



Dual Extracts of Star Fruit Leaves and *Toddalia accuelata* Leaves as Antiobesity in Rats

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ABSTRACT

The imbalance between food intake and energy expenditure can lead to obesity. Obesity is a major risk factor for chronic diseases such as cardiovascular disease and can cause premature death worldwide. This study is aimed to investigate the effect of Star Fruit Leaves Extract (SLE) and *T. accumulate* Leaves Extract (TLE) combination on Body Weight (BW), Body Mass Index (BMI), and Fat Content (FC) of obese rats. Male Wistar rats aged 5 weeks, with BW of 100-150 g were induced with a High Fat Diet (HFD) and 10% fructose solution for 30 days. A total of 18 rats were randomly divided into 3 groups, namely Negative Control (NC), Therapy (T), and Normal (N). After 35 days of treatment compared to the NC group, the mean BW of the T group was significantly lower ($p=0.014$) and the mean decrease was significantly higher ($p<0.001$). Similarly, the mean BMI and FC of the T group were significantly lower and the decrease in the mean of both groups was significantly higher than that of the NC group ($p<0.001$). Therefore, the combination of SLE and TLE can be used as a treatment for obesity because it reduces BW, BMI, and FC better than only a low-calorie diet.

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Kata kunci:

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ABSTRAK

Ketidakeimbangan antara asupan makanan dan pengeluaran energi dapat menyebabkan obesitas. Obesitas merupakan faktor risiko utama terjadinya penyakit kronis seperti penyakit kardiovaskular dan dapat menyebabkan kematian dini di seluruh dunia. Penelitian ini bertujuan untuk mengetahui pengaruh kombinasi Ekstrak Daun Belimbing (EDB) dan Ekstrak Daun *T. accuelata* (EDT) terhadap Berat Badan (BB), Indeks Massa Tubuh (IMT), dan kandungan lemak tikus obesitas. Tikus wistar jantan umur 5 minggu, BB 100-150 g diinduksi dengan pakan tinggi lemak dan larutan fruktosa 10% selama 30 hari. Sebanyak 18 ekor tikus dibagi secara acak menjadi 3 kelompok yaitu Kontrol Negatif (KN), Terapi (T), dan Normal (N). Setelah 35 hari perlakuan dibandingkan dengan kelompok KN, rata-rata BB kelompok T secara signifikan lebih rendah ($p=0,014$) dan rata-rata penurunannya lebih besar secara signifikan ($p<0,001$). Demikian pula, rata-rata IMT dan kandungan lemak kelompok T secara signifikan lebih rendah dan penurunan rata-rata kedua kelompok tersebut secara signifikan lebih besar dibandingkan dengan kelompok KN ($p<0,001$). Oleh karena itu, kombinasi EDB dan EDT dapat digunakan sebagai pengobatan obesitas karena dapat menurunkan BB, IMT, dan kandungan lemak lebih baik daripada diet rendah kalori saja.

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INTRODUCTION

Obesity is a major risk factor for chronic diseases such as Type 2 Diabetes Mellitus and cardiovascular disease and is one of the top five causes of premature death worldwide (Hakkak, R., Bell, 2016). Obesity is an abnormal and excessive accumulation of body fat with a BMI > 30 kg/m² (WHO, 2020). Factor contributing towards obesity are multifactorial and not yet fully understood (Narciso J, Silva AJ, Rodrigues V, Monteiro MJ, Almeida A, Dias RV, 2019). Worldwide, 650 million (13%) people over the age of 18 were obese in 2016. The prevalence has tripled since 1975 and is estimated to reach 57.8% by 2030 (WHO, 2020).

