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| RESEARCH ARTICLE

Male Sprague Dawley Rats with Type 2 Diabetes Mellitus and Obesity on Body Weight, Body Mass Index, and Fat Content as Affected by Alkaloid Fraction of *Litsea Glutinosa Leaves* (AFLG)

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| ABSTRACT
There were 463 million people living with diabetes around the world, and it is expected to increase by 48% in 2045. Dipeptidyl Peptidase 4 (DPP4) *inhibitor* is asecond-line anti-diabetic drug, that frequently causes weight gain for long term treatment. Alkaloid fraction of *L. glutinosa* leaves (AFLG) is able to inhibit DPP4 activity in diabetic rats. Therefore, this study aimed to evaluate the effect of AFLG on body weight (BW), BMI, and fat percentage in male rats with model type 2 diabetes mellitus (T2DM) and obesity. Male Sprague Dawley rats, which weighed 200-250 g, were used in this experimental study. Twelve rats were fed with a high-fat diet for 30 days and then were intraperitoneally injected with 230 and 65 mg/kg BW nicotinamide and streptozotocin, respectively. Those rats were randomly divided into 4 groups, control (C) and three treatment (T1-T3) groups, which were orally given 20, 40, and 80 mg/kg BW/day AFLG for 28 days. Collected data were analyzed using *a paired t-test,* the one-way ANOVA test. Significant differences in BW in T2 and T3 ($p = 0.001$), Rohrer index in T2 ($p = 0.001$) and fat content in C ($p = 0.013$) were observed between before and after treatments. The average of BW, Lee, Rohrer, and TM indexes and fat content in treatment groups did not significantly differ from the C group after 28 days of treatment ($p > 0.005$). In conclusion, Alkaloid fraction of *L. glutinosa* leaves does not influence body weight, nutrition status, or fat content of male rats' models of T2DM and obesity.

| KEYWORDS

Type 2 diabetes mellitus and obesity, *Litsea glutinosa* leaves, Sprague Dawley rat, Body mass index.

| ARTICLE INFORMATION

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1. Introduction

Given the rapidly increasing nature of the obesity epidemic, much research has focused on lifestyle and pharmaceutical interventions [Asyifa, 2021]. Weight loss remains the most effective approach to obesity and reduces the risk of associated diseases; However, weight loss can be difficult to achieve and maintain [Botchlett, 2018]. Based on the 2018 Basic Health Research, the prevalence of obesity in adults was 21.8% from 14.8% in 2013 [Kemenkes, 2018].

The biggest factor leading to overweight and the development of obesity is overnutrition. Changes in eating patterns that contain high carbohydrates, fat, and protein, as well as a decrease in physical activity in the form of work and excessive mobility, can ultimately lead to fat synthesis and accumulation [Dludla, 2018].

These conditions can cause various health problems, one of which is Type 2 Diabetes Mellitus (T2DM) [Fruh, 2017]. T2DM, a non-insulin dependent form of DM, accounts for more than 80% of the total number of diabetes cases and is often accompanied by obesity [Raman, 2012]. The incidence of obesity and T2DM which is characterized by excessive fat accumulation in the body causes changes in body composition and metabolism, such as endothelial dysfunction. Measurement of body composition is based on atomic, molecular, cellular, organ and tissue components, as well as water, protein, and fat content [Van-Loan, 2005].

Dipeptiydl Peptidase - 4 (DPP4) Inhibitor is a second line treatment of T2DM [Okur, 2018]. An alternative to DPP-4 inhibitor from Indonesian plants, is litsea glutinosa leaves [Sukma, 2018]. From in vitro and in silico tests, actinodaphine in litsea glutinosa leaves has an effect similar to that of the DPP4 inhibitor [Nityasewaka, 2015] and administration of Alkaloid fraction of L. glutinosa leaves (AFLG) at a dose of 80 mg/kgBW/day in a diabetic rat model can decrease DPP-4 enzyme activity, increase glucagob-like peptide-1 and lower fasting blood sugar [Kisnawaty, 2019]. The administration of AGLF was more effective than the administration of sitagliptin for 14 days. However, the increase in body weight of T2DM rats given AGLF was higher than sitagliptin 1.8 mg/KgBW/day for 14 days. Therefore, the purpose of this study was to determine the administration of AGLF on body weight, body max index and fat content in Male Sprague Dawley Rats with T2DM and Obesity.

