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RESEARCH ARTICLE

The Effects of Methanol Extract of Rue Leaves (*Ruta graveolens*) on Body Weight, Lipid Profile, Adipokine Levels, TCPTP, and Perilipin-1 Expression in Obesity Model Rats

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Abstract

Obesity is one of the non-communicable diseases that becomes a health problem with high prevalence every year. Rue leaves (*Ruta graveolens*) containing a rutacridone active compound can computationally interact with T cell protein tyrosine phosphatase (TCPTP) and have anti-obesity activity properties. Therefore, this study aimed to investigate the Methanol Extract of Rue Leaves (MERL) on Body Weight (BW), Obesity Index (OI), Subcutaneous Fat Thickness (SFT), lipid profile, Fasting Blood Glucose (FBG), Leptin (LEP) and Adiponectin (ADP) levels, TCPTP and Perilipin-1 expression in obesity model rats (OR). This study was an animal experiment with a pre-posttest control group design, which used 48 male rats of the Sprague-Dawley strain and was randomly divided into 6 groups. The normal Group (N) was male rats + rat standard feed (BR-2). The negative and positive Groups (NC and PC) were obese rats (ORs) + BR-2 + 1 mL of 0.9% NaCl and 0.6 mg/kg BW pure rutacridone, respectively. The treatment groups (T1-T3) were ORs + BR-2 + 80, 100, or 200 mg/kg BW MERL for 30 days. Our results demonstrated that MERL administration significantly reduced BW, OI, SFT, TG, TC, LDL-C, FBG, and LEP levels and significantly increased HDL-C and ADP levels with $p < 0.05$, compared to the NC group in a dose-dependent manner. Our data also showed low expression of TCPTP and Perilipin-1 in all treatment groups. In conclusion, MERL administration reduces leptin, TG, TC, LDL-C, FBG levels, SFT, BW, and OI while increasing ADP and HDL-C levels in male obese rats.

Keywords: Rue leaves, Body weight, Adipokine, Lipid profile, Obesity

Introduction

Obesity is one of the significant public health problems worldwide due to its continuously increasing prevalence across all age groups. According to the World Obesity Atlas 2023 report, the prevalence of obesity (BMI ≥ 30 kg/m^2) is estimated to rise from 14 to 24%, or approximately 2 billion adults, children, and adolescents, by 2035. Furthermore, the double prevalence in adults, from 4 to 10% in men and 8 to 16% among women, will occur in this period. Indonesia was the country with the most rapid growth, at the rate of 5.8% for adults and 7.9% for children [1]. Obesity is now a major risk factor for metabolic and degenerative diseases such as cardiovascular diseases, diabetes, dyslipidemia, sleep apnea, and various cancers [2]. Diabetes has become one

of the leading causes of premature death and disability for people of all ages and sexes in the world [3]. By 2045, the International Diabetes Federation estimates that 260 million people in the Western Pacific region, including Indonesia, will be afflicted with diabetes. Available data indicate that in 2021, the prevalence of diabetes in adults in Indonesia was 10.8% [4].

Obesity results from a constant imbalance between food intake and energy expenditure, tightly regulated by the central nervous system and by peripheral hormones such as T cell protein tyrosine phosphatase (TCPTP), leptin, adiponectin, and insulin. Leptin is an appetite-regulating hormone released by adipocytes. In obese people, leptin secretion is overzealous, causing leptin resistance. Currently, leptin resistance has become a key factor in obesity pathophysiology, which reduces anorexic response to leptin stimulus, thus causing hyperphagia [2]. In this condition, uncontrolled appetite leads to overnutrition and fat accumulation in the adipose tissues and impairs insulin action to further uptake blood glucose. As a result, it continues insulin secretion and insulin resistance, characterized by raised blood insulin levels (hyperinsulinemia). In addition, overnutrition leads to fat deposits that can elevate blood triglycerides, total cholesterol, and LDL-C levels, paired with a simultaneous fall in HDL-C levels. Perilipin-1 proteins will increase to engage elevated triglycerides for lipolysis, releasing free fatty acids in the bloodstream. Elevated fat accumulation promotes subcutaneous fat development, increases body weight and obesity index, and induces inflammation, marked by higher ROS and MDA levels in the blood circulation. This inflammatory process is followed by reductions of anti-inflammatory cytokines, including adiponectin [5,6].

Obesity diagnosis in clinical and community settings traditionally relies on anthropometric measurements like body weight, body mass index, waist circumference, and waist circumference to height ratio. In animals, various measurements were used, such as Rohrer, Lee, and TM indexes, body fat content, and visceral fat measurement [7]. However, combining anthropometric measurements with radiological approaches like dual-energy X-ray absorptiometry (DXA) and computed tomography (CT) scans is crucial to assess fat accumulation effectively. The DXA allows for a precise evaluation of fat mass, fat-free mass, and bone density with minimal error rates, while CT scans aid in cardio-metabolic risk analysis by examining different fat types [8,9]. Despite their effectiveness, both DXA and CT involve ionizing radiation that can pose risks like gene mutations and cancer [10]. Ultrasound imaging is an alternative technique to observe tissue morphology and blood flow without radiation exposure [11].

There are several treatment modalities for managing obesity, but the current treatments are either ineffective or have several side effects [12]. Lifestyle changes and dietary modifications have weight loss effects, but many obese individuals do not adhere to dietary rules, leading to weight regain [13]. The use of medication for obesity is only prescribed for obese patients with moderate to high risks. Some of the anti-obesity drugs currently used include orlistat, lorcaserin, and naltrexone. However, the long-term use of those drugs remains unknown, and it has been reported to have several side effects, such as dizziness, nausea, hypertension, insomnia, and hypoglycaemia [14]. Therefore, it requires an alternative therapy to control body weight and modulate adipose tissues in obese people.

Medicinal therapy using plant-based drugs is a growing trend in current obesity management. *In silico* studies have shown that a rutacridone phytochemical in the Rue (*Ruta graveolens*) plant can interact with the TCPTP, thus potentially reducing leptin resistance and addressing obesity [15]. Rue leaves are one of the medicinal plants known in Indonesia, containing over 231 active compounds with various pharmacological effects [16]. For example, quercetin, an important flavonoid, is reported to decrease obesity by increasing adiponectin and decreasing leptin levels [17]. A previous research study states that the administration of 240 mg/kg/day quercetin for 11 weeks in obese rats can control obesity by reducing intracellular oxidative stress, and chronic low-grade inflammation, inhibiting adipogenesis and lipogenesis, and suppressing the differentiation of preadipocytes into mature adipocytes [18]. It seems that natural supplements obtained from traditional medicinal plants are a rational approach to treating obesity, and recent experiments have revealed many useful herbal products for treating obesity [19]. Therefore, the aim of this study was to investigate the methanol extract of Rue leaves (MERL) in addressing

obesity through the TCPTP inhibition mechanism in an obese animal model.

Materials and methods

Preparation of methanol extract of rue leaves (MERL)

Fresh rue leaves were obtained from the Center for Research and Development of Medicinal Plants and Traditional Medicine, Tawangmangu, Karanganyar, Central Java, Indonesia**.** The leaves were dried in an oven at 60 °C for 24 h and then ground using a blender to produce a powder**.** The extraction of Rue powder was soaked in 70**%** methanol at room temperature for 3 days**.** The filtrate of rue powder was then concentrated using a rotary evaporator at 60 °C, 80 rpm, and 175 mbar **[**18**].** MERL was stored at 4 °C before further analysis**.**

Phytochemical analysis using liquid chromatography high-resolution mass spectrometry (LC-HRMS)

The methanol extract of rue leaves was chemically analyzed using an LC-HRMS (Dionex™ Ultimate 3000 RSLC nano, Thermo Scientific™, USA) at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia. The MERL sample was dissolved in 1.000 µL of methanol and then vortexed for 3 min. The homogenized sample was filtered using a 0.2 µM Millex filter, and 5 µL MERL was injected into the analytical column: Phenyl Hexyl 100 mm × 2.1. The LC-HRMS consisted of mobile phase A (water + 0.1% Formic Acid) and B (Acetonitrile + 0.1% Formic acid) with a flow rate of 0.20 mL/min for 30 min. Identification of bioactive compounds was performed using Thermo Scientific™ Compound Discoverer Software.

Development of obese rat model

Male white rats (Rattus norvegicus), the Sprague Dawley strain, were adapted for 7 days in a cage with 12 h of light, 12 h of darkness at 25 °C, and controlled humidity. The rats were given Comfeed Br-2 feed and aquadest. After adaptation, all male rats were evaluated for their body weight, body length, and subcutaneous fat thickness. A total of 40 rats were developed to become obese through the administration of a high-fat high-fructose (HFHFr) diet for 30 days. The HFHFr consisted of beef fat, duck egg yolk, chicken liver, and butter, along with 10% fructose in their drinking water, which had nutrient values of 100 g and consisted of 610 kcal of total energy and 16.6% of total carbohydrates. 13.11% of total protein and 54.64% of total fat. The HFHFr was given 10% of the rats' body weight every day [20]. BW, BL, and SFT were regularly monitored every week. The male rats with obesity were established using Lee's obesity index ≥ 30 g/cm^3 [7]. The rat's SFT was measured using a portable ultrasonography with ≥ 1.54 mm obese value [39].