Obesity is caused by the imbalance between energy intake and energy expenditure. Excessive food intake will cause Free Fatty Acid (FFA) to be stored in adipose tissue and absorbed by the liver thereby increasing the secretion of Very Low-Density Lipoprotein (VLDL) which is rich in triglycerides (TG) by the liver. After this process, FFA is regenerated which is taken up by muscle and adipose tissue for storage (Klop B, Elte JW, 2013). The excess energy that is created also results in hyperplasia and hypertrophy of adipose tissue which can ultimately increase BW and leptin secretion (Fonseca DC, Sala P, Ferreira BA, Reis J, Torrinas RS, Bendavid I, 2018). Hyperleptinemia which occurs in excess actually induce leptin resistance, triggering hyperphagia which further accelerates the increase in BW (Gruzdeva O, Borodkina D, Uchasova E, Dyleva Y, 2019).

Pharmacological therapy is often planned in obese patients since long-term low-calorie diet and physical activity often fail due to loss of compliance (Ruban A, Stoenchev K, Ashrafian H, 2019). However, administration of Orlistat (lipase inhibitor) is less effective in reducing BW and often causes side effects such as increased heart rate and blood pressure, as well as gastrointestinal symptoms (Sahebkar A, Simental-Mendía LE, Reiner Z, Kovanen PT, Simental-Mendia M, Bianconi V, 2017). Therefore, the use of phytochemicals can be used as an alternative to pharmacological therapy for obesity. Several previous studies have used herbal plants for obesity therapy such as green tea (Huang J, Wang Y, Xie Z, Zhou Y, Zhang Y, 2014) and Dutch teak leaves (Hidayat M, Soeng S, Prahastuti S, Erawijantari PP, 2015), however, the results are inconsistent. A study by Hardani & Lestariana (Hardani, E., Lestariana, W., 2014), showed that green tea can reduce BW and increase HDL levels, but does not significantly reduce cholesterol, TG, and LDL levels in obese adult women. Thus, it is necessary to look for alternative obesity therapy from other herbal plants.

Starfruit leaves (*Averrhoa carambola*) and *T. aculeata* leaves are plants that grow in tropical areas such as Asia, that have not been widely exploited for their potential as anti-obesity (Aladaileh SH, Saghir SA, Murugesu K, Sadikun A, Ahmad A, 2019; RK, 2016). Star fruit leaves contain phytochemical ECG (Moresco HH, Queiroz GS, Pizzolatti MC, 2012), similar to green tea (Huang J, Wang Y, Xie Z, Zhou Y, Zhang Y, 2014) which is known to inhibit cholesterol synthesis by *in silico* (Isnawati A, 2015) and has antioxidant activity (Aladaileh SH, Saghir SA, Murugesu K, Sadikun A, Ahmad A, 2019). Likewise, *T. accuelata* leaves also shows antioxidant activity (Irudayaraj SS, Sunil C, Duraipandiyar V, 2013) and contain dictamine phytochemicals which according to molecular docking studies, can increase cholesterol excretion and prevent atherosclerosis (Nurfitri FB, Wulandari RAJS, 2017; RD, 2019).

Providing Star Fruit Leaves Extracts (SLE) 1000 mg/kgBW/day once a day orally for 35 days in

hyperlipidemic rat models showed a decrease in BW, BMI, serum lipid profile, and activity of the enzyme *HMG Co-A reductase*, as well as a significant increase in antioxidant enzymes compared to the control group (Aladaileh SH, Saghir SA, Murugesu K, Sadikun A, Ahmad A, 2019). Similar to SLE, oral administration of *Toddalia accuelata* Leaves Extracts (TLE) 400 mg/kgBW/day once daily for 28 days in hyperlipidemic rats also significantly reduced lipid profile levels and increased HDL levels compared to the control group (Irudayaraj SS, Sunil C, Duraipandiyar V, 2013). However, to this day no study has been conducted to assess the effect of the aforementioned extracts, which is expected to increase the effectiveness of both extracts as an anti-obesity due to the different contained phytochemicals with different mechanisms in reducing BW, BMI and FC (Irudayaraj SS, Sunil C, Duraipandiyar V, 2013; Saghir, S. A. M., Sadikun, A., Al-Suede, F.S.R., Majid, A. M. S. A., Murugaiyah, 2016).

