ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Effects of Soursop (*Annona muricata*) Leaf Water Extract (SLWE) on Body Weight, Leptin and TNF α Plasma Levels of Rats with High Fat and High Fructose (HFHF) Diet

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Abstract

Background: Soursop leaves believed to be able to lose weight. The aim of the study was to prove that SLWE had an effect on body weight, leptin and TNF α plasma level in mice with the HFHF diet. Methods: The study used post test group design only. Male Wistar rats aged 8-10 weeks weighing 175-200g, healthy, 25 were divided into 5 groups by simple random sampling. Normal groups (N) , Positive groups (P) were given a HFHF 10% diet, treatment group (T1), T2, and T3 (HFHF diet + SLWE at dose of 100 mg / kg bw, 200 mg / kg bw, and 400 mg / kg bw). The diet and extract were given for 10 weeks. Measurement of body weight and weighing the amount of food consumed were carried out every week. Measurement of plasma leptin and TNF α levels using the ELISA method. Statistical analysis using ANOVA and Kruskal Wallis test with a confidence level p <0.05.Results : SLWE at all doses increased the amount of intake compared to P and N groups (p <0.05).Giving SLWE at dose of 400 mg/kgbw causes a slowdown in weight gain (p> 0.05), increase levels of plasma leptin than P group. There was a tendency to decrease TNF α plasma levels with an increase in the dose of the extract (p>0.05). Conclusion: Giving SLWE in HFHF diet-induced mice increased appetite, increased Leptin levels, and tendency to decreased weight gain and TNF α levels.

Keywords: *Soursop leaf, Weight gain, Leptin, TNF α, High Fat-High Fructose Diet.*

Introduction

Obesity is a condition of increasing body weight due to an imbalance between food intake and energy expenditure, causing fat accumulation in adipose tissue, liver, muscle, islet langerhans and other organs involved in metabolic processes. Criteria for obesity in adults based on body mass index (BMI) which describes the amount of fat deposits in adipocyte tissue. Someone was obese if the BMI value is more 30 [1, 2].

The incidence of obesity is increasing. An increasing number of populations who are obese occur at the age level of children and adults, men and women, as well as middle and lower socioeconomic levels. At present almost 1/3 of the world population in adulthood is overweight.

In 2030 it is estimated that the number of adults who are overweight is around 38% and 20% of them are obese [2, 3]. Obesity increases the risk of diabetes mellitus, coronary heart disease, hyperlipidemia, hypertension, stroke, breast and colon malignancies, and osteoarthritis [4].

The high obesity morbidity and mortality causes a decrease in work productivity, quality of life and high medical costs that must be borne by the government [2].A high-fat diet in mice causes' obesity. The

mechanism of obesity is caused by leptin resistance, increased ghrelin, increased appetite, and fat accumulation in adipose tissue [5]. In addition to a high-fat diet, a high-fructose diet also induces obesity. Fructose has an obestogenic effect because it stimulates the center of hunger, increases appetite, and increases fat accumulation in adipocyte tissue [6, 7].

The regulation of hunger and satiety occurs in the short and long term. Short-term regulation of hunger and satiety is due to intestinal hormone secretions such as Glucagon Like Peptide-1 (GLP-1), Cholecystokinin (CCK), Neuro Peptide Y (NPY), and Ghrelin which regulates the response to hunger and satiety. The regulation of long-term hunger and satiety is more played by the Leptin [8].Obesity complications arise due to disintegration of intestinal epithelial cells. Some researchers say that high-fat and high-fructose diets cause dysbiosis in the intestine. Most phylum in mammalian intestine are Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria.

Bacteroidetes and Firmicutes are the two most dominant bacterial phylum. In obese mice found a decrease in the ratio of Bacteroidetes colonies to Firmicutes colonies [9].Intestinal micro biota plays a role in the metabolism of short chain fatty acids such as propionate, acetate, and butyrate.

Propoinonic acid as the substrate for the formation of cholesterol de novo in the liver. While acetat plays a role in reducing the production of cholesterol de novo in the liver. Thus the ratio of acetat and propionate play a role in lipid metabolism and cholesterol. Butirate is the main energy source for intestinal cells.

Butirate deficiency causes intestinal epithelial damage resulting in increased intestinal permeability [9]. The decrease in the number of commensal bacterial colonies will increase the number of bacterial colonies that are pathogenic, thereby increasing the expression of lipopolysacharides which induce inflammation.

Increased inflammatory mediators especially TNF α and IL-1ß cause a decrease in claudin-1 protein, damages tigh junction between intestinal cells, increases the production of free radicals in the intestine, and increases the amount of endotoxin which all damage the barrier system and intestinal cell integrity [10, 11]. This condition allows the spread of sistemic inflammation and induces inflammation in various organs such as blood vessels, liver, skeletal muscle, pancreas and adipocyte tissue [11]. The use of herbs as a traditional medicine has been widely used by people in the world to treat various diseases, especially chronic diseases. The community considers traditional medicine to be safer and cheaper than modern medicine, easy to obtain, and has been trusted by generations to treat diseases.