Study design

This study was animal experiment research with a pre-posttest control group design for body weight, obesity index, subcutaneous fat thickness, fasting blood glucose, lipid profile, leptin, adiponectin, malondialdehyde levels, and a posttest only group design for protein expression of TCPTP and Perilipin-1. Male white rats (*Rattus norvegicus*), Sprague Dawley strain, were used in this study, and they were aged 8 - 10 weeks and weighed approximately 300 g. The sample size was calculated using the formula: (n×t) – t, where n = the number of rats and p = the number of rat groups, establishing a minimum of 4 rats per treatment group. In anticipation, if there are rats that drop out, an estimate of up to 50% is added [21]. The normal group or non-obese rats (N) was given *BR-2 comfeed* and aquadest drinking water for 30 days. Meanwhile, obese rats were divided into 5 groups. The negative (NC) and positive control (PC) groups were given a standard diet and 1 mL 0.9% NaCl and 0.6 mg/kg BW/day rutacridone solution (Sigma, USA), respectively, for 30 days. Treatment groups (T1, T2 and T3) were given a standard diet and 80, 100 and 200 mg/kg BW/day MERL, respectively, for 30 days. The protocol of this research study was approved by the Health Research Ethics Committee, Dr. Moewardi, General Hospital, Surakarta, Indonesia, number 100/I/HREC/2023, on January 30, 2023.

Measurement of BW, BL, OI and SFT

BW and BL measurements were conducted weekly in the morning using a body weight scale (Sonic, Jakarta) and a stadiometer (Measure King, Jakarta). The OI was calculated using the Rohrer Index formula = {BW (g)/Naso − anal length (cm)^3} × 10^3, where rats were considered obese if they had an index ≥ 30 g/cm^3. Before measuring SFT, the rats' hair in the abdominal

area was shaved to approximately 5 cm in diameter. The SFT measurement was conducted weekly in the morning before giving the standard feed. The shaved abdomen was evenly covered with ultrasound gel (Onemed, Sidoarjo). Rats were considered obese if they had an SFT ≥ 1.54 mm.

Measurement of FBG, lipid profiles, LEP and ADP levels

Blood samples were collected thrice on days 0, 15 and 30 in the morning before intervention**.** All collected blood samples were centrifuged at 4**.**000 rpm for 10 min to obtain serum for measuring fasting blood glucose **(**FBG**)** levels, lipid profiles**:** Triglyceride **(**TG**)**, total cholesterol **(**TC**)**, Low**-**Density Lipoprotein Cholesterol **(**LDL**-**C**)**, High Density Lipoprotein Cholesterol **(**HDL**-**C**)** levels, leptin **(**LEP**)**, and adiponectin **(**ADP**)** levels**.** FBG levels were determined using the GOD**-**PAP method, TG levels using the GPO**-**PAP method, and TC, LDL**-**C, and HDL**-**C levels using the CHOD**-**PAP method**.** The measurements of LEP and ADP levels used ELISA assays provided by Elabscience®, China (E-EL-R0582 and E-OSEL-R0006, respectively). The ELISA protocols for measuring LEP and ADP levels were based on the manufacturer.

Western blotting analysis of TCPTP and perilipin-1

The examination of TCPTP and Perilipin-1 protein expression used rats' brains and adipose tissues, which were collected on day 30 of intervention and lysed using RIPA buffer (Thermo Scientific, USA) [22]. Before SDS PAGE Electrophoresis, total protein concentrations of the cell lysate of all rats were measured using a BCA Protein Assay Kit (Thermo Scientific, USA catalog number: 23227). The following antibodies were used: Primary Antibody (Perilipin1 Recombinant Rabbit Monoclonal Antibody (Invitrogen, ARC1122; 1: 3.000), beta Actin Antibody (Abcam a68227; 1: 10.0000), and PTPN2 antibody (Sigma-Aldrich SAB4200249; 1: 3.000)) and Secondary antibody (Goat Anti-Rabbit IgG H&L (HRP), Abcam ab6721; 1:3.000). The SDS-PAGE process began with the preparation of reagents and samples, electrophoresis using the Mini Protean Tetra System-Biorad, loading the samples and protein marker (Precision Plus Protein Dual Color Standards, Biorad cat #1610734) into the wells, and running the gel at 150 V for 45 - 60 min. The proteins were then transferred to wells, 50 µg for the brain sample and 200 µg for the lipids sample, each well (Mini Transblot-cell), followed by incubation with primary and secondary antibodies. Visualization was conducted by transferring the PVDF membrane, adding ECL, incubating for 5 min, and then drying the membrane before placing it on a sample tray and visualizing using the ChemiDoc Touch Imaging System (Biorad).

Statistical analysis

The obtained data were then analyzed using IBM SPSS (Statistical Package for the Social Sciences) version 25 and presented as mean ± standard deviation. Parametric test of BW, OI, SFT, TG, TC, LDL-C, HDL-C, FBG, LEP, ADP levels, expression of TCPTP and PLIN-1 using 1-way ANOVA followed by the LSD post hoc test. To compare means within the same group on different days, we used the dependent T-test, and the data were considered significantly different if the p-value was < 0.05.

Results and discussion

The results of the data analysis indicated that the administration of 80, 100 and 200 mg/kg BW MERL declines SFT, BW, OI, TG, TC, LDL-C, FBG, and leptin levels, and low relative expression of TCPTP and Perilipin-1 in obese male rats in a dose-dependent manner, except for Leptin, Perilipin-1, TG, TC, and LDL-C. Therefore, our findings suggest that MERL will become a potential anti-obesity drug in the future.

Phytochemical screening of methanol extract of rue leaf

Based on the results of the phytochemical screening of the MERL, we selected the top 20 compounds based on their peak areas. Of the top 20 compounds, several appeared more than once. The data of the phytochemical screening results of the MERL are presented in **Table 1**.

From **Table 1**, we found 13 compounds that have various health-related activities. Based on their activities, these compounds are divided into antidiabetics such as 4,7,8-trimethoxyfuro[2,3-b] quinoline, Rutamarin, Osthol, Psoralen, Isoimperatorin, 4-Hydroxycoumarin, Scoparone, L-Tyrosine, Bis(2-ethylhexyl) phthalate, and Quercetin. Antioxidants such

as Osthol, Rutin, 4-Hydroxycoumarin, L-Tyrosine, and Quercetin. Antihyperlipidemic such as Quercetin and anti-obesity such as Osthol, Psoralen, Rutin, Scoparone, and Quercetin.

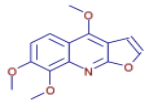
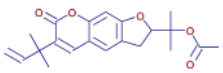
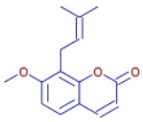
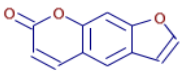
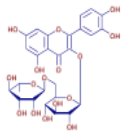
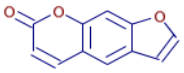
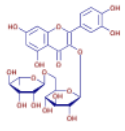
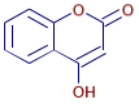
The results of the MERL phytochemical test revealed 121 compounds contained in the extract. We targeted one compound, rutacridone, but the results of the phytochemical test did not show the presence of this compound, because rutacridone is only slightly soluble in water. In our study, the solvent used when making rue leaf extract was methanol, so it is suspected that rutacridone cannot dissolve in this solvent. Based on previous research, it was stated that rutacridone dissolves very well in ethyl lactate solvent. Meanwhile, rutacridone dissolved in ethanol cannot be dissolved like ethyl lactate [23]. Although we did not find the rutacridone compound, we found several compounds with the largest area that had good activity in overcoming obesity. There are 13 compounds (**Table 1**) with various activities, such as 4,7,8-trimethoxyfuro[2,3-b] quinoline, rutamarin, osthol, psoralen, isoimperatorin, 4-Hydroxycoumarin, scoparone, L-tryosine, Bis(2-ethylhexyl) phthalate, asparagin, quercetin which have antidiabetic activity. In addition, there are compounds that have antiobesity activity, such as scoparone, quercetin, rutin, psoralen, and osthol, so these 5 compounds are suspected to play a role in overcoming obesity in our study. **Figures 1(A) - 1(E)** is the LC-HRMS result of MERL compounds with anti-obesity activity. Osthol compound (**Figure 1(A)**) is a compound with anti-obesity activity that has the largest area compared to other compounds, which is 7.4×10^9 (**Table 1**). However, the compound with the highest intensity is Psoralen compound at RT 12.012 (**Figure 1(B)**).