METHODS

All procedures generating rat model with obesity and treatment with SLE and TLE combination followed the ethics of experimental animals and it was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret Surakarta under number 15/UN27.06.6.1/KEP/EC/2021.

Extract preparation of Star Fruit and *T. accuelata* Leaves

Fresh star fruit and *T. accuelata* leaves were obtained from Demak and Tawangmangu, Central Java, respectively and the later plant was authenticated by The Center for Research and Development of Medicinal Plants and Traditional Medicine Tawangmangu, Karanganyar Regency, Central Java Province. The extraction of star fruit leaves used a method from Aladaileh et al. (Aladaileh SH, Saghir SA, Murugesu K, Sadikun A, Ahmad A, 2019) with modification while the *T. accuelata* leaves were extracted using the modified method from Rasamison et al. (Rasamison VE, Brodie PJ, Merino EF, Cassera MB dan Ratsimbason MA, 2016). In brief, both leaves were washed with tap water and then dried using a food hydrator (Getra, Indonesia) at 60°C for 9.5 h. Once they have completely dried, it was grounded using a disk mill (Fomac, Indonesia) and then was sieved using an 80 mesh sieve. Simplicia of star fruit leaves were macerated using 96% (volume/volume) methanol with 1:6 ratio for 48 h at 28±2°C while 70% (v/v) ethanol solvent was used to macerate simplicia *T. accuelata* with 5:1 ratio for 48 h at 28±2°C. The maceration process of star fruit and *T. accuelata* leaves was repeated one more with 1:3 ratio. Collected filtrates were concentrated using a rotary vacuum evaporator at 60°C with 80 rpm and 175 mbar and followed by drying in an oven at 45-50°C for 10-15 minutes. Finally, SLE and TLE were kept at 4°C before further analysis.

Generating Rat Model With Obesity

Male albino rats used in this study were provided by the Pharmacology and Toxicology Laboratory, Universitas Setia Budi (USB), Surakarta. A total of 18 male rats (*Rattus norvegicus*) Wistar strain met the inclusion criteria: aged 4-5 weeks old and weighted 100-150g and were then adapted for 7 days in a hygienic *polypropylene* cage with 6 rats/cage. The cages were placed in a room with 22-25°C and 12 h dark and light cycles. During adaptation, they were fed with 100 g

standard 594 diet and were provided drinking water *ad libitum*. To generate rats model with obesity, we used the method developed by Zarghani et al. (Zarghani SS, Soraya H, Zarei L, 2016) and added a mixture of Broiler-2(BR-2) feed, beef fat, duck egg yolk, chicken liver, and butter. We also used 10% (w/v) fructose added in drinking water and were given every morning and evening for 30 days. HFD induced obesity in rat was determined using the Rohrer Index $\{BW (g)/\text{naso-anal length (cm)}^3\} \times 10^3$ with BMI >30. In addition, rat'sFC was calculated using the formula = $(0.581 - \text{TM index}) \times 22.03$, where the TM index was obtained from the calculation = $BW (g)/\text{aso-anal length (cm)}^{2.823} \times 10^3$ (Lee S-I, Kim, J-W, Lee Y-K, Yang SW, Lee I-A, Sah J-W, 2011).

Research Design

After adaptation, 18rats were randomly divided into 3 groups (N, NC, and T). The N group was given normal diet and did not received any treatment. The NC group was only given 200 g/day the standard diet while the T group was given 200 g/day the standard diet and was treated with a combination of 750 mg/kgBW/day SLE and 100 mg/kgBW/day TLE for 35 days. We measured BW and length in the day 14 and 35 and calculated rat BMI and FC.

