



The Effects of Star Fruit (*Averrhoa carambola* Linn.) Extract on Body Mass Index, Fasting Blood Glucose, and Triglyceride Levels in Male Rats with Obesity and Type 2 Diabetes Mellitus

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Abstract

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BACKGROUND: Obesity is the main risk factor of diabetes by which induces insulin resistance. Epicatechin gallate can virtually interact with sodium-glucose co-transporter 2 as same as dapagliflozin and is found in green tea and star fruits.

AIM: This study aimed to investigate the effects of methanol extract of star fruit (MES) on body weight (BW), body mass index (BMI), fasting blood glucose (FBG), and triglyceride levels in male rats with obesity and type 2 diabetes mellitus (T2DM).

METHODS: Twenty-four male Sprague-Dawley rats were randomly assigned to normal and high-fat diet (HFD) groups. Obesity was induced with a HFD diet for 5 weeks and followed by induction of T2DM with 230 mg/kg BW nicotinamide and 65 mg/kg BW streptozotocin injections. Twenty-one obesity and T2DM rats were randomly assigned to negative control (n = 3) and the remaining rats in the MES1-3 groups, which were given 250, 500, and 1000 mg/kg BW/day MES. Data of BW, BMI, FBG, and triglyceride levels were collected at day 1, 14, and 28 interventions. Data were statistically analyzed using parametric and non-parametric tests with p < 0.05 considered significant.

RESULTS: The MES3 group (282.56 ± 10.75 g) had significantly lower mean BW than the MES2 group (331.33 ± 13.17 g, p = 0.035). The duration of MES administration significantly decreased BW (p = 0.009) and BMI (p = 0.034) compared with the negative control. The mean triglyceride levels in MES1 (93.72 ± 53.69 mg/dl, p = 0.020), MES2 (71.98 ± 35.72 mg/dl, p = 0.025), and MES3 (56.68 ± 16.37 mg/dl, p = 0.020) groups significantly lower than the control group (1042.13 ± 681.74 mg/dl) on day 14. The mean FBG levels in MES1 (437.85 ± 33.04 mg/dl) and MES2 (353 ± 33.04 mg/dl) groups were also lower than the control group (470.97 ± 33.04 mg/dl).

CONCLUSION: Administrations of 250, 500, and 1000 mg/kg BW/day MES decrease BW, BMI, and triglyceride level but increase FBG level in male rats with obesity and T2DM for 14 and 28 days.

Introduction

The prevalence of obesity and Type 2 Diabetes Mellitus (T2DM) has been rising in the last decades. Between 1975 and 2016, the global prevalence of obesity nearly tripled. Overweight and obesity prevalence has increased dramatically among children and adolescents aged 5 to 19 years, rising from 4% in 1975 to nearly 18% in 2016. In 2019, some countries in the Asia region had nearly half of all children under the age of 5 who were overweight or obese. Overweight adults who aged 18 and more accounted for 39% of cases, 13% among them were obese. In the majority of the world population, overweight and obesity commonly cause premature death in adult people [1]. Almost 60% and 25% people in 34 out of 36 countries from Organization for Economic Co-operation and Development are overweight and obesity, respectively [2].

Obesity also increases the risk of non-communicable diseases such as diabetes. Diabetes prevalence increased among adults over 18-year-old from 4.7% in 1980 to 8.5% in 2014 [3]. Three of four diabetic adults live in low- and middle-income countries [4]. In a meta-analysis study, obese group has 4.1 higher risk of T2DM development, compared with lean group [5]. Diabetes was the seventh leading cause of death in 2019, accounting for an estimated 1.5 million deaths [3] and it is expected to increase by 6.7 million people in 2021 (1 every 5 s) [4]. Fat accumulation in the adipocytes induces inflammation through increase of pro-inflammatory cytokines such as tumor necrosis factor α and interleukin 6, which contributes significantly to insulin resistance and T2DM progression [6].