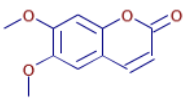
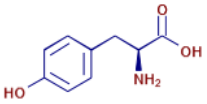
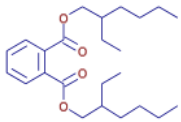
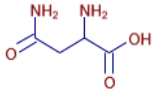
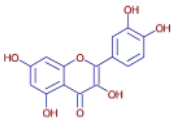
Effect of MERL on body weight, obesity index, and subcutaneous fat thickness

Obesity is characterized by an increase in body weight (BW) due to food consumption exceeding the body's required nutrient intake. Excess energy is then converted into fat, leading to the accumulation of fat cells under the skin. **Figure 2(A)** shows that after 30 days of intervention, the PC, T1, T2, and T3 groups showed a significant decrease in BW (p = 0.001, 0.036, 0.010 and 0.023). The largest decrease in BW was observed in the T3 group (42.75 ± −5.06 g), while the smallest decrease in BW was observed in the NC group (12.5 ± −2.44 g). The mean BW levels among rat groups after 30 days of intervention were significantly different (p = 0.001). The highest mean BW levels were found in the NC group (378.75 ± 8.54 g), while the lowest mean BW levels in obese rats were found in the T3 group (348.88 ± 18.09 g). In our study, we observed a significant reduction in BW in the PC, T1, T2 and T3 groups, although the T3 group (treated with 200 mg/kg BW of MERL) showed the greatest average BW reduction among the obese rat groups (42.75 ± 5.06 g). However, the 200 mg/kg BW dose of MERL was unable to restore the BW of obese rats to normal levels within the 30-day intervention period. The decreased BW in our study was in accordance with a previous study that used methanol extract of *R. graveolens* and a high-fat diet, although the largest decrease in our study (42.75 ± 5.06 g) was lower than the previous study (558 g). This difference is related to the use of the animal model, which we used male Sprague Dawley rats, while the previous study used rabbits [24].

There was a significant decrease in OI in the PC, T1, T2, and T3 groups (p = 0.012). The largest and the smallest decrease in OI was observed in the T3 and NC groups (−9.07 ± 0.8 and −3.43 ± −0.3 g/cm^3). The highest and the lowest mean OI levels were found in the NC and T3 groups (30.64 ± 0.53 and 24.16 ± 1.52 g/cm^3) (**Figure 2(B)**). The OI is one of the methods used to detect obesity. A significant reduction was recorded for the obesity index in the PC, T1, T2, and T3 groups after the intervention period of our study, thus proving that MERL can reduce OI by lowering their body weight and length ratio. A previous study reported a significant reduction of OI in obese rats receiving 1 g/kg/day of *Safoof Mohazzil*, a mixture *of Ptychotis ajowan*, *Origanum majorana*, *Foeniculum vulgare*, *Carum carvi*, *Coccus lacca*, and *Ruta graveolens*, for 2 weeks, compared to the obese control group. However, our results showed a greater reduction in OI (−9.07 ± 0.8 g/cm^3). The previous research study used Mohazil, which consists of a mixture of several plant extracts, and one of them is *Ruta graveolens*. In addition, the animal models were Female Sprague Dawley rats with 100 - 150 g BW, while our study used Male Sprague Dawley rats with ± 300 g BW [25].

Table 1 Results of LC-HRMS testing of methanol extract of rue leaf.

No.	Name	RT (min)	Area (max)	Formula	Molecular Weight (g/mol)	Structure
1	4,7,8-trimethoxyfuro[2,3-b] quinoline	11.85	2.2×10^{10}	$C_{14}H_{13}NO_4$	259.0839	
2	4,7,8-trimethoxyfuro[2,3-b] quinoline	11.96	1.7×10^{10}			
3	Rutamarin	18.16	1.3×10^{10}	$C_{21}H_{24}O_5$	356.1616	
4	Osthol	16.33	7.4×10^{9}	$C_{15}H_{16}O_3$	244.10972	
5	D-(+)-Proline	1.03	4.3×10^{9}	$C_5H_9NO_2$	115.0634	
6	Psoralen	12.35	4.2×10^{9}	$C_{11}H_6O_3$	186.0314	
7	Psoralen	12.01	3.1×10^{9}			
8	Psoralen	11.90	1.6×10^{9}			
9	Psoralen	12.73	1.5×10^{9}			
11	Rutin	7.75	8.1×10^{8}	$C_{27}H_{30}O_{16}$	610.152	
12	Rutin	8.28	5.7×10^{8}			
13	Psoralen	11.68	5.3×10^{8}	$C_{11}H_6O_3$	186.0315	
14	Rutin	7.26	3.5×10^{8}	$C_{27}H_{30}O_{16}$	610.152	
15	4-Hydroxycoumarin	8.67	2.9×10^{8}	$C_9H_6O_3$	162.031	

No.	Name	RT (min)	Area (max)	Formula	Molecular Weight (g/mol)	Structure
16	Scoparone	12.74	2.6×10^8	$C_{11}H_{10}O_4$	206.0577	
17	L-Tyrosine	1.06	2.6×10^8	$C_9H_{11}NO_3$	181.0737	
18	Bis(2-ethylhexyl) phthalate	23.48	2.6×10^8	$C_{24}H_{38}O_4$	390.2765	
19	Asparagine	1.15	2.4×10^8	$C_4H_8N_2O_3$	132.0534	
20	Quercetin	10.86	2.4×10^8	$C_{15}H_{10}O_7$	302.0424	

Figures 3(A) - 3(F) shows the SFT measurements representing each treatment group after treatment. It can be seen that the SFT with the highest thickness is in NC (**Figure 3(B)**), and the thinnest SFT is in PC (**Figure 3(C)**). A significant decrease in SFT was observed in the NC, PC, T1, T2, and T3 groups (p = 0.012, 0.011, 0.012, 0.011, and 0. 012, respectively). The largest decrease in SFT was observed in the PC group (0. 71 ± −0.01 mm). Meanwhile, the smallest decrease in SFT in obese rats was observed in the NC group (0. 33 ± 0. 01 mm). The highest mean SFT levels were found in the NC group (1.36 ± 0.10 mm), while the lowest mean SFT levels were found in the PC group (0. 97 ± 0. 05 mm) (**Figure 3**). Continuously increasing BW leads to the distribution of excess lipids to various parts of the body. Subcutaneous adipose tissue stores the majority of these lipids. Based on the research findings, administration of 80, 100, and 200 mg/kg BW MERL significantly reduced SFT (1.15 ± 0.06, 1.06 ± 0.08, and 1. 04 ± 0. 02 mm, respectively), although it did not match the reduction observed in the positive control group (0.97 ± 0. 05 mm). This indicates that the pure compound rutacridone is more effective in reducing subcutaneous fat thickness. Until now, there is no similar study that uses ultrasound to measure SFT in obese rats, so this parameter is an update on our study.

The mean TG, TC, LDL-C, HDL-C, and FBG levels among obese rat groups on day 0 showed no significant differences (p = 0.908, 0.906, 0.102, 0.988, and 0.906). **Figure 4(A)** shows that after 30 days of intervention, there was a decrease in mean TG levels in all rat groups. A significant decrease in TG was observed in the PC, T1, T2, and T3 groups ($p = 0.012$). The smallest and the highest decrease in TG levels in obese rats was observed in the NC and T2 groups (−13.69 ± 0.18 and −59.86 ± 9.38 mg/dL). The mean TG levels among rat groups after 30 days of intervention were significantly different ($p = 0.001$). The highest

mean TG levels in obese rats on day 30 were found in the NC group (104.02 ± 25.95 mg/dL), while the lowest mean TG levels were found in the T3 group (61.63 ± 14.34 mg/dL).