This condition causes the need for regulations on the use of traditional medicine so that it is safe, effective, efficient, rational and standardized [12, 13].One herb that is often used for treatment is soursop (*Annona muricata*) leaves. Soursop leaves have many active ingredients. The main active compound of soursop leaves is acetogenin [14]. Soursop leaves also contain phenols, alkaloids, terpenoids, flavonoids, coumarins, steroids, fatty acids, phlorotannins, tannins, and saponins [15]. As an herb, soursop leaves are consumed in the form of decoction or infusion [3].

Previous researchers proved that soursop leaves have an effect as anti-diabetic [16] antihypertensive [17], antioxidants [18], antibacterial [19], anti-hyperlipidemia [20], anti-inflammatory [21], anti-cancer [22], anti-obesity [23, 24], anti-depressants [25], and weight loss [26].Based on the facts above and the absence of a mechanism for soursop leaves to lose weight, the researchers were interested in finding the mechanism of the active ingredients contained in SLWE to work to lose weight in mice given a HFHF diet. The research has been approved by the Commission for Research Ethics Feasibility Medicine Faculty of Unversitas Brawijaya, Malang, Indonesia No.110 / EC / KEPK-S3 / 04/2018.

Material and Methods

Types and Design of Research

The study was a true experimental laboratory of study in animal experiments. The study design used a post test control group only.

Place and Time of Research

The study was conducted at the Biochemistry Laboratory of Medicine Faculty of Universitas

Islam Malang, Central Laboratory of Universitas Ma Chung, Malang, Central Laboratory of Universitas Negeri Malang, Inbio of Universitas Brawijaya, Malang and Parasitology Laboratory of Medicene Faculty of Universitas Brawijaya Malang. The study was conducted for 70 days starting August 1th, 2018 until October 10th, 2018.

Collection and Identification of Plant Materials

Soursop leaf samples were identified and originated from the Medika Batu Materia Center.

Identification of SLWE Active Compounds

Sample Preparation

Making SLWE using the infusion method. Soursop leaf powder is put in a pan and dissolved in water with a ratio of 1: 10, then heated over boiling water for 15 minutes. The temperature in the pan is maintained at 60◦C. Filtering the extract using Whatmann no 1. Filtrates that have been accommodated are then vacumed and evaporated at 60◦C to produce concentrated extracts. Concentrated extracts were carried out freez drying to obtain water extract in powder form [27].

Identification Active Compound Using GCMS method

SLWE in powder form is dissolved with aceton with a ratio of 1: 1 and filtering is carried out. The filtrate was identified as the active compound using GC-MS tools (Shimadzu 10).

Identification of active compounds based on the resting time and mass spectrum that are converted with the data base contained in Wiley8.Lib [28]. The higher the percentage of similarity, it is assumed that the active ingredients identified are close to the truth.

Table 1: GC-MS optimization

Collum Oven Temperature	**80°C**
Injection Temperature	250 ° C
Injection mode	Split
Flow control mode	Pressure
Total Flow	588.8 mL/min
Collum flow	1.46 mL/min
Linier velocity	45.5 cm/sec
Purge flow	3.0 mL/min
Split ratio	400
Oven temperature program Ratte / Temperature / Hold time (min) - / 80.0 / 1.00 10.00 / 250.0 / 1.00	
Equilibrium time	3.0 min

Materials and Methods

The animals tried using male Wistar rats, aged 8-10 weeks, weighing around 175-200 g, local breeding from the Pharmacology Laboratory of Medicine Faculty of Universitas Brawijaya, Malang. The selection and distribution of experimental animal groups was carried out by random sampling. 25 rats were divided randomly into 5 groups namely N: Normal group, P: Positive group given HFHF diet, T1: SLWE at dose of 100mg / kgbw + HFHF diet, KP2: SLWE at dose of 200mg / kgbw + HFHF diet, T3: SLWE at dose of 400mg / kgbw + HFHF diet. Each group consists of 5 rats. Mice were placed in cages and covered in wire mesh. Each cage consists of 1 rat. Room temperature 25◦C, lighting for 12 hours, good air circulation.

Replacement of husk was done every day. N group got standard laboratory food. The normal diet composition consists of PARS and wheat flour with a ratio of 200g PARS, 100g wheat flour and 81 cc water (for 10 rats). The amount of feed consumed was calculated based on the formula for the amount of food

consumed (g) = the weight of feed given (g) - the remaining feed per day (g). Drinking was given in ad libitum. To give the same stressor effect, KN also received 2 cc / day of distilled water for 70 days. P group and all treatment groups get HFHF diet. The composition of the HFHF diet consisted of 200g PARS, 100 g wheat flour, fructose 40g, 10 cc pork oil, 8g cholesterol, 0, 8g colic acid and 81 cc water (for 10 rats). The composition of this diet contains 2% cholesterol, 0.2% colic acid, and 5% pork oil, 10% fructose and 17% water.

The composition of a high-fat diet refers to research conducted by [29] and the addition of fructose by 10% is a modification of a high-fructose diet in humans around 55g / day or more than 10% of total calories [30]. Giving fructose was mixed in food so that mice continue to consume fructose. The diet was given for 70 days. To get the same stressor effect, both N and P group are given 2 cc / day aquadest by gastric sonde.