Data analysis

All collected data were presented in mean \pm standard error. Before carrying out the multivariate statistical test, the

data were firstly tested for normality using the Shapiro-Wilk test and for homogeneity using the Leuvene test. The One-Way Analysis of Variance (ANOVA) test was used to compare different means among groups and followed by the LSD post hoc test with p value <0.05. To analyze the difference in the mean effect between groups which was carried out repeatedly was used the repeated measures ANOVA test. Meanwhile, the independent t-test was used to compare nutrition status between rats treated with SLE and TLE combination and rats without treatment.

RESULTS AND DISCUSSIONS

Body Weight, BMI, and Fat Content Changes After Feeding with HFD and Fructose Solution

Before feeding with HFD and fructose solution, all rats had similar BW (Table 1). The mean BW in T group (131.33 \pm 3.84 g) was higher than the mean BW in NC group (132.17 \pm 3.41 g) and N group (131.33 \pm 3.84 g) but it was not significantly different (p=0.164). Thirty days later, the mean BW in all groups increased but only NC and T groups reached significantly (p<0.001). In addition, increased BW in T group (79.80 \pm 7.70 g) was significantly higher than increase BW in NC group (p=0.005).

Table 1
Average Body Weight of Rats Before and After Obesity Induction

Group	Average BW (g)			p ^a
	Before Induction	After Induction	Δ BW	
NC	132.17 \pm 3.41	183.17 \pm 7.43	51.00 \pm 4.37	<.001*
T	140.80 \pm 3.32	220.60 \pm 9.27	79.80 \pm 7.70	<.001*
N	131.33 \pm 3.84	152.67 \pm 8.11	21.33 \pm 5.90	.069
p ^b	.164	.001*	<.001*	
p ^b NC-T	.092	.007*	.005*	
p ^b NC-N	.882	.040*	.009*	
p ^b T-N	.122	<.001*	<.001*	

Notes:

NC: Negative Control; T: SLE and TLE combination; N: Normal. Δ BW: Increase in mean BW before and after induction, *p<.05), a) Statistical test with paired t-test; b) Statistical test with One Way ANOVA and continue followed by LSD post hoc test.

BMI and FC were important parameters for evaluation of obesity. Table 2 and 3 indicated that the mean BMI and FC increased in rats which were fed with HFD and fructose solution. In Table 2, similar BMI was observed in all groups before feeding with HFD and fructose solution. The highest mean BMI was found in the T group (34.22 \pm 0.89 g) after 30 days induction and it was followed by the NC (31.76 \pm 0.56). These increased mean BMI ratios were significantly higher than that of the N group (p=<0.001).

The same pattern of obesity parameter was also found in the FC parameter (Table 2). Before induction of obesity, T group (1.72 \pm 0.75) had the lowest FC, compared to the N group (2.23 \pm 0.21) and NC group (2.71 \pm 0.85). However, the FC differences did not reach significantly (T: N p= 0.37 and T:NC p=0.051). After 30 days feeding with HFD and fructose solution, the T group had the highest FC (11.33 \pm 0.90) and followed by the NC group (8.73 \pm 0.57). Although the mean FC in the N group increased (3.29 \pm 0.24), it did not differ from the mean FC before induction. Moreover, the increased FC in NC and T groups reached significantly compared to the increased FC in N group.

In a previous study, administration of 53% HFD and 20% fructose solution for 40 days could increase the average BW

of experimental animals by almost 50 g (Zarghani SS, Soraya H, Zarei L, 2016). The composition of the fructose solution in this study was lower (10%) than the previous study, however, the FC was slightly higher (55%). However, this composition caused the mice to become obese more quickly with a larger average increase in BW of 65.4 g in 30 days.