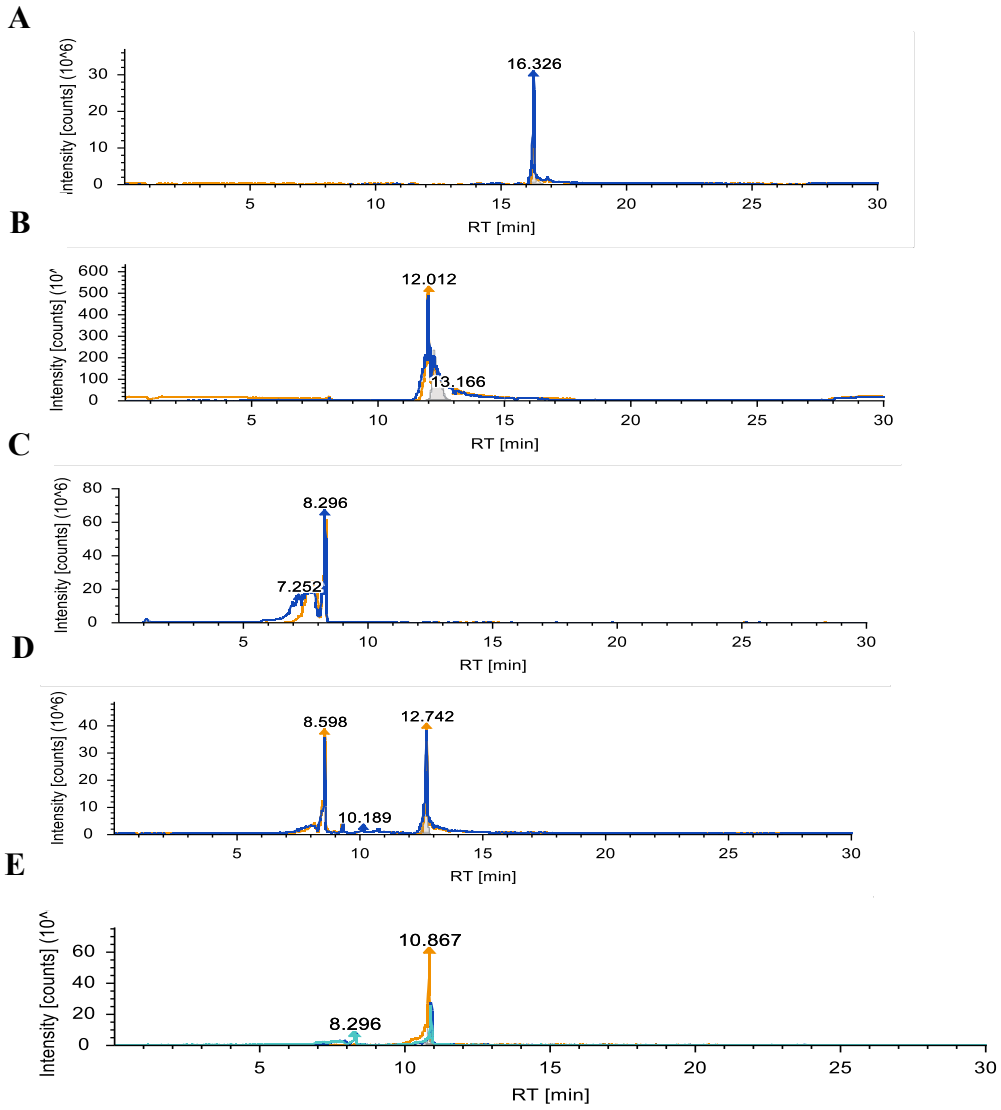


Figure 1 Results of LC-HRMS testing of compounds (a) osthol, (b) psoralen, (c) rutin, (d) scoparone, and (e) quercetin in the MERL.

The triglycerides are a group of lipids that are absorbed in the intestinal tract. Blood vessels, muscle tissues, and adipose tissues then utilize these lipids. Following digestion by the lipases, triglycerides are conveyed to the liver, which then catalyzes conversion into LDL cholesterol. The level of triglycerides increases because of intake that exceeds what the body can utilize, which causes an increase in the level of carbohydrates in the body. High levels of triglycerides and cholesterol cause the deposition of these compounds on blood vessel walls, forming plaques. The study's results showed that leaf extract of ginkgo, at doses of 80, 100, and 200 mg/kg BW, significantly reduced the triglyceride levels, with the best dose being T2 (100 mg/kg BW). A previous research study reported that the administration of 0.25 mg/kg *R. graveolens* and flavonoids extract was able to reduce TG levels among all groups in rats with a diabetic model. The results of TG levels in that study were even lower (42.3 ± 1.9 mg/dL) than the TG levels in our study (61.63 ± 14.34 mg/dL). This different result may be related to the use of an animal model, which used male Wistar rats with 200 ± 20 g BW, while our study used male Sprague Dawley with 300 ± g BW [26]. A previous study

reported that administration of 50 mg/kg rutin and induction of hyperlipidemia resulted in lower TG levels compared to rats with a hyperlipidemia model by reducing the HMG-CoA reductase activity as well as increasing fecal sterols. The results of TG levels in that study were higher (3,495.5 ± 874.7 mg/dL) than TG levels in our study (61.63 ± 14.34 mg/dL). This difference may suggest that the previous study used pure quercetin, while the MERL in our study contained several bioactive compounds [27].

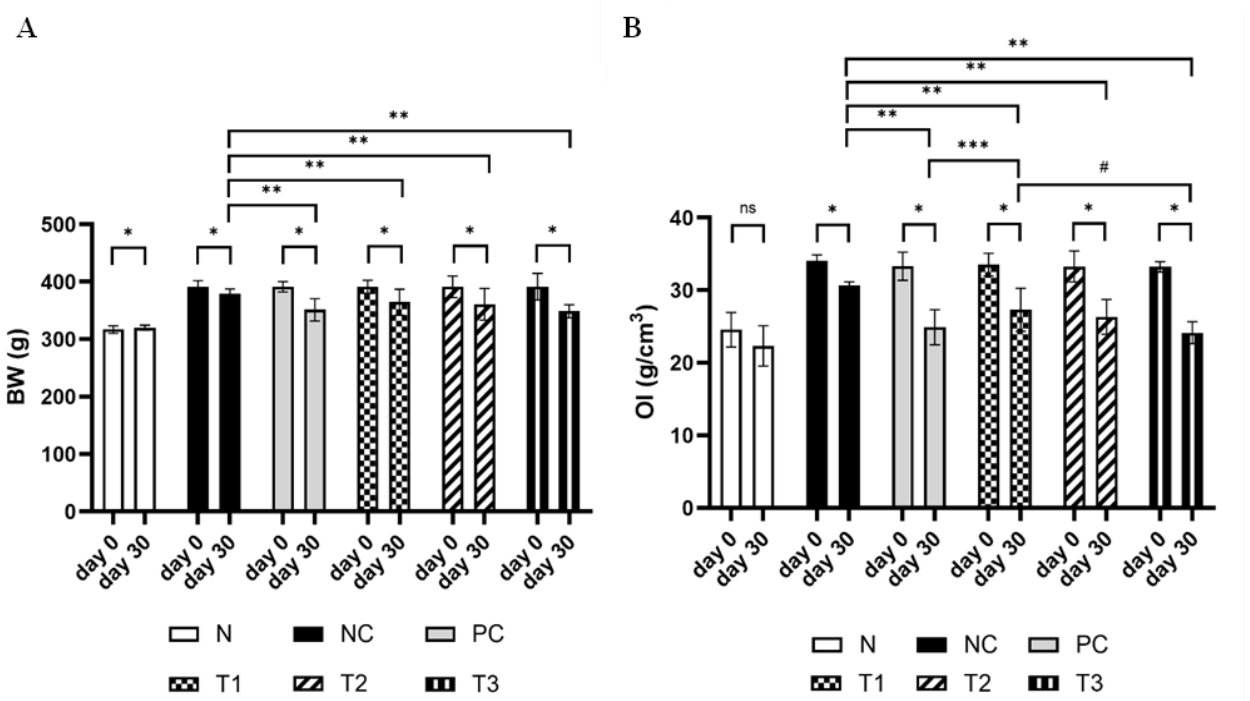


Figure 2 Mean (A) BW and (B) OI in male obese rat models on day 0 and day 30, presented as mean ± SD. [ns]) Independent t-test not significant ($p > 0.05$) *) Independent t-test significant ($p < 0.05$) **) Post hoc test significant compared to the negative control (NC). ***) The post hoc test was significant compared to the positive control (PC). [#]) Post hoc test was significant compared to treatment 1 (T1). [##]) The post hoc test was significant compared to treatment 2 (T2). Statistical analysis was performed using Repeated-Measures ANOVA (for normally distributed data) and the Friedman test (for non-normally distributed data). N = Normal group (non-obese rats), NC = Negative control group (OR + standard diet + 0.9% NaCl), PC = Positive control group (OR + BR-2 + 0.6 mg/kg BW/day pure rutacridone), T1, T2 and T3 were OR + BR-2 + 80, 100 and 200 mg/kg BW/day MERL).

A significant decrease in TC (**Figure 4(B)**) was observed only in the PC and T3 groups ($p = 0.012$). The smallest and the highest decrease in TC was observed in the T1 and T2 groups (19.64 ± −3.9 and 23.86 ± −2.43 mg/dL). Although T2 had the highest decrease in TC, this decrease was not significant ($p = 0.05$). The highest mean TC levels in obese rats on day 30 were found in the NC group (93.59 ± 14.47 mg/dL), while the lowest mean TC levels were found in the T2 group (68.84 ± 16.07 mg/dL). Cholesterol is a highly influential factor that is an amphipathic compound from fat. In our study, 200 mg/kg BW MERL significantly reduced total cholesterol level by 23.15 ± 11.29 mg/dL in the study on obese rats. In a previous study, it was reported that the decrease in TC levels was 72.1% in the 40 mg/kg of *Ruta chalepensis* ethanolic extract group compared to the high cholesterol diet control. The results of TC levels in the study were still higher than the TC levels in our study (68.84 ± 16.07 mg/dL). This different result is that the previous study made a hyperlipidemia rat model by administering Triton WR-1339 intraperitoneally, while the rats in our study were made obese models by administering HFHFr [28].