The sodium-glucose co-transporter 2 (SGLT2) is a glycoprotein that is highly expressed in the proximal renal tubules. In these renal cells, the SGLT1 protein

is also expressed but it has a low capacity to reabsorb glucose (10%), compared to the SGLT2 protein (90%) [7]. In patients with T2DM, the SGLT2 expression is higher than that of healthy people, leading to increase of hyperglycemia [8]. Therefore, administration of SGLT2 inhibitor (SGLT2i) is expected to lower plasma glucose levels and which results in glucosuria (calorie loss) by inhibition of glucose and sodium reabsorption in the kidneys. In addition, T2DM patients who took SGLT2i, have reduction of body weight (BW) and visceral adiposity [9], [10], [11]. Dapagliflozin, for instance, is the first generation of SGLT2i [12] that is able to lower plasma leptin levels and BW, and to increase lipolysis in white adipose tissues in rats with T2DM [13], [14]. However, long-term uses of this drug can cause genital infections, ketoacidosis, and dehydration [13], [15]. Therefore, it requires alternative therapies in other to inhibit SGLT2 with minimal side effects.

Averrhoa carambola L. or star fruit is a family member of *Oxalidaceae*, which has a peculiar shape. This plant is easily found in India, Malaysia, Indonesia, China, Philippines, and Brazil [16]. Star fruits contain 132 phytochemicals including flavonoids, terpenes, phenylpropanoids, and glycosides, which have antioxidant activity and modulate the gut microbiota [17], [18]. Hosoi *et al.* reported that star fruits extracted with ethyl acetate contained epicatechin, but not Epicatechin Gallate [19]. Meanwhile, the ECG compound is widely found in green tea, grapes, strawberries, and buckwheat [20]. From an *in silico* study, the ECG molecule is able to interact with the SGLT2 protein at Asn⁷⁵, Gly⁷⁹, and His⁸⁰ residues as same as dapagliflozin but has lower binding affinity than dapagliflozin [21]. To extract ECG compounds from any parts of those medicine plants, some polar and semi polar solvents have been used. Methanol is the most effective solvent for ECG extraction of grape seeds [22]. Based on *in vitro* study conducted by Singapore scientists, it has reported that administration of 10, 100, 500, and 1000 mg/L ethanol extracts of star fruit peels for 8 days effectively suppress adipocyte differentiation in 3T3-L1 preadipocytes [23]. However, only few studies reported the effects of star fruit extract on BW, body mass index (BMI), fasting blood glucose (FBG), and triglyceride levels. Therefore, this research aimed to investigate the effects of methanol extract of star fruits (MES) on BW, BMI, FBG, and triglyceride levels in male rats with obesity and T2DM.

Methods

Extraction of star fruits

The extraction method of star fruits referred to our previous study [24]. In general, ripened star fruits were purchased from a farmer in Ngangkrang, Wonosari,

Bonang, Demak regency, Central Java Province, which were dried using an oven at 40°C for 36 h. Dried star fruits were macerated using a high-grade methanol solvent and the filtrate were evaporated in a vacuum evaporator at 45°C for 4 h. The methanol extract was stored at 4°C before further analysis. Phytochemicals content in star fruits are shown in Table 1. Star fruits extracts had 6.2 ± 0.6 mg L⁻¹ ECG concentrations.

Table 1: Chemical compositions in the methanol extract of star fruit

Compounds	Formula	Retention time (min)	MW
2,3-Butanediol, [S-(R*, R*)]-	C4H10O2	4.26	90
Glycerin	C3H8O3	7.55	92
4H-Pyran-4-one,	C6H8O4	10.12	144
2,3-dihydro-3,5-dihydroxy-6-methyl-5-Hydroxymethylfurfural	C6H6O3	11.14	126
D-Glucose, 6-O- α -D-galactopyranosyl-	C12H22O11	12.13	342
Sucrose	C12H22O11	13.28	342
n-Hexadecanoic acid	C16H32O2	19.01	256
4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-	C6H6O4	13.13	142
Epicatechin gallate	C22H18O10	28.33	442

MW: Molecular Weight.