Fat is a food with high calories, so consuming it in large quantities may cause an imbalance between excessive intake and energy expenditure. In conditions of excess calories, FFA will be stored again in adipose tissue in the form of TG. Due to the excessive amount of FFA in the circulation, it will be absorbed by the liver and can increase the secretion of TG-rich VLDL by the liver. After that, the TG is hydrolyzed to produce FFA which is taken up by muscle and adipose tissue for storage (Klop B, Elte JW, 2013). The occurrence of fat accumulation can lead to hyperplasia and hypertrophy of adipose tissue thereby increasing BW. Fat accumulation can also reduce adinopectin levels so that TG oxidation in adipose tissue is reduced which further increases BW (Fonseca DC, Sala P, Ferreira BA, Reis J, Torrinhas RS, Bendavid I, 2018). Giving fructose also has a greater obesogenic potential than other sugars. This is because almost no fructose is absorbed by pancreatic beta cells,

because the expression of GLUT2 and GLUT5 in the pancreas is small thus unable to trigger insulin release. In addition, fructose does not stimulate leptin release and does not suppress ghrelin release, resulting in decreased energy expenditure and glucose uptake (Hannou SA, Haslam DE, McKeown NM, 2018). The liver has a lot of GLUT 2, but the transport of fructose into the cytoplasm of hepatocytes is rapidly phosphorylated to fructose 1-phosphate (fructose 1-P) which causes an increase in fatty acid synthesis, inhibits

lipid transport to mitochondria, and stops β -oxidation resulting in adipocyte hypertrophy resulting an increase in BW (Caton PW, Nayuni NK, Khan NQ, Wood EG, 2011). Therefore, such rat models is appropriate for biochemical and pharmacological studies which is aimed to evaluate the potential of SLE and TLE combination as an antiobesity therapy.

Table 2
Average BMI and Fat Content of Rats Before and After Obesity Induction

Group		NC	T	N	p ^b
Parameters Obesity					
Average BMI	Before Induction	25.73±.87	24.54±.76	25.21±.21	.074 pNC-T=.025* pNC-N=.356 pT-N=.252
	After Induction	31.76±.56	34.22±.89	26.13±.20	<.002* pNC-T=.022* pNC-N<.001* pT-N<.001*
	Δ BMI	6.04±.59	9.68±.74	.92±.26	<.001* pNC-T=.001* pNC-N<.001* pT-N<.001*
Average FC	Before Induction	2.71±.85	1.72±.75	2.23±.21	.135 pNC-T=.051 pNC-N=.381 pT-N=.370
	After Induction	8.73±.57	11.33±.90	3.29±.24	<.001* pNC-T=.018* pNC-N<.001* pT-N<.001*
	Δ BMI	6.02±.56	9.60±.74	1.05±.29	<.001* pNC-T=.001* pNC-N<.001* pT-N<.001*
p ^a		<.001*	<.001*	.068	

Notes: Δ BMI and FC: Increase in mean BMI and FC before and after induction, *p<.05), a) Statistical test with paired t-test; b) Statistical test with One Way ANOVA and followed by LSD post hoc test.

Combination Of SLE and Tle Reduced Rats BW, BMI, and Fat Content During Intervention

Combination of SLE and TLE had been established for reduction of BW in rats with obesity. Based on Table 4, the administration of the SLE and TLE combination reduced rats BW depending on time manner. Before treatment of SLE and TLE combination, higher mean BW was found in the T group, compared to mean BW in NC group. By contrast, the mean BW in the NC group increased by (12.5 g) while reduction of mean BW occurred in the T group from (220.60±9.27 g to 175.60±5.02 g) during 14 days intervention. Changes in BW in both groups reached significantly only in T group (p=0.001), but not in NC group (p=0.294). In the end of intervention, the mean BW in NC (182.83±8.69 g) and T (150.80±4.70 g) groups reduced significantly compared to the 14 days intervention with p<.001. Moreover, reduction of mean BW in T group significantly differed from the mean BW in NC group (p<.001).