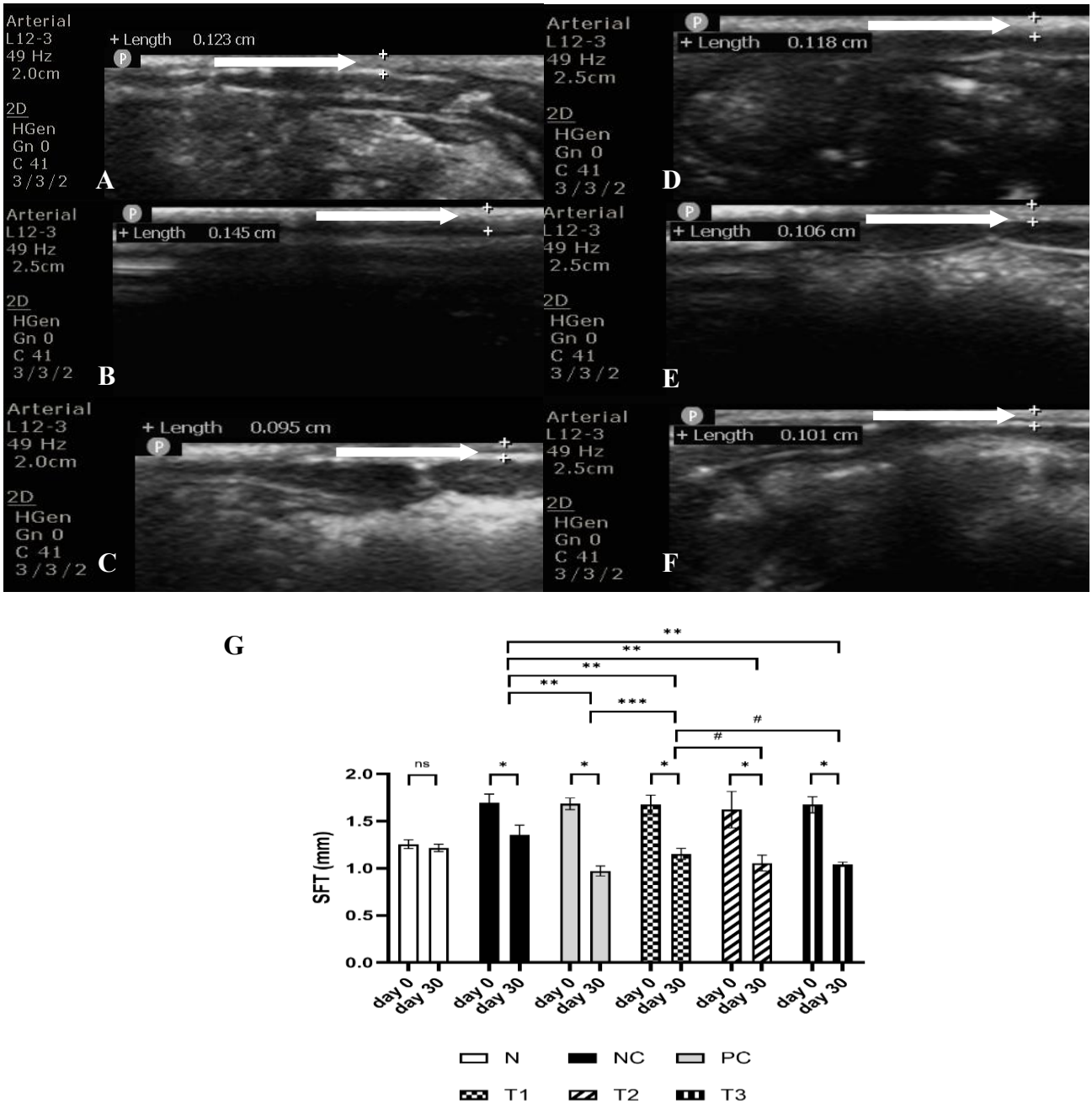


Figure 3 Results of 30-day SFT intervention period using USG (A) N (B) NC (C) PC (D) T1 (E) T2 and (F) T3, each image shown is a SFT representing each group, (G) mean SFT in male obese rat models on day 0 and day 30, presented as mean ± SD. [ns]) Independent t-test not significant ($p > 0.05$) *) Independent t-test significant ($p < 0.05$) **) Post hoc test significant compared to the negative control (NC). ***) The post hoc test was significant compared to the positive control (PC). [#]) The post hoc test was significant compared to the T1 group. [##]) The post hoc test was significant compared to the T2 group. Statistical analysis was performed using Repeated-Measures ANOVA. N = Normal group (non-obese rats), NC = Negative control group (OR + standard diet + 0.9% NaCl), PC = Positive control group (OR + BR-2 + 0.6 mg/kg BW/day pure rutacridone), T1, T2 and T3 were OR + BR-2 + 80, 100 and 200 mg/kg BW/day MERL).

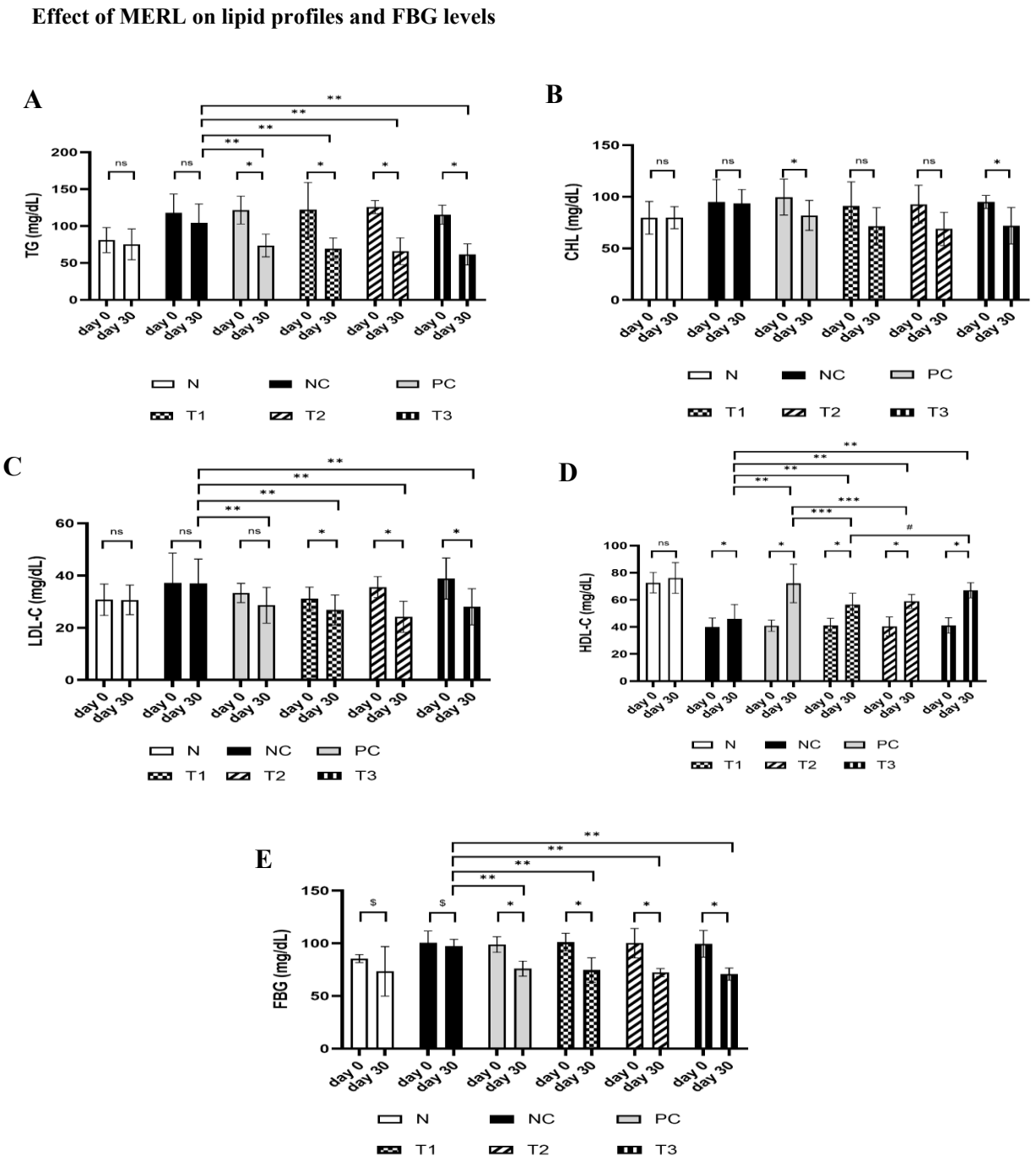


Figure 4 Mean levels of (A) TG, (B) TC, (C) LDL-C, (D) HDL-C, and (D) FBG in male obese rat models on day 0 and day 30, presented as mean ± SD. [ns]) Independent t-test not significant ($p > 0.05$) *) Independent t-test significant ($p < 0.05$) **) Post hoc test significant compared to the negative control (NC). ***) The post hoc test was significant compared to the positive control (PC). [#]) Post hoc test was significant compared to treatment 1 (T1). [##]) Post hoc test was significant compared to treatment 2 (T2). Statistical analysis was performed using Repeated-Measures ANOVA. N = Normal group (non-obese rats), NC = Negative control group (OR + standard diet + 0.9% NaCl), PC = Positive control group (OR + BR-2 + 0.6 mg/kg BW/day pure rutacridone), T1, T2 and T3 were OR + BR-2 + 80, 100 and 200 mg/kg BW/day MERL).