2. Literature Review

The increasing prevalence of T2DM is influenced by a combination of genetic factors related to impaired insulin secretion, insulin resistance, and environmental factors such as obesity, unhealthy diet, physical activity, and patient quality of life [Sami, 2017].

In obese individuals, pancreatic β-cells cannot compensate for decreased insulin sensitivity. *Non-Esterified Fatty Acids* (NEFAs) induce insulin resistance and impair pancreatic β-cell function [13]. The accumulation of excess fat tissue due to obesity modulates metabolism by releasing NEFAs and *glycerol*, hormones (leptin and adiponectin), and proinflammatory cytokines. *Retinol-binding protein-4* (RBP4) induces insulin resistance through decreasing *phosphatidylinositol-3-OH kinase* (PI(3)K) signaling in muscle and increasing the expression of the glucogenic enzyme *phosphoenolpyrucatecarboxykinase* in the liver through a *retinol-dependent* mechanism. Conversely, adiponectin acts as an insulin sensitizer by stimulating fatty acid oxidation. Increased secretion of *tumor necrosis factor-α* (TNF-α), *interleukin-6* (IL-6), *monocyte chemoattractant factor-1* (MCP-1), macrophage adducts, and other cells found in fat tissue also have a role in the development of insulin resistance [Kahn, 2006].

Mice can be used as animals to study human biological conditions because mice and humans have genetic and physiological similarities (Perlman, 2016). A commonly used experimental animal in scientific research is the rat. Rats (*Rattus norvegicus*) have perfectly known properties, are easy to maintain, and are relatively healthy animals suitable for various studies. The morphological characteristics of Rattus norvegicus include a weight of 150–600 grams, a blunt nose, a large body with a length of 18–25 cm, a head and body shorter than the tail, relatively small ears, and no more than 20–23 mm [Kemenkes, 2018].

The rats used in the study were male Sprague Dawley strains aged 2 months. Female Sprague Dawley rats were not used because of the highly fluctuating hormonal conditions at the onset of adulthood, so it is feared that they will give different responses and may affect the results of the study [International Diabetes Federation. 2019]. The Sprague-Dawley (SD) rat line was developed from the Wistar rat line. This strain comes from the Sprague-Dawley farm, Madison, Wisconsin. The characteristics of the SD strain are a long body with a narrower head, thick and short ears, and fine hair. The eyes of white rats are red, and the most visible feature is the tail that is longer than the body. Rats have a life span of 4-5 years, with a general body weight of males ranging from 267-500 grams and females 225-325 grams [Kisnawaty, 2019]. This breed has rapid growth, a good temperament, and high lactation ability [Carere, 2013]. The mice were housed in light and temperature-controlled cages, and their health was monitored daily by the laboratory assistant [Alexandru, 2011].

Obesity in rats is defined as an increase in body weight or energy content in rats above normal, assuming that all control animals maintained in the laboratory are in normal condition. One of the parameters used is the BMI; the normal value of rats ranges from 0.45 to 0.68 g/cm2. Calculation of BMI in rats using the formula of the ratio of body weight (g) to the square of the length of the rat (cm2). The length of the rat is calculated from the nose to the anus [Shiyan, 2017]. Making obese model rats were given a high-fat diet with a composition of 45% fat (beef fat 39.4% and soybean oil 5.6%), 20% protein, and 35% carbohydrates for 2 weeks [Zhao, 2012]. Furthermore, the rat model was induced by streptozotocin (STZ), according to Tripathi and Verma [20]. STZ is a natural chemical that can be used to produce T2DM in experimental animals at low doses or in combination with other chemicals. According to Ghasim et al. [2014], the induction of experimental animals using a combination of STZ and Nicotinamide (NA) shows that the T2DM disease model is more favorable because it can test the potential pharmacological antidiabetic effects and the effects of natural compounds. The T2DM rat model with STZ and NA induction is based on the protective effect of NA against the cytotoxic effects of STZ. Administration of NA before STZ has an effect on pancreatic β-cells that can reduce DNA methylation. NA

is dissolved in normal saline and administered intraperitoneally. Besides that, NA also has a role in reducing ROS production in pancreatic β cells, which is known to increase due to the presence of STZ and low protection from antioxidants [Kishore, 2017].