To evaluate the effect of SLE and TLE combination, we measured BMI and FC before, during and after intervention. The combination of SLE and TLE given once a day to group T at a dose of 750:100mg/kgBW/day caused a decrease in the mean BMI and FC of group T after 14 and 35 days of treatment (Tables 3 and 4). Likewise, the KN group also showed a decrease in the average BMI and FC, but with a smaller decrease. After 14 and 35 days of treatment, the

mean BMI and FC in group T decreased significantly compared to the mean BMI and FC before treatment (p<.001), but the KN group only experienced a significant decrease after 35 days of treatment (p≤0.008). The mean BMI and FC of the T group were also significantly lower than the KN group after 14 and 35 days of treatment with p<.001 (Figures 2 and 3). At the end of the treatment, the decrease in mean BMI in group T was significant (p<.001) greater than the KN group, which was -14.51±0.65 g vs -4.78±1.06 g. Similarly, the T group also showed a significantly greater decrease in the mean FC compared to the KN group, which was -13.95±0.63 vs -4.38±1.04.

In this study, group T which was given a combination of SLE and TLE at a dose of 750:100 mg/kgBW/day once a day for 35 days, in addition to being given a standard diet, showed a significantly greater reduction in BW, BMI, and FC compared to the NC group whose diet was only returned to standard diet. The results of this study are in accordance with previous studies which showed that giving 1000 mg/kgBW/day of EDB once a day for 35 days was effective in reducing the BW and BMI of Sprague Dawley rats significantly compared to the hyperlipidemic control group (Aladaileh SH, Saghir SA, Murugesu K, Sadikun A, Ahmad A, 2019). There are no studies showing the effect of TLE on BW, but research of Irudayaraj et al. (Irudayaraj SS, Sunil C, Duraipandian V, 2013) supports the results of this study which showed that a decrease in cholesterol, TG, and LDL

levels, and an increase in HDL serum level of Wistar rats is significantly after giving TLE with a dose of 400mg/kgBW, once daily for 28 days, compared to the hyperlipidemic

control group. Decreased lipid levels in the blood eventually can reduce BW (Od-Ek H, Deenin W, Malakul W, Phoungpetchara I, 2020).

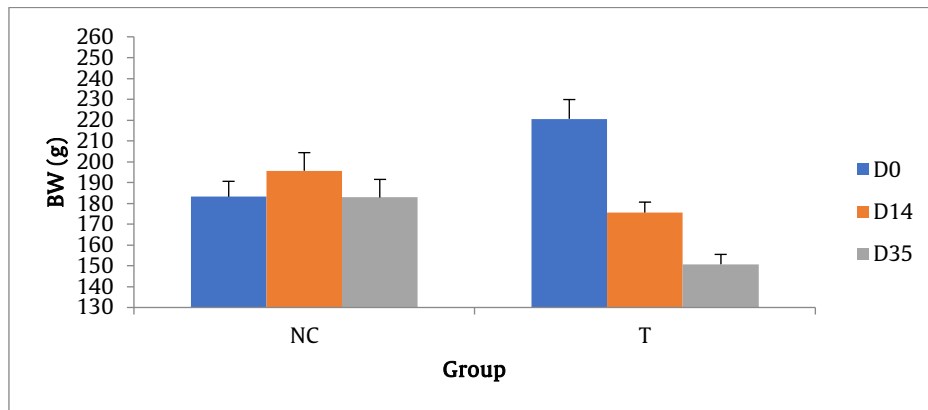


Figure 1.

Effect of SLE and TLE combination on body weight male Wistar rats model obesity. Body weight was measured on day 0, 14, and 35 and datas are presented in the form of mean±SE. *significant between H0-H14 and H0-H35 in each group with repeated measures ANOVA. **significant with KN between H0-H14 and H0-H35 with independent sample t-test.