The smallest increase in HDL-C in obese rats was observed in the NC group (6.11 ± 3.82 mg/dL). The highest increase in HDL-C was observed in the PC group (31.15 ± 10.1 mg/dL). The mean HDL-C levels among rat groups after 30 days of intervention were significantly different ($p < 0.001$). The highest mean HDL-C levels in the methanol extract of Rue leaf groups were found in the T3 group (56.54 ± 8.30 mg/dL), while the lowest mean HDL-C levels were found in the T1 group (25.95 ± −0.21 mg/dL) (Figure 4C). In humans, high-density lipoprotein (HDL-C) removes excess harmful cholesterol from the blood, directing it back to the liver for removal by the body. Unlike total cholesterol levels that rise in obesity, HDL-C levels decrease in the case of obese individuals. This is consistent with our research results, where administration of 80, 100 and 200 mg/kg BW MERL significantly increased HDL-C levels (15.38 ± 3.09, 18.46 ± 1.82 and 25.95 ± 0.21 mg/dL, respectively), although not as much as the increase observed in the PC group (31.15 ± 10.1 mg/dL). A previous study reported that administration of 50 mg/kg BW quercetin increased HDL-C levels in obese rats compared to the control groups. Increased HDL-C levels in our study (56.54 ± 8.30 mg/dL) were higher than HDL-C levels in the previous study (48.10 ± 13.8 mg/dL). Surprisingly, the previous study used pure quercetin while our study used MERL, containing various bioactive compounds [29].

After 30 days of intervention, there was a decrease in mean FBG levels in all rat groups. The smallest decrease in FBG was observed in the NC group (−3.12 ± −4.68 mg/dL). The highest decrease in FBG was observed in the T3 group (−28.88 ± −7.08 mg/dL). The mean FBG levels among rat groups after 30 days of intervention were significantly different ($p < 0.001$). The highest mean FBG levels were found in the NC group (97.24 ± 6.60 mg/dL), while the lowest mean FBG levels were found in the T2 group (70.71 ± 5.58 mg/dL) (**Figure 4(D)**). A previous study reported that administration of 200 mg/kg BW chloroform extract of *R. graveolens* reduced FBG levels, compared to other groups. Reduced FBG levels in our study (70.71 ± 5.58 mg/dL) were lower than the previous study (100 ± 4.54 mg/dL). This result discrepancy may be due to differences in using the extract solvent and the rat strain. The previous study used chloroform solvent and Wistar rats, while our study used methanol solvent and Sprague Dawley rats [30]. Research findings suggest that *Ruta graveolens* and rutin possess qualities that can lower blood glucose levels and reduce blood lipid levels. This can occur by stimulating insulin secretion, reducing glucose and cholesterol absorption in the intestines, enhancing insulin efficacy in the body, influencing factors contributing to insulin resistance, increasing glucose uptake in peripheral tissues, and reducing glucose production by the liver. Flavonoids also could regulate carbohydrate digestion, insulin production, insulin signaling, glucose absorption, and adipose deposition, contributing to their antidiabetic effects. Quercetin has been shown to reduce blood glucose levels in diabetic rats by enhancing Akt phosphorylation and glycogen synthase kinase 3 (GSK-3) activity [31].

Effects of MERL on leptin and adiponectin levels

Figure 5(A) shows that the highest and lowest mean leptin levels on day 30 were found in the NC group (21.80 ± 1.85 mg/dL) and PC group (14.38 ± 2.51 mg/dL), respectively. Based on the measurement of leptin levels, the T2 showed the most significant reduction compared to other groups; however, the difference among the 80 and 200 mg/kg BW doses was not significant. The reduction in leptin in the MERL-treated groups was better and significantly different from that of the PC group treated with pure rutacridone. It implies that many of the activities in MERL are better than those in pure rutacridone, which has a mechanism of inhibition of TCPTP rise under conditions of overnutrition, which improves leptin receptor activation and maintenance conditions. High levels of leptin indicate a greater body weight and body fat. The main physiological functions of leptin are regulating food intake and energy expenditure and controlling blood glucose and insulin levels. An earlier study has shown that oral administration of 1 g/kgBW/day *Safoof Mohazzil* herbal prescription, which consists of *Ptychotis ajowan*, *Origenum majorana*, *Foeniculum vulgare*, *Carum carvi*, *Coccus lacca*, and *R. graveolens*, significantly reduced the leptin levels of overweight and obese rats in a 2-week period, compared to the untreated obese rats [25]. The highest reduced leptin levels in our study (15.90 ± 0.79 mg/dL) were lower than those in the previous study (477.25 ± 160.82 pg/mL). This difference may be due to the different sex of the animal

model. The previous researchers used female Sprague Dawley rats, while our study used male rats of the same strain. Previous research showed that administering 50 mg/kg BW quercetin to obese mice over 14 weeks elevated leptin levels in the mice by increasing the expression of quinone oxidoreductase 1 (NQ01) in the liver [32].

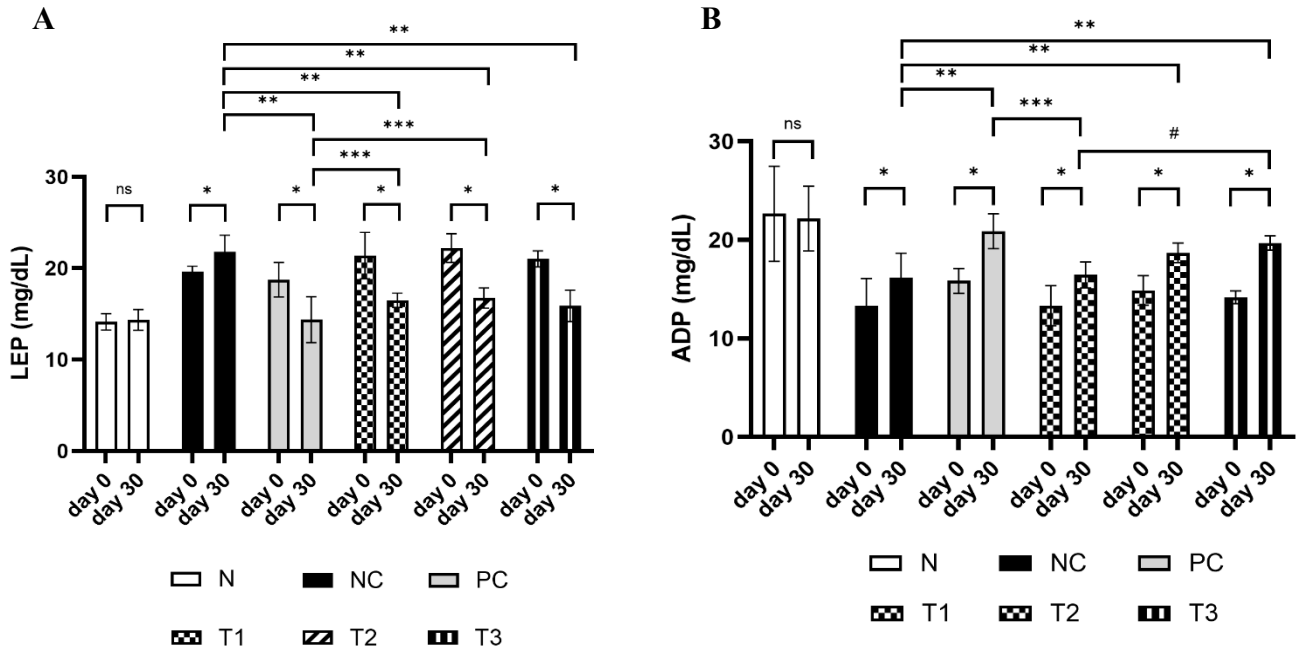


Figure 5 Mean hormone levels of (A) leptin and (B) adiponectin in male obese model rats on day 0 and day 30, presented as mean ± SD. [ns]) Independent t-test not significant ($p > 0.05$) *) Independent t-test significant ($p < 0.05$) **) Post hoc test significant compared to the negative control (NC). ***) The post hoc test was significant compared to the positive control (PC). [#]) Post hoc test was significant compared to treatment 1 (T1). [##]) Post hoc test was significant compared to the treatment 2 (T2). Statistical analysis was performed using Repeated-Measures ANOVA. N = Normal group (non-obese rats), NC = Negative control group (OR + standard diet + 0.9% NaCl), PC = Positive control group (OR + BR-2 + 0.6 mg/kg BW/day pure rutacridone), T1, T2 and T3 were OR + BR-2 + 80, 100 and 200 mg/kg BW/day MERL).

Figure 5(B) shows a significant increase in mean ADP levels in the PC, T1, T2, and T3 groups. The highest increase in mean ADP was observed in T3 at 5.50 ± 0.90 mg/dL. The smallest increase in mean ADP was observed in T1 at 3.13 ± −0.72 mg/dL. The mean ADP levels among rat groups after 30 days of intervention were significantly different ($p < 0.001$). The highest and lowest mean ADP levels in obese rats on day 30 were found in the PC group (20.88 ± 1.78 mg/dL) and NC group (16.16 ± 2.51 mg/dL), respectively (**Figure 5(B)**). Elevating reactive oxygen species and malondialdehyde levels would induce IL-6, which acts alone or in concert to elevate C-reactive protein, causing endothelial dysfunction. The regulatory role of Apelt in inflammation in endotypes of obesity is that of an anti-inflammatory cytokine; it helps by going against leptin. An increase in the mass of adipose tissue results in decreased activity of ADP and heightened leptin levels, thereby increasing the chances of developing cardiovascular disease. This agrees with the study that leptin levels decreased and ADP levels increased over the 30 days. The highest increase in levels of ADP above a basal level in the PC group was seen in T3, which received 200 mg/kg BW MERL. A previous study reported that 40 µg/mL rutin treatment *in vitro* reduced leptin levels but increased adiponectin levels in cell culture using 3T3-L1 adipocytes [33]. Another study reported that quercetin inhibited MAPKs and inflammatory cytokines. Additionally, quercetin induced ADP in macrophages while inhibiting the expression of MCP-1 and TNF-α [34].

