidence of a possible new therapeutic approach. *Pharmaceuticals* (*Basel*) 2021; 14: 995. doi: 10.3390/ph14100995
2. Eslam M, Newsome PN, Sarin SK, *et al*. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol* 2020; 73: 202-209.
3. Sakurai Y, Kubota N, Yamauchi T, Kadowaki T. Role of insulin resistance in MAFLD. *Int J Mol Sci* 2021; 22: 4156. doi: 10.3390/ijms22084156
4. Gofton C, Upendran Y, Zheng MH, George J. MAFLD: how is it different from NAFLD? *Clin Mol Hepatol* 2023; 29 (Suppl): 17-31.
5. Tilg H, Effenberger M. From NAFLD to MAFLD: when pathophysiology succeeds. *Nat Rev Gastroenterol Hepatol* 2020; 17: 387-388.
6. Pipitone RM, Ciccioli C, Infantino G, *et al*. MAFLD: a multisystem disease. *Ther Adv Endocrinol Metab.* 2023;14. 20420188221145549. doi: 10.1177/20420188221145549
7. Xu X, Yi H, Wu J, *et al*. Therapeutic effect of berberine on metabolic diseases: Both pharmacological data and clinical evidence. *Biomed Pharmacother.* 2021; 133: 110984. doi: 10.1016/j.biopha.2020.110984
8. Ilyas Z, Perna S, Al-Thawadi S, *et al*. The effect of berberine on weight loss in order to prevent obesity: a systematic review. *Biomed Pharmacother.* 2020; 127: 110137. doi: 10.1016/j.biopha.2020.110137
9. Wang L, Ye X, Hua Y, Song Y. Berberine alleviates adipose tissue fibrosis by inducing AMP-activated kinase signaling in high-fat diet-induced obese mice. *Biomed Pharmacother* 2018; 105: 121-129.
10. Wei X, Wang C, Hao S, Song H, Yang L. The therapeutic effect of berberine in the treatment of nonalcoholic fatty liver disease: a meta-analysis. *Evid Based Complement Alternat Med* 2016; 2016: 3593951. doi: 10.1155/2016/3593951
11. Sumida Y, Yoneda M, Tokushige K, *et al*. FIB-4 first in the diagnostic algorithm of metabolic-dysfunction-associated fatty liver disease in the era of the global metabodemic. *Life* (*Basel*) 2021; 11: 143. doi: 10.3390/life11020143
12. Han AL. Validation of fatty liver index as a marker for metabolic dysfunction-associated fatty liver disease. *Diabetol Metab Syndr* 2022; 14: 44. doi: 10.1186/s13098-022-00811-2
13. Yi X, Zhu S, Zhu L. Diagnostic accuracy of the visceral adiposity index in patients with metabolic-associated fatty liver disease: a meta-analysis. *Lipids Health Dis* 2022; 21: 28. doi: 10.1186/s12944-022-01636-8
14. Xue Y, Xu J, Li M, Gao Y. Potential screening indicators for early diagnosis of NAFLD/MAFLD and liver fibrosis: Triglyceride glucose index-related parameters. *Front Endocrinol* (*Lausanne*) 2022; 13: 951689. doi: 10.3389/fendo.2022.951689
15. Ndrepepa G. De Ritis ratio and cardiovascular disease: evidence and underlying mechanisms. *J Lab Precis Med* 2023; 8: 1-24. doi: 10.21037/jlpm-22-68
16. Wadolowska L. Validation of food frequency questionnaire-FFQ Reproducibility assessment. *Bromat Chem Toksykol* 2005; 38: 27-33.
17. Biernat E, Stupnicki R. International Physical Activity Questionnaire (IPAQ) - Polish version. *Physical Education and Sport* 2008; 52: 46-52.
18. Norton KI. Standards for anthropometry assessment. In: Kinanthropometry and Exercise Physiology 2018, pp. 68-137.
19. Kyle U. Bioelectrical impedance analysis part I: review of principles and methods. *Clin Nutr* 2004; 23: 1226-1243.
20. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome - a new world-wide definition. A consensus statement from the International Diabetes Federation. *Diabet Med* 2006; 23: 469-480.
21. Zhu X, Bian H, Gao X. The potential mechanisms of Berberine in the treatment of nonalcoholic fatty liver disease. *Molecules* 2016; 21: 1336. doi: 10.3390/molecules21101336
22. Luo Y, Zeng Y, Peng J, *et al*. Phytochemicals for the treatment of metabolic diseases: Evidence from clinical studies. *Biomed Pharmacother* 2023; 165: 115274. doi: 10.1016/j.biopha.2023.115274