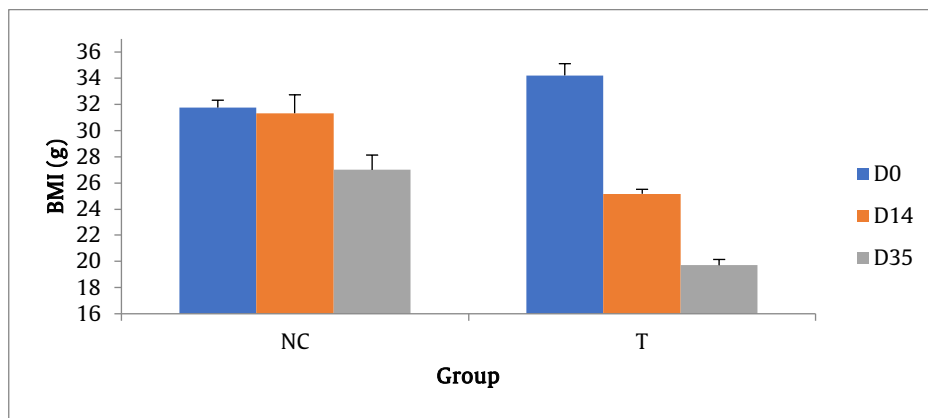


Figure 2.

Effect of SLE and TLE combination on BMI male Wistar rats model obesity. BMI was measured on day 0, 14, and 35 and datas are presented in the form of mean±SE. *significant between H0-H14 and H0-H35 in each group with repeated measures ANOVA. **significant with KN between H0-H14 and H0-H35 with independent sample t-test.

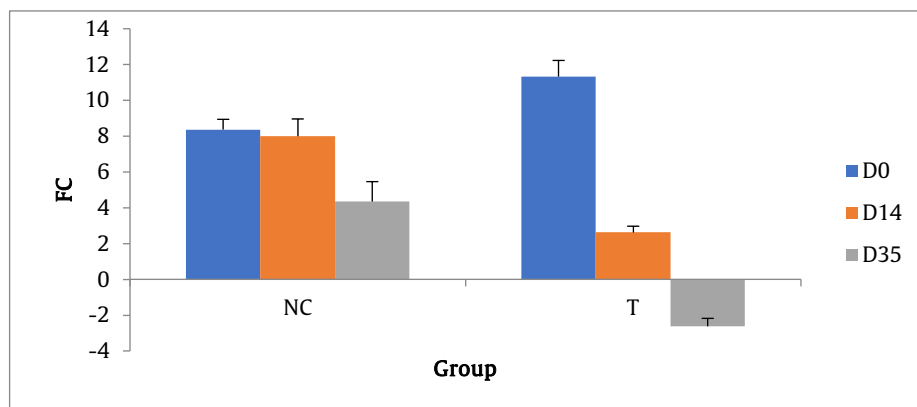


Figure 3.

Effect of SLE and TLE combination on fat content male Wistar rats model obesity. Fat content was measured on day 0, 14, and 35 and datas are presented in the form of mean±SE. *significant between H0-H14 and H0-H35 in each group with repeated measures ANOVA. **significant with KN between H0-H14 and H0-H35 with independent sample t-test.

These results show that the addition of SLE and TLE combination can produce a better effect in the treatment of obesity. This may be due to ECG (flavonoid derivatives) contained in SLE, which based on molecular docking studies can inhibit the HMG-CoA reductase enzyme with the strongest binding among other catechin derivatives (Isnawati A, 2015; Moresco HH, Queiroz GS, Pizzolatti MC, 2012). Inhibition of the HMG-CoA reductase enzyme results in no formation of L-mevalonate so that cholesterol synthesis does not occur (Isnawati A, 2015). In addition, ECG also functions as an SGLT-2 inhibitor based on molecular docking studies so which may increase lipolysis (RA, 2015). Dictamine contained in TLE can also play a role in decreasing BW, BMI, and FC in this study through inhibition of the FMO₃ enzyme based on *in silico* studies (Nurfitria FB, Wulandari RAJS, 2017; RD, 2019). With the inhibition of the FMO₃ enzyme, *Trimethylamine N-Oxide* (TMAO) is not formed so that it can increase cholesterol excretion through the biliary and intestinal systems and can inhibit lipogenesis in the liver (Canyelles M, Tondo M, Cedo L, Farras M, Escola-Gil JC, 2018).