Effect of MERL on expression of TCPTP and perilipin-1

Based on **Figure 6(A)**, the NC group had the highest expression of TCPTP (1.18 ± 0.39), while the PC group had the lowest relative expression of TCPTP (0.96 ± 0.60). The T1 group (MERL 80 mg/kg BW) had the lowest relative expression of TCPTP among the MERL groups and was not significantly different from the PC group. Additionally, the PC and T1 groups had lower ratios compared to the N group. Meanwhile, the T2 (1.16 ± 0.51) and T3 (1.13 ± 0.54) groups had higher relative TCPTP expression than T1, indicating that T1 was the most effective MERL dose in providing low expression in obese rats. TCPTP appears to be an attractive candidate for thinking about drug development for obesity. Beige adipocytes can transform white adipose tissue to enact energy storage or calorie burning, which is putatively under the control of the hypothalamus [35]. TCPTP expression was observed to be inducible by fasting, and in the case of glucocorticoids, adiponectin is one hormone that is known to inhibit the signaling of leptin and insulin into AgRP/NPY neurons. The results conclude that MERL at a dose of 80 mg/kg BW proves best to provide such low TCPTP expression in obese rats. The PC group had the lowest relative TCPTP expression due to the influence of rutacridone, which acts as a TCPTP inhibitor [15]. Although the MERL used did not contain rutacridone, the 80 mg/kg BW dose of MERL lowered TCPTP expression, but not significantly different from the PC group. This is likely due to the presence of several compounds in MERL. A previous study has shown that quercetin could interact with the TCPTP protein with −7.40 kcal/mol optimal conformational affinity [36]. Rutamarin also effectively inhibits the TCPTP protein with 6.4 μM IC50, acting as a competitive inhibitor that enhances insulin-induced glucose transporter 4 (GLUT4) translocation in CHO/GLUT4 cells [37].

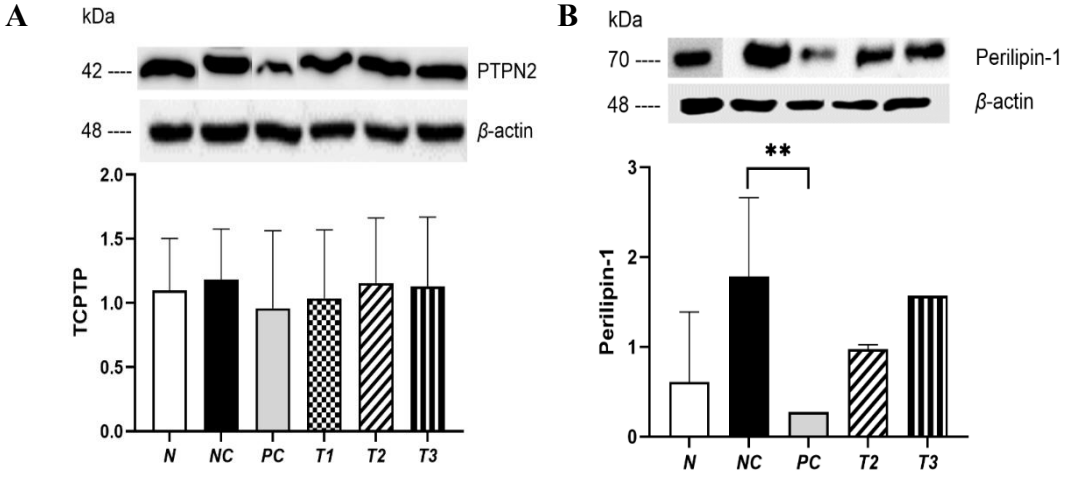


Figure 6 Mean expression ratios of (A) TCPTP and (B) Perilipin-1 in male obese rat models on day 0 and day 30, presented as mean ± SD. [ns]) Independent t-test not significant ($p > 0.05$) *) Independent t-test significant ($p < 0.05$) **) Post hoc test significant compared to the negative control (NC). ***) The post hoc test was significant compared to the positive control (PC). [#]) Post hoc test was significant compared to treatment 1 (T1). [##]) Post hoc test was significant compared to the treatment 2 (T2). Statistical analysis was performed using Repeated-Measures ANOVA. N = Normal group (non-obese rats), NC = Negative control group (OR + standard diet + 0.9% NaCl), PC = Positive control group (OR + BR-2 + 0.6 mg/kg BW/day pure rutacridone), T1, T2 and T3 were OR + BR-2 + 80, 100 and 200 mg/kg BW/day MERL). Note: In the PC and T3 groups, there was no repeat of Perilipin-1 expression; in the T1 group, Perilipin-1 expression did not appear.

Based on **Figure 6(B)**, the NC group had the highest relative expression ratio of Perilipin-1 (1.78 ± 0.87), while the PC group had the lowest relative expression ratio of Perilipin-1 (0.27). The T2 group (0.97 ± 0.04) had the lowest relative expression of Perilipin-1 among the MERL groups and was not

significantly different from the PC and N groups (0.61 ± 0.78). The perilipin is a protective coating against the body's lipases, including lipases that break down triglycerides into glycerol and free fatty acids during lipolysis in metabolism. T1 is found not to express Perilipin-1-linked lipid alteration. The reason behind this could be due to the pure fat sample itself, the hard extraction methods, and the production of very high detergent levels. T2, compared to T3, has a lower PLIN-1 expression, selecting T2 (100 mg/kg BW) as the most effective dose in Rue leaf extract. Rutin could reduce PLIN-1 protein by inhibiting the release of pro-inflammatory cytokines, lowering ROS production, and inhibiting p-JNK translocation from the cytoplasm to the nucleus in intestinal cells [38].

Although MERL administration has some benefits in treating obesity, our study has some limitations. Firstly, there were several parameters that were not affected by the dose response, such as LEP, PLIN-1, TG, TC, LDL-C levels, and TCPTP expression. Secondly, the phytochemical analysis in our study did not demonstrate the presence of the rutacridone compound in MERL, which is a potential inhibitor of TCPTP protein. Thirdly, we did not conduct molecular docking to the MERL bioactive compounds; the mechanism by which the compounds in MERL content can overcome obesity is unknown. Fourthly, PLIN-1 expression did not show up in the obese rats treated with the lowest MERL dose because the sample used was adipocytes, which required a specific method in the extraction process.

Conclusions

Administration of MERL effectively reduces leptin, TG, TC, LDL-C, FBG levels, SFT, BW, and OI while increasing ADP and HDL-C levels in male obese rats. Furthermore, the MERL downregulates TCPTP and Perilipin-1 expression. Reduced ADP, HDL-C, GDP, SFT, BW, and OI in obese rats in a dose-dependent manner, but not for Leptin, TG, TC, and LDL-C levels. However, 100 mg/kg BW/day MERL is a more effective dose than 80 and 200 mg/kg BW/day doses for reducing and increasing all parameters of obesity. Further investigations are needed to conduct molecular docking to elucidate the mechanism of action of MERL, change the solvent in the extraction process, pay further attention to protein extraction methods, conduct toxicity experiments, and explore potential clinical applications for obesity management.

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Declaration of Generative AI in Scientific Writing

We declared that we do not use generative AI and AI-Assisted technologies in writing our original research article.

CRediT Author Statement

Sulistyani Kusumaningrum: Investigator, assessment of SFT, data acquisition, data trial administration, writing original draft preparation. **Ambar Mudigdo**: conceptualization, methodology, and validation. **Bambang Purwanto**: Conceptualization, formal analysis, and validation. **Dono Indarto**: Conceptualization, methodology, data analysis, and interpretation, writing-reviewing, and editing.

References

[1] World Obesity Atlas, Avaliable at: https://data.worldobesity.org/publications/?cat=19, accessed July 2024.

[2] B Masood and M Moorthy. Causes of obesity: A review. *Clinical Medicine* 2023; **23**, 284-291.

[3] GBD 2021 Diabetes Collaborators. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: A systematic analysis for the Global Burden of Disease Study 2021. *The Lancet* 2023; **402**, 203-234.

[4] International Diabetes Federation. IDF diabetes atlas, Available at: https://www.diabetesatlas.org, accessed July 2024.

[5] AM Giudetti. Editorial: Lipid metabolism in obesity. *Frontiers in Physiology* 2023; **14**, 1268288**.**

[6] A Jais and JC Bruning. Arcuate nucleus-dependent regulation of metabolism - pathways to obesity and diabetes mellitus. *Endocrine Reviews* 2023; **43(2)**, 314-328.

[7] SI Lee, JW Kim, YK Lee, SH Yang, I Lee, JW Suh and SD Kim. Anti-obesity effect of *Monascus pilosus* mycelial extract in high fat diet-induced obese rats. title of the article. *Journal Applied Biological Chemistry* 2011; **54(3)**, 195-205.