The recommended dose of STZ for the manufacture of DMT2 animal models is 60 mg/kgBW (Radenkovic, 2016). Furthermore, according to Ghami et al. [2014], the optimal STZ dose recommended for the manufacture of T2DM experimental animals is 55–65 mg/kgBW. NA induction of 230 mg/kgBW and STZ induction of 65 mg/kgBW can cause rats to experience hyperglycemia with blood glucose levels > 150 mg/dL starting from 3 days after induction until day 35 [Palupi, 2023].

3. Methodology

3.1. Preparation on AFLG.

Litsea Glutinosa leaves were obtained from farmers at the Research Center for Medicinal Plant Development (BPTO) of Tawangmangu, Karanganyar City and extracted using the Voight (1994) soxhletation method with 96% ethanol as solvent. Furthermore, the alkaloid fractionation process was carried out using hexane, 25% NH4OH and chloroform.

3.2 Generation of Animal Model with T2DM and Obesity

Male Sprague Dawley rats, which weighed 200-300 g, were used in this experimental study. Each Sprague Dawley rat from 12 selected rats was adapted for 7 days in a cage made of stainless steal measuring 20 cm long, 30 cm wide and 17 cm high. Drinking water is provided ad libitum. The humidity in the room is between 70-75%, while the temperature is between 25-280C. Light reception is set to 12 hours of light and 12 hours of darkness. The following day, sprague dawley rats were fed a high-fat diet for 30 days and on day 31 were injected with a single dose of nicotinamide 230 mg/KgBW. Fiften minutes later sprague dawley rats were injected streptozotocin 65 mg/KgBW intraperitoneally. All stages of research experiments followed animal ethics and the research protocol got permission from the Health Research Ethics Committee, Integrated Research And Testing Laboratory, Gadjah Mada University with number 00019/04/LPPT/VII/2020.

3.3 Experimental Design

Once sprague dawley rats have become T2DM and Obesity, 12 rats were randomly divided in four groups. 4 groups, control (C) and three treatment (T1-T3) groups were orally administered given 20, 40 and 80 mg/KgBW/day AFLG for 28 days. BW of sprague dawley T2DM and obesity rats were recorded before, during and after intervention.

3.4 Statistical Analysis

All collected data were presented in mean ± standard deviation. Before running statistical analysis, homogeneity and normality data of BW, body max index and fat content analyzed using *paired t-test* and *one way ANOVA*.

4. Results and Discussion

The measurement results for each group are shown in Tables 4.1 and 4.2. The average BW on day 0 for groups C, T1, T2 and T3 were 305.67, respectively; 316.67; 305.67 and 314.00. The results of the ANOVA test showed that there was no difference in the average BW on day 0 between groups (p=0.950). The average BW of rats on day 28 was 188.67; 194.00; 288.00 and 294.67. The four groups experienced weight loss after the intervention, but there was no difference in the mean BW on day 28 between groups (p=0.939). In group C, this can be caused by the effect of insulin in the metabolism of sugar into cells, which is not perfect so blood sugar remains high. These conditions can be toxic and cause a feeling of weakness and unwellness and cause complications and other metabolic disorders. If the body is not able to get enough energy from sugar, the body will process other substances to be converted into energy such as fat. The use or destruction of fat and protein causes a decrease in BW [Indriyani, 2016].