By decreasing cholesterol levels in the blood, there will be a decrease in LDL which carries cholesterol to peripheral tissues for storage. This causes a decrease in the process of lipogenesis and differentiation of pre-adipocyte cells into mature adipocytes, and an increase in beta-oxidation and thermogenesis that can cause adipocyte cell atrophy. The effect of decreasing circulating cholesterol also occurs in skeletal muscle by increasing thermogenesis (Od-Ek H, Deenin W, Malakul W, Phoungpetchara I, 2020; Sun N-N, Wu T-Y, 2016). Increased glucosuria due to SGLT-2 inhibition by ECG in SLE can also increase fasting and post-meal glucagon concentrations so that it can reduce plasma glucose levels and cause mobilization of lipid stores (DeFronzo RA, Hompesch M, Kasichayanula S, Liu X, Hong Y, 2013; Ferrannini E, Baldi S, Frascerra S, Astiarraga B, Heise T, 2016). Utilization of lipids in adipose tissue for energy production (lipolysis) results in reduced subcutaneous and visceral adipose tissue (Ferrannini E, Baldi S, Frascerra S, Astiarraga B, Heise T, 2016). All of these mechanisms can ultimately lead to a decrease in BW, BMI, and FC of mice.

Epicatechin gallate which is a flavonoid group also has antioxidant activity by scavenging radicals and donating H⁺ ions. This antioxidant activity will effect adipositokin regulation in white fat tissue in the form of increasing adiponectin and a decrease in leptin and TNF- α (Tun S, Spainhower CJ, Cottrill CL, Lakhani HV, Pillai SS, 2020). Increased adiponectin can stimulate fatty acid oxidation in peripheral tissues and energy expenditure by targeting the central nervous system (Ma W, Huang T, Zheng Y, Wang M, Bray GA, 2016). Leptin, which is secreted in excess will be suppressed by antioxidants, thereby increasing tryptophan levels which can induce satiety (Mangge H, Summers K, Almer G, Prassl R, Weghuber D, 2013). The decrease in TNF- α due to antioxidants can also inhibit the degradation of morphologically altered POMC neurons, in which POMC is anorexigenic (Patsalos O, Dalton B, Leppanen J, Ibrahim MAA, 2020). Dictamine, which is an alkaloid group, also has antioxidant activity by inhibiting the synthesis, activation, or translocation of NADPH-oxidase which is a key enzyme for ROS production at the cellular level through activation of the nuclear factor Nrf2 pathway (Macakova K, Afonsob R, Sasoc L, 2019). The mechanism of suppression of oxidative stress can inhibit hyperphagia and trigger lipolysis and decrease in blood lipid levels which in turn can cause a decrease in BW, BMI, and FC of mice.

LIMITATION OF THE STUDY

It is not yet fully known the mechanism of the combination of EDB and EDAK in reducing obesity parameters in mice in this study, whether through inhibition of HMG Co-A reductase by ECG and FMO3 by dictamine or caused by other compounds with different mechanisms because in this study no enzyme examination was carried out. and no experimental animal groups were given a combination of the two pure compounds.

CONCLUSIONS AND SUGGESTIONS

Obese mice given the SLE and TLE combination at a dose of 750:100 mg/kgBW/day once a day for 35 days had a significantly lower mean BW, BMI, and FC compared to the NC group, as well as a significantly greater mean reduction. Further research is needed to confirm the results obtained in this study, using pure ECG and dictamine compounds from SLE and TLE in order to understand whether the combination of both compounds indeed may result in reduction of BW, BMI and FC. It is necessary to examine the concentration of ECG in SLE and dictamine in TLE then give the combination of the two pure compounds to experimental animal models of obesity based on their concentration and compare their effects with the combination of SLE and TLE. In addition, it is also necessary to examine the enzyme HMG Co-A reductase and FMO3 in obese rats.

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ETHICAL CONSIDERATIONS

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Conflict of Interest Statement

There are no conflicts of interest.

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