Research design

This randomized controlled trial study used the pre-post-tests group design. Sample size was calculated using the comparison repeated-measures analysis of variance (ANOVA) design and resource equation approach [25]. This study compared five groups: normal diet, negative control, and treatment groups (MES1-3) with three repeated measurements (before, during, and after interventions). Therefore, we got three rats in normal and negative control groups while the MES groups consisted of six rats per group. Twenty-four rats were randomly distributed into two groups: normal (n = 3) and HFD diets (n = 21). Once rats became obese in the HFD treatment, the rats were randomly divided into four groups: Three rats for negative control and the remaining rats into MES1, 2, and 3 groups (Figure 1).

Generating rat models with obesity and T2DM

This study used male Sprague-Dawley rats (*Rattus norvegicus*), which aged 3 months old and had 250–400g BW. Four rat groups were adapted in a hygienic polypropylene cage for 1 week by controlling light condition (12 h/day) and 22°C ± 0.5°C temperature. The normal group received a standard food containing 66.88% carbohydrate, 14.19% lipids, and 18.93% protein, providing 3.17 kcal/kg food (normo-caloric). The HFD group received a pelleted food, which was adopted from a previous study with some modification (Table S1) [26] and contained 30.45% carbohydrate, 59.08% lipids, and 10.47% protein, providing 3.58 kcal/kg for 5 weeks. After reaching obesity with Lee's index (>300 mg^{1/3}/cm), rats in the HFD group were intraperitoneally injected with 230 mg/kg BW nicotinamide (NA) and followed by second injection with a 65 mg/kg BW streptozotocin (STZ) with the same route [27], [28].

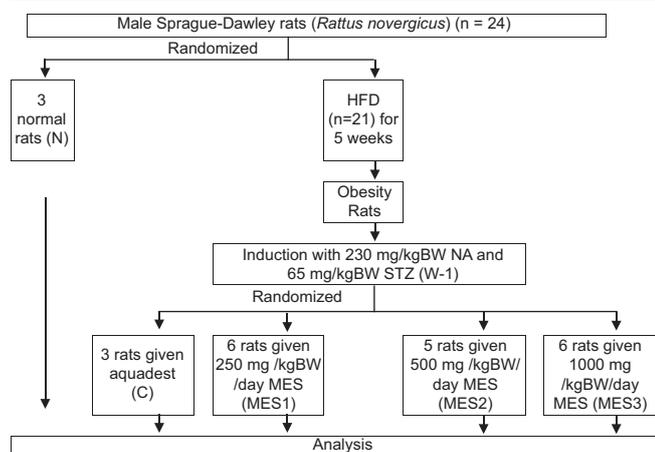


Figure 1: Schematic of research design of the effects of star fruit extract on BW, BMI, FBG, and triglyceride levels in male rats with obesity and T2DM (N: Normal Diet; HFD: High-Fat Diet; C: Control; MES: Methanol Extract of Star fruit; BW: Body weight; BMI: Body mass index; FBG: Fasting blood glucose; T2DM: Type 2 Diabetes Mellitus)

Administration of MES in rats with obesity and T2DM

One week after injection with NA and STZ, FBG levels of rats in the HFD group were measured using the Glucose Oxidase Phenol Aminophenazone (GOD-PAP) method. Diabetes rats indicated hyperglycemia with FBG levels ≥ 150 mg/dL [29]. The rats in MES1-3 groups were orally administered with 250, 500, and 1000 mg/kg BW/day MES for 28 days.

Negative control and MES1-3 groups received HFD until the end of the intervention while water was provided *ad libitum*. All stages of the research experiments followed animal ethics and which the research protocol was approved by the Ethical Clearance Committee, Integrated Research and Testing Laboratories, Gadjah Mada University Yogyakarta with number 00018/04/LPPT/IV/2020.

Measurement of BW, BMI, FBG, and triglyceride levels

BW and length were monitored every week using an animal BW scale and standardized ruler respectively. BMI was calculated using the Lee's index formula: $(BW [g]^{1/3} / \text{Naso-anal length [cm]}) \times 103$. Whole blood was taken from orbitalis sinus at day 1, 14 and 28 interventions and further process to obtain rat's serum. FBG levels were processed using the GOD-PAP method and triglyceride levels used the Glycerol-3-Phosphate Oxidase-Para Amino Phenazone (GPO-PAP) method, which were spectrophotometrically read at 546 nm [30], [31], [32].