All treat men groups were given SLWE in the form of powder which has been dissolved in aquadest until the volume reaches 2 cc. The dose given to the T1 group was 100mg / kgbw per day, T2 was 200mg / kgbw per day, and T3 was 400mg / kgbw per day. The dosage determinations of extract were a modification of the research conducted by Yuniarti [23]. Weighing the weight and weighing the rest of the meal are done every week. Giving extract for 70 days along with giving a diet. At the end of the study, animals were sacrificed in anesthetized conditions using ketamine at a dose of 4 mg / kg body weight. Surgery through the incision at the medial line from above the blader to the diaphragm, and continued with the opening of the thorax. Taking blood through the left ventricular puncture was 3 cc and included in the vacuntener in which EDTA already exists.

Measurement of Leptin and TNF α Plasma Levels

Leptin and TNF α plasma levels were examined according to the procedure of the kit used (E0561 Ra Rat Leptin Elisa Kit 96T (BT Lab), E0764Ra Rat TNF α Elisa Kit 96T (BT Lab)). Reading the absorbance results using ELISA reader at a wavelength of 450nm for Leptin and TNF α.

Statistical Analysis

Data was carried out normality test and homogeneity test. Data on food intake, Leptin and TNF α plasma levels were analyzed using ONE WAY ANOVA followed by Duncan test $p < 0.05$. Data on weight gain were tested using the Kruskal Wallis, followed by the Mann Whitney Test, $p < 0.05$.Statistical analysis using the SPSS 20 program.

Results and Discussion

Active Compounds of SLWE

The results of identification of active compounds in SLWE using the aceton as solven are shown in Figure (1) and Table (2) below.

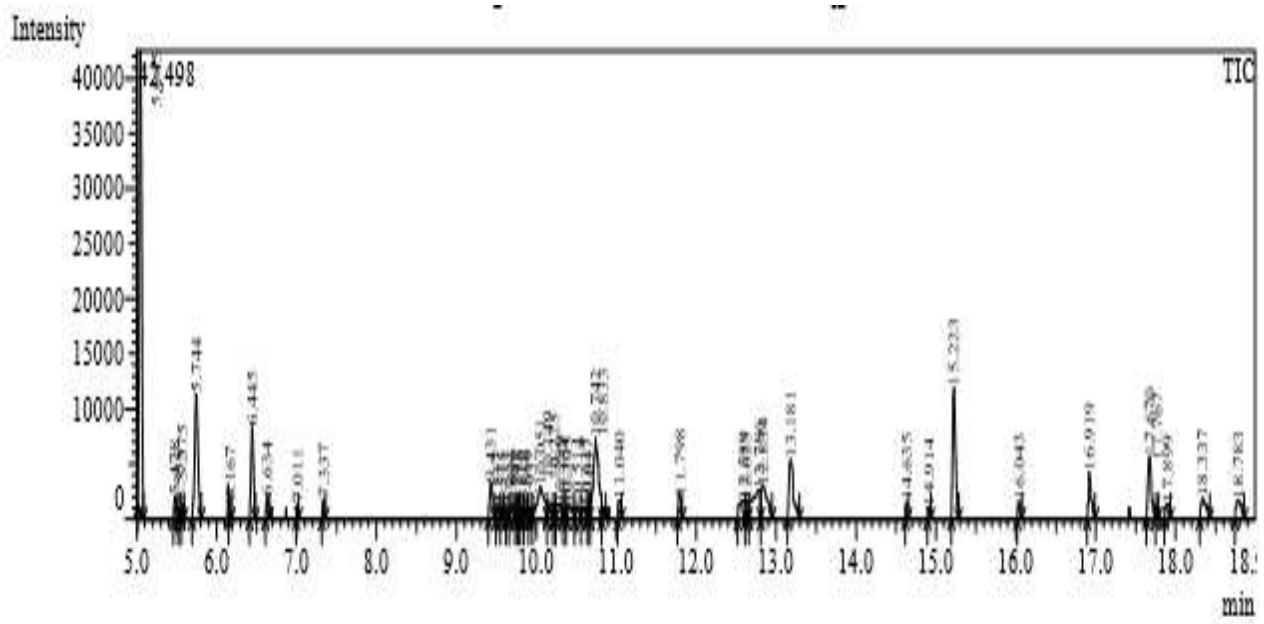

Figure 1: Identification of SLWE active compounds using the GCMS method

The results of identification of SLWE active compounds obtained 48 peaks. Of the 48 peaks there are 5 peaks with the highest area, namely peaks with resting time (RT) 5,045 (19.27%), RT 5.74 (7.40%), RT 10,742 (7.44%), RT 15,223 (6.66%), RT 10,051 (4.42%).

Table 2: Identification of active compounds in SLWE

Peak Number	RT	SI	MW	Name of active compound	Structure
1	5.045	94%	134	Benzene, 1,2,3,4-tetramethyl	C10H14 CAS: 488-23-3
2	6.44	98%	228	2-Oxazolidinone, 3,3'-ethylidenebis[5-methyl,	C10H16N2O4 CAS:3889-54-