Table 4.1 Average Body Weight, Body Mass Index (Lee and Rohrer)

Group	Body Weight			Body Mass Index					
				Lee			Rohrer		
	Day 0	Day 28	p[a]	Day 0	Day 28	p[a]	Day 0	Day 28	p[a]
C	305,67 ± 11,59	188.67 ± 164,73	0,330	299,47±9,12	188,66±164,73	0,346	26,76±2,51	17,03±14,82	0,323
T-1	316,67 ± 29,36	194,00± 169,57	0,371	304,96±7,05	193,21±167,53	0,378	28,39±1,94	16,27±14,24	0,308
T-2	305,67±16,92	288,00±16,70	0,001*	299,02±7,83	286,84±11,18	0,111	26,77±2,12	23,67±2,77	0,000*
T-3	314,00±11,53	294,67±10,50	0,001*	299,50±8,22	287,28±7,29	0,062	26,90±2,23	23,74±1,81	0,061
P[b]	0,950	0,939		0,901	0,828		0,939	0,828	

description : [a] paired t – test, [b] one way anova test, * significant (p<0,05)

Table 4.2. Average Body Mass Index (TM) and Fat Content

Group	Body Mass Index			Fat Content		
	TM			Day 0	Day 28	p[a]
	Day 0	Day 28	p[a]			
C	46,42±4,22	29,57±25,73	0,326	4,94 ± 2,45	2,49 ± 2,61	0,013*
T-1	49,20±3,44	28,30±24,78	0,311	6,55 ±1,99	3,38 ± 2,94	0,360
T-2	46,45±3,46	41,22±4,62	0,094	4,95 ± 2,00	1,92 ± 2,68	0,095
T-3	46,75±3,70	41,40±3,07	0,058	5,13 ± 2,15	2,02 ± 1,78	0,058
P[b]	0,945	0,838		0,945	0,369	

description : [a] paired t – test, [b] one way anova test, * significant (p<0,05)

The results of the paired t-test on BW T2 and T3 experienced significant weight loss after being given AFLG. At T2 orally given 40 mg/KgBW/day AFLG (p = 0.001) and T3 orally given 80 mg/KgBW/day AFLG (p = 0.001), this is why there is an increase in the decrease in BW through an increase in the satiety hormone secreted by L -cells in the gastrointestinal tract are GLP-1. This hormone also has secondary production in the central nervous system, in the nucleus tractus solitarius. GLP-1 functions to reduce appetite by influencing the vagus afferent nerves that reach the NTS and reducing AMPK activity in the hypothalamus. In the gastrointestinal system, GLP-1 slows down intestinal motility thereby reducing the absorption of carbohydrates and glucose. Actinodaphine whose action steps are similar to sitagliptin functions to increase the amount of GLP-1 in the body by inhibiting dipeptidyl peptidase-IV (DPP-IV), an enzyme that degrades GLP-1 [Drucker, 2007].

We evaluated the weight of rats at day 28 after AFLG treatment. In general, the average weight loss occurred in rats treated with AFLG at a dose of 40 mg/KgBW/day and a dose of 80 mg/KgBW/day (Table 4.1). Compared to group C, a significantly higher mean BW was found in treatment groups T1 and T2. The mean weight of rats in group T2 was significantly higher than the mean weight of rats in group C (p=0.001). From our results, it is clear that administration of AFLG decreases the satiety response in the metabolism of T2DM rats. Our findings are in agreement with the findings of Shiyan et al. study that diabetic rats treated with.

Compounds with high phytochemical content such as flavonoids, tannins and saponins which averaged between groups experienced a decrease in BW, a decrease in lee index and a decrease in fat content even though we used different models to generate a diabetic rats model. We used 230 mg/kg BW of NA and 65 mg/kg of STZ to induce T2DM while in Qin made with high dose STZ to produce T2DM. Therefore, the decrease in BW of rats in this study demonstrated a protective effect, leading to an increase in the control of food intake of patients with T2DM [Shiyan, 2017].

In this study, the decrease in BW in rats treated with T2 and T3 (AFLG at a dose of 40 mg/KgBW/day and a dose of 80 mg/KgBW/day) was higher than the increase in BW in groups C and T1. This implies that AFLG has a better effect in the treatment of T2DM because Litsea Glutinosa leaves contain actinodapine as a DPP4 inhibitor [Krishnamurthy, 2016].

5. Conclusion

In conclusion, there was no significant difference in BW, Body Max Index, and fat content of male rats' model T2DM and obesity. Even though there was a decrease in the T2 and T3 groups, further research needs to be carried out to confirm whether the weight loss occurred due to the administration of alkaloid fractionation from Litsea glutinosa leaves.

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