Data collection and statistical analysis

All collected data were presented as mean \pm standard deviation. Normality and homogeneity

data were examined using the Shapiro–Wilk and Levene's tests before statistical analysis. The mean differences before and after intervention of BW, BMI, FBG, and triglyceride were compared using Kruskal–Wallis and the Mann–Whitney *post hoc* tests. The repeated measures ANOVA and followed by LSD *post hoc* tests were used for comparison of BW and FBG. The Games Howell *post hoc* test was used for comparison of BMI while the Friedman followed by Wilcoxon *post hoc* tests was used to compare the average of triglyceride levels among groups with $p < 0.05$ as statistically significance.

Results

Administration of MES significantly reduced BW in rats with obesity and T2DM

We evaluated rat's BW on days 14 and 28 after MES treatment (Figure 2). The mean rat BW in the MES1 group significantly decreased from day 1 (307.50 ± 12.41 g) to days 14 (291.50 ± 13.46 g) and 28 (288.00 ± 12.73 g, $p = 0.046$). The lowest mean BW was found in the control group (280.13 ± 25.43 g), followed by MES3 (282.67 ± 16.75 g), MES1 (297.86 ± 14.76 g), and MES2 (310 ± 28.66 g) groups. On the day 28 interventions, mean rat BW in the MES2 and 3 groups increased (Figure 2a).

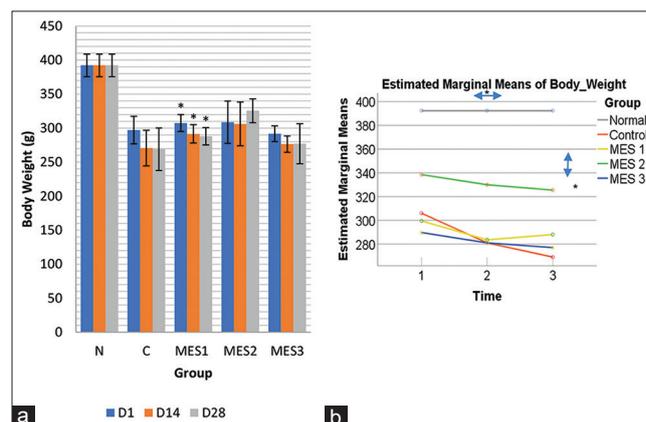


Figure 2: The effect of MES administration on BW in male rats with obesity and T2DM. (a) BW measurements were taken on days 1, 14, and 28 of intervention and were presented as mean \pm standard deviation. The rat BW differences among groups were compared using the Kruskal–Wallis and Mann–Whitney *post hoc* tests with $p < 0.05$. *a significant difference within groups. (b) Estimated marginal means for BW was determined using repeated-measures analysis of variance and the LSD *post hoc* test. The asterisk (*) on the horizontal or vertical arrows denoted within- or between-subject effects were significant. (N: normal; C: control; MES1-3: Administrations of 250; 500; and 1000 mg/kg BW MES; BW: Body weight; BMI: Body mass index; T2DM: Type 2 Diabetes Mellitus)

Further statistical analysis, Figure 2b indicated that the duration of MES administration had a significant effect on reduction of rat's BW ($p = 0.009$). Significant decreases of rat BW were observed in the

day 14 (293.88 ± 6.92 g) and 28 (289.88 ± 8.54 g) intervention, compared with the day 1 (308.42 ± 4.74 g) with $p = 0.003$ and $p = 0.021$, respectively. The mean rat BW in the MES3 group (282.56 ± 10.75 g) was significantly lower than that of in the MES2 group (331.33 ± 13.17 g, $p = 0.035$).

Administration of MES reduced BMI in rats with obesity and T2DM

In general, decreased mean rat BMI occurred in rats treated with MES (Figure 3a and b). On the day 28 interventions, the mean rat BMI in the MES1-3 groups decreased while the mean rat BMI in the C group increased. The MES3 group had the lowest mean rat BMI (287.03 ± 10.17 mg^{1/3}/cm), followed by C (287.43 ± 13.4 mg^{1/3}/cm), MES1 (289.85 ± 6.21 mg^{1/3}/cm), and MES2 (291.13 ± 7.12 mg^{1/3}/cm) groups (Figure 3a).