23. Yan HM, Xia MF, Wang Y, *et al*. Efficacy of berberine in patients with non-alcoholic fatty liver disease. *PLoS One* 2015; 10: e0134172. doi: 10.1371/journal.pone.0134172
24. Banach M, Patti AM, Giglio RV, *et al*. The role of nutraceuticals in statin intolerant patients. *J Am Coll Cardiol* 2018; 72: 96-118.
25. Guo J, Chen H, Zhang X, *et al*. The effect of berberine on metabolic profiles in type 2 diabetic patients: a systematic review and meta-analysis of randomized controlled trials. *Oxid Med Cell Longev* 2021; 2021: 2074610. doi: 10.1155/2021/2074610
26. Zhang Z, Zhang H, Li B, *et al*. Berberine activates thermogenesis in white and brown adipose tissue. *Nat Commun* 2014; 5: 5493. doi: 10.1038/ncomms6493
27. Yan T, Yan N, Wang P, *et al*. Herbal drug discovery for the treatment of nonalcoholic fatty liver disease. *Acta Pharm Sin B* 2020; 10: 3-18.
28. Li QP, Dou YX, Huang ZW, *et al*. Therapeutic effect of oxyberberine on obese non-alcoholic fatty liver disease rats. *Phytomedicine* 2021; 85: 153550. doi: 10.1016/j.phymed.2021.153550
29. Wu L, Xia M, Duan Y, *et al*. Berberine promotes the recruitment and activation of brown adipose tissue in mice and humans. *Cell Death Dis* 2019; 10: 468. doi: 10.1038/s41419-019-1706-y
30. Reljic D, Konturek PC, Herrmann HJ, Siebler J, Neurath MF, Zopf Y. Very low-volume interval training improves nonalcoholic fatty liver disease fibrosis score and cardiometabolic health in adults with obesity and metabolic syndrome. *J Physiol Physiol* 2021; 726: 927-938.
31. Kovar J, Dusilova T, Sedivy P, *et al*. Acute responses of hepatic fat content to consuming fat, glucose and fructose alone and in combination in non-obese non-diabetic individuals with non-alcoholic fatty liver disease. *J Physiol Pharmacol* 2021; 721: 45-53.
32. Crocetto F, Barone B, Manfredi C, *et al*. Are insulin resistance and non-alcoholic fatty liver disease associated with Peyronie's disease? a pilot study. *J Physiol Pharmacol* 2022; 731: 53-62.
33. Achamrah N, Colange G, Delay J, *et al*. Comparison of body composition assessment by DXA and BIA according to the body mass index: a retrospective study on 3655 measures. *PLoS One* 2018;13: e0200465. doi: 10.1371/journal.pone.0200465
34. Nejati L, Movahedi A, Salari GR, Moeineddin R, Nejati P. The effect of berberine on lipid profile, liver enzymes, and fasting blood glucose in patients with non-alcoholic fatty liver disease (NAFLD): A randomized controlled trial. *Med J Islam Repub Iran* 2022; 36: 39. doi: 10.47176/mjiri.36.39
35. Wang Y, Tai YL, Zhao D, *et al*. Berberine prevents disease progression of nonalcoholic steatohepatitis through modulating multiple pathways. *Cells* 2021; 10: 210. doi: 10.3390/cells10020210
36. Chen YM, Sun GP, Sun YG. Effects of berberine hydrochloride on body weight of obese prediabetic patients and the outcome after treatment. *Chin J Prev Control Chronic Dis* 2017; 25: 54-56.
37. Wang L, Yan Y, Wu L, Peng J. Natural products in non-alcoholic fatty liver disease (NAFLD): Novel lead discovery for drug development. *Pharmacol Res* 2023; 196: 106925. doi: 10.1016/j.phrs.2023.106925
38. Poli A, Barbagallo CM, Cicero AFG, *et al*. Nutraceuticals and functional foods for the control of plasma cholesterol levels. an intersociety position paper. *Pharmacol Res* 2018; 134: 51-60.

Received: March 28, 2024
Accepted: June 24, 2024

Author for correspondence: Anna Koperska, Department of Treatment of Obesity, Metabolic Disorders and Clinical Dietetics, Poznan University of Medical Sciences, 82/84 Szamarzewskiego Street, 60-569 Poznan, Poland.
E-mail: anna.koperska@student.ump.edu.pl