[8] KW Baek, JS Kim, JS Park, SJ Kim, YC Ha, OY Jeong and JI Yoo. Validation of dual energy x-ray absorptiometry and nuclear magnetic resonance in the analysis of body composition in mice. *Journal of Bone Metabolism* 2020; **27(4)**, 291.

[9] SV Eastwood, T Tillin, A Wright, J Heasman, J Willis, IF Godslanc, N Forouhi, P Whincup, AD Hughes and N Chaturvedi. Estimation of CT-derived abdominal visceral and subcutaneous adipose tissue depots from anthropometry in Europeans, South Asians and African Caribbeans. *PLoS One* 2013; **8(9)**, e75085.

[10] S Cornacchia, LL Tegalo, A Maldera, E Pierpaoli, U Tupputi, G Ricatti, L Eusebi, S Salerno and G Guglielmi. Radiation protection in non-ionizing and ionizing body composition assessment procedures. *Quantitative Imaging in Medicine and Surgery* 2020; **10(8)**, 1723**.**

[11] Y Wang, X Ge, H Ma, S Qi, G Zhang and Y Yao. Deep learning in medical ultrasound image analysis: A review. *IEEE Access* 2021; **9**, 54310-54324**.**

[12] P Gonzalez-Muniesa, MA Mártinez-González, FB Hu, JP Després, Y Matsuzawa, RJF Loos, LA Moreno, GA Bray and JA Martinez. Obesity. *Nature Reviews Disease Primers* 2017; **3**, 17034.

[13] GA Bray, G Frühbeck, DH Ryan and JPH Wilding. Management of obesity. *The Lancet* 2016; **387(10031)**, 1947-1956**.**

[14] N Kishore, D Marques, Mahmud, MV Kiang, I Rodriquez, AJD Fuller, P Ebner, C Sorensen, F Racy, J Lemery, L Maas, J Leaning, RA Irizarry, S Balsari, CO Buckee and D Phil. Mortality in Puerto Rico after Hurricane Maria. *The New England Journal of Medicine* 2018; **379(2)**, 162-170.

[15] Y Fitrianingrum, D Indarto, R Kusumawati and YH Suselo. Actinodaphnine and rutacridone as new T-cell protein tyrosine phosphatase inhibitors for drug development of obesity. *IOP Conference Series: Materials Science and Engineering* 2019; **546(6)**, 062007**.**

[16] H Ali, A Ahmed and AQ Abdulwahab. Potential of some therapeutic effect of *Ruta graveolens* plant and their bioactivities: A review. *Medical Journal of Ahl al-Bayt University* 2024; **3(1)**, 174-189.

[17] A Hosseini, BM Razavi, M Banach and H Hosseinzadeh. Quercetin and metabolic syndrome: A review. *Phytotherapy Research* 2021; **35(10)**, 5352-5364**.**

[18] Y Zhao, B Chen, J Shen, L Wan, Y Zhu, T Yi and Z Xiao. The beneficial effects of quercetin, curcumin, and resveratrol in obesity. *Oxidative Medicine and Cellular Longevity* 2017; **2017(1)**, 1459497**.**

[19] E Valizadeh, A Ostadrahimi, D Fazli, H Khodaei and H Akbari. Efficacy of a traditional herbal mixture as an anti-obesity supplement in obese individuals: A randomized controlled trial. *Journal of Nutritional Sciences and Dietetics* 2017; **3(4)**, 1-8.

[20] I Sundari, D Indarto and P Dirgahayu. Dual extracts of star fruit leaves and *Toddalia accuelata* leaves as antiobesity in rats. *Jurnal Aisyah: Jurnal Ilmu Kesehatan* 2022; **7(1)**, 93-100.

[21] MN Ilyas, NB Simbak, AB Atif and MKR Adzim. Sample size calculation for animal studies using degree of freedom (E); an easy and statistically defined approach for metabolomics and genetic research. *Current Trends in Biomedical Engineering & Biosciences* 2017; **43**, 207-213.

[22] RD Marin, S Crespo-Gracia, AM Wilson and P Sapieha. RELi protocol: Optimization for protein extraction from white, brown and beige adipose tissues. *MethodsX* 2019; **6**, 918-928.

[23] LR Vaquero, GA Rivera, JA Mendiola, AADV Martinez, E Ibanez and M Bueno. Utilizing green solvents in compressed fluids technologies for

extracting bioactive compounds from *Ruta graveolens* L. *Industrial Crops and Products* 2024; **216**, 118717.

[24] I Munawar, SSU Hassan, M Abbas, M Abbas, M Saeed, MA Ghaffari and H Ahmad. Phytochemical evaluation and lipid profile significance of *Ruta graveolens* through obesity-induced animal model. *Bioscience Research* 2023; **20(2)**, 557-564.

[25] P Gupta, J Mehla and YK Gupta. Antiobesity effect of *Safoof Mohazzil*, a polyherbal formulation, in cafetaria diet induced obesity in rats. *Indian Journal of Experiment Biology* 2012; **50(11)**, 776-784.

[26] M Noori, M Jafari, H Azimi and M Node-Farahani. Effects of *Ruta graveolens* total and flavonoids extracts on rat blood glucose, cholesterol, triglycerides and urea comparing synthetic drugs. *Nusantara Bioscience* 2019; **11**, 23-29.

[27] AG Manzoni, DF Passos, JLG da Silva, VM Bernardes, JM Bremm, MH Jantsch, JS de Oliveira, TR Mann, CM de Andrade and DBR Leal. Rutin and curcumin reduce inflammation, triglyceride levels and ADA activity in serum and immune cells in a model of hyperlipidemia. *Blood Cells, Molecules and Diseases* 2019; **76**, 13-21.

[28] AR Althaher, M Alwahsh, A Hasan, D Al-Majali, MW Awadallah and T Al-Qirim. Anti-hyperlipidemic effect of *Ruta chalepensis* ethanolic extract in triton WR-1339-induced hyperlipidemia in rats. *Applied Sciences* 2024; **14(19)**, 9017.

[29] C Gur, S Ozkanlar, S Gedikli, E Sengul, V Gelen and A Kara. The effects of quercetin administration on heart tissue and serum parameters in the rats with experimental obesity. *Eurasian Journal of Molecular and Biochemical Sciences* 2022; **1(1)**, 16-21**.**

[30] C Velmurugan, I Sakthivel and V Subramaniyan. Evaluation of anti-diabetic and wound healing potential of Ethiopia plant '*Ruta graveolens*' in diabetic induced rat. *Natural Volatiles & Essential Oils* 2022; **9(1)**, 1571-1582.

[31] Z Peng, X Gong, Y Yang, L Huang, Q Zhang, P Zhang, R Wan and B Zhang. Hepatoprotective effect of quercetin against LPS/d-GalN induced acute liver injury in mice by inhibiting the IKK/NF-κB and MAPK signal pathways. *International Immunopharmacology* 2017; **52**, 281-289.

[32] A Abdullah, A Atia and N Alrawaiq. The effect of administration of an equal dose of different classes of dietary chemicals on NQO1 expressional level in mice liver. *Pharmacophore* 2017; **8(5)**, 1-9.

[33] MS Ganjayani, RS Karunakaran, S Gandham and B Meriga. Quercetin-3-O-rutinoside from *Moringa oleifera* downregulates adipogenesis and lipid accumulation and improves glucose uptake by activation of AMPK/glut-4 in 3T3-L1 cells. *Revista Brasileira de Farmacognosia* 2023; **33(2)**, 334-343.

[34] SY Seo, WS Ju, K Kim, J Kim, JO Yu, JS Ryu, JS Kim, HA Lee, DB Koo and YK Choo. Quercetin induces mitochondrial apoptosis and downregulates ganglioside GD3 expression in melanoma cells. *International Journal of Molecular Sciences* 2024; **25(10)**, 5146.

[35] Y Wang, S Liu, T Jia, Y Feng, X Xu and D Zhang. T cell protein tyrosine phosphatase in glucose metabolism. *Frontiers in Cell and Developmental Biology* 2021; **9**, 682947.

[36] X Bai, X Zhao, K Liu, X Yang, Q He, Y Gao, W Li and W Han. Settings order article reprints open accessarticle mulberry leaf compounds and gut microbiota in alzheimer's disease and diabetes: A study using network pharmacology, molecular dynamics simulation, and cellular assays. *International Journal of Molecular Sciences* 2024; **25(7)**, 4062.

[37] C Bailly. *Ruta angustifolia* pers. (narrow-leaved fringed rue): Pharmacological properties and phytochemical profile. *Plants* 2023; **12(4)**, 827.

[38] A Ran, C Shi, Y Tang, Z Cui, Y Li, Z Chen, M Xiao and L Xu. Chitosan/rutin multifunctional hydrogel with tunable adhesion, anti-inflammatory and antibacterial properties for skin wound healing. *Carbohydrate Polymers* 2024; **343**, 122492.

[39] S Kusumaningrum. 2025, Effects of Methanol Extract of Rue Leaves (*Ruta graveolens*) on Leptin Resistance, Fat Metabolism, and Weight Loss in Obese Male Rats. Ph.D. Dissertation. Universitas Sebelas Maret, Surakarta, Indonesia.