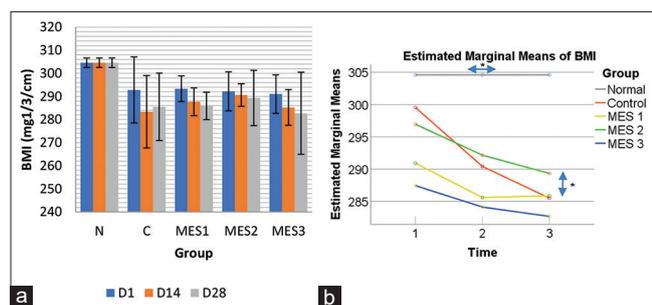


Figure 3: The effect of MES administration on BMI in male rats with obesity and T2DM. (a) BMI was measured on days 1, 14, and 28 of intervention and was presented as mean \pm standard deviation. The differences of mean rat's BMI among groups were analyzed using the Kruskal–Wallis and Mann–Whitney post hoc tests with $p < 0.05$. (b) Estimated marginal means for BMI was determined using repeated-measures analysis of variance and the Games Howell post hoc test. The asterisk (*) on the horizontal or vertical arrows denoted within- or between-subject effects were significant. BW: Body weight; BMI: Body mass index; T2DM: Type 2 Diabetes Mellitus

The duration of MES administration significantly influenced mean rat BMI ($p = 0.034$). Significantly decreased mean BMI (5.64 ± 0.63 mg^{1/3}/cm) occurred on the day 14 intervention (288.08 ± 3.30 mg^{1/3}/cm), compared with the day 1 (293.72 ± 3.09 mg^{1/3}/cm, $p < 0.001$). The mean difference of rat BMI in the MES1 (287.47 ± 7.37 mg^{1/3}/cm) and 2 (292.82 ± 7.37 mg^{1/3}/cm) groups was lower than that of in the C group (291.83 ± 7.37 mg^{1/3}/cm) (Figure 3b).

Administration of MES increased FBG levels in rats with obesity and T2DM

The mean FBG levels in C and MES groups after interventions increased compared with the mean FBG levels before intervention. However, FBG levels of the same groups in the C, MES1 and three groups decreased in the day 28 compared with the day 14 intervention. The MES2 group had the lowest FBG levels (398.04 ± 122.86 mg/dl), followed by MES1 (448.16 ± 66.56 mg/dl), control (479.56 ± 55.71 mg/dl), and MES3 (488.38 ± 48.13 mg/dl) (Figure 4a).

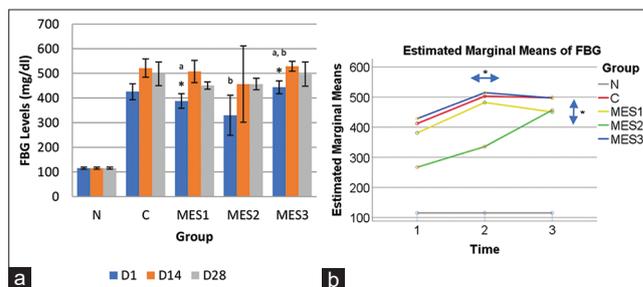


Figure 4: The effect of MES administration on FBG levels in male rats with obesity and T2DM. (a) FBG levels were measured on days 1, 14, and 28 of treatment and were shown as mean \pm standard deviation. The difference in rat FBG levels was determined using the Kruskal–Wallis and Mann–Whitney post hoc tests with $p < 0.05$. *a significant difference within groups. ^a $p < 0.05$, comparisons of MES1 and MES3. ^b $p < 0.05$, comparisons of MES2 and MES3. (b) Estimated marginal means for FBG levels was determined using repeated-measures ANOVA and the LSD post-hoc test. The asterisk (*) on the horizontal or vertical arrows denoted within- or between-subject effects were significant. BMI: Body mass index; FBG: Fasting blood glucose; T2DM: Type 2 Diabetes Mellitus

After statistical analysis with the repeated measure ANOVA, the duration of MES administration significantly increased FBG levels ($p = 0.016$). A significant increase of FBG levels was observed in the days 14 (458.73 ± 34.24 mg/dl, $p = 0.009$) and 28 interventions (475.52 ± 13.46 mg/dl, $p = 0.016$) compared with the day 1 (372.10 ± 18.96 mg/dl). However, the mean FBG levels in MES1 (437.85 ± 33.04 mg/dl) and MES2 (353 ± 33.04 mg/dl) groups were lower than the C group (470.97 ± 33.04 mg/dl). The MES2 group had lower FBG levels than the MES3 group (479.99 ± 26.98 mg/dl, $p = 0.031$) (Figure 4b).

Administration of MES increased triglyceride levels in rats with obesity and T2DM

On the day 14 intervention, triglyceride levels in the C group significantly increased ($p = 0.031$) compared to the N group. However, the mean triglyceride levels in MES1 (93.72 ± 53.69 mg/dl, $p = 0.020$), MES2 (71.98 ± 35.72 mg/dl, $p = 0.025$), and MES3 (56.68 ± 16.37 mg/dl, $p = 0.020$) groups were significantly lower than the mean triglyceride levels in the C group (1042.13 ± 681.74 mg/dl) on the day 14 intervention (Figure 5a).

After statistical analysis with the Friedman test, MES administration increased triglyceride levels ($p = 0.016$). A significant increase of triglyceride levels was observed in the day 28 intervention, compared with the day 1 intervention ($p = 0.008$) (Figure 5b).

Discussion

In this study, we have documented the effects of MES administration on BW, BMI, and FBG

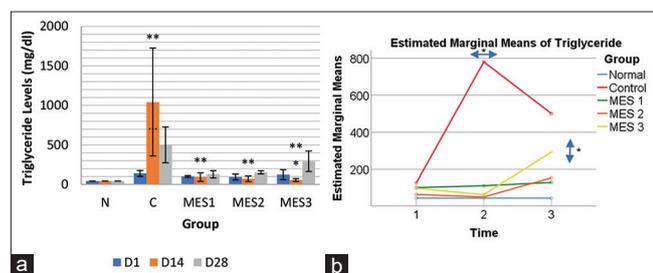


Figure 5: The effect of MES administration on triglyceride levels in male rats with obesity and T2DM. (a) Triglyceride levels were measured on days 1, 14, and 28 of treatment and were shown as mean \pm standard deviation. The differences in rat triglyceride levels were determined using the Kruskal–Wallis and Mann–Whitney post hoc tests with $p < 0.05$. *a significant difference within groups. ** $p < 0.05$ when compared to the control. (b) Estimated marginal means for triglyceride levels was determined by Friedman followed by Wilcoxon post hoc test. The asterisk (*) on the horizontal or vertical arrows denote if within- or between-subject effects were significant. T2DM: Type 2 Diabetes Mellitus

and triglyceride levels of male rats with obesity and T2DM. MES administration significantly decreased the mean BW and BMI. Our findings are consistent with a previous study that male obese mice which were treated with 240 mg/kg BW green tea diet (11.69 mg ECG) for 4 weeks induced weight loss [33]. Catechins in green tea have been shown to inhibit BW gain and adipose tissue formation by modification of appetite, decrease of nutrient absorption, increase of energy expenditure and utilization of fat oxidation, protein deterioration, and muscle wasting [34], [35]. The catechin compound may also reduce BW and food intake by reduction of leptin resistance, appetite, and calorie consumption. In obesity, fat accumulation causes oxidative stress, which disrupts insulin signals and reduces insulin-stimulating glucose uptake [36]. Pham *et al.* found that administration of metformin and *A. carambola* juice for 21 days induced BW loss in diabetic mice. *A. carambola* juice has beneficial effects by inhibiting stress oxidative and enhancing endogenous anti-oxidants [37]. Therefore, this study suggests that MES administration has protective effects, leading to an improvement in health outcomes.

Our second findings are that duration of MES administration significantly decreased mean BMI. Aladaileh *et al.* study revealed that administration of 1.000 mg/Kg BW methanolic extract of *A. carambola* leaves for 5 weeks decreased the BMI and hyperlipidemia in HFD fed rats [38]. From a human study, healthy Japanese men who were given one bottle of green tea extract (690 mg catechins) for 12 weeks had reduction of their BW and BMI. Overall, these findings suggest that catechins increase energy expenditure and decrease body fat through inactivation of catechol-*O*-methyltransferase, resulting in increases of thermogenesis and body fat loss [39].

Surprisingly, the mean FBG levels increased all MES1-3 groups after 28 days intervention,

compared to the 1st day intervention. However, MES1 and 2 groups had the mean FBG level lower than the C and MES3 groups. These results are different from a recent study that administration of 100, 200, and 400 mg/kg BW green tea for 3 weeks decreased FBG, LDL-C, cholesterol, triglyceride, and insulin resistance in T2DM rats [40]. Catechins in green tea can inhibit carbohydrate absorption through intestinal sucrose, alpha-glucosidase, and alpha-amylase. Catechins may also inhibit hepatic gluconeogenesis by up regulating gluconeogenic gene expression and protein-tyrosine phosphorylation in the liver [41]. We speculate that catechin and its derivatives in our extract might be not enough to modulate carbohydrate metabolism.

In contrast to FBG levels, triglyceride levels were significantly lower in the MES1-3 groups during intervention, compared to the control group. However, the triglyceride levels tend to increase in the MES2&3 groups in the end of intervention. These findings are in accordance with Pang's study that db/db mice were treated with *A. carambola* free phenolic extract (10, 20, and 30 g/kgBW) for 8 weeks reduced triglyceride levels. *A. carambola* extract inhibited lipid synthesis, effectively decreased blood lipids and ameliorated liver steatosis. The possible mechanisms are through downregulated the expression levels of miRNA-33 and microRNA-34a, which modulate the phosphorylation of AMPK α (a catalytic subunit of AMPK) that suppresses the expressions of SREBP-1c, SCD1, and FAS signaling [42].

We have some limitations of our study, which cause inconsistency data of BMI, FBG, and triglyceride levels. Perhaps, reduction of mean BW and BMI is not only caused by MES administration but also by T2DM induction with STZ and NA compounds. MES fruits contain low ECG level, which is unable to inhibit carbohydrate and lipid metabolisms as the green tea extract does. MES also does not contain other derivatives of catechin, which may have synergetic effects with the ECG compound.

Conclusion

Administrations of 250, 500, and 1000 mg/Kg BW/day MES decrease BW, BMI, and triglyceride level but increase FBG level in male rats with obesity and T2DM for 14 and 28 days. More research studies are needed to produce methanol extract from other parts of star fruit plant, which have more content of ECG, to measure catechins and its derivatives in star fruit extract and to figure out the action mechanism of MES administration in reduction of BW and triglyceride levels. Pure ECG and SGLT2 inhibitor compounds are necessary in the next research setting in order to provide further evidence whether or

not MES fruits are more effective in inhibition of glucose and lipid metabolisms than those two compounds.

The authors declare no conflict of interest.

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Supplement Table

Table S1: Dietary formulations and energy distribution

Ingredients	ND			HFD		
	g/kg	kcal/kg	%	g/kg	kcal/kg	%
A.D. II (complete feed, normal pellet diet)						
Carbohydrate	530	2120	66.88	254.4	1017.60	28.44
Protein	150	600	18.93	72	288.00	8.05
Fat	50	450	14.19	24	216.00	6.04
Fiber	60			28.8		
Calcium	11			5.28		
Phosphorus	9			4.32		
Ash	70			33.6		
Water	120			57.6		
Vitamins and mineral mix				60		
DL-Methionine				3		
Yeast powder				1		
Sodium chloride				1		
Fish flour				245	324.76	9.08
Beef tallow				189	1542.24	43.11
Soybean oil				21	189.00	5.28
Total	1000	3170	100.00	1000	3577.60	100.00
Content analysis						
Carbohydrate	530	2120	66.88	272.4	1089.5	30.45
Protein	150	600	18.93	93.7	374.7	10.47
Fat	50	450	14.19	234.8	2113.5	59.08
Total		3170	100.00		3577.6	100.00

ND: Normal diet, HFD: High-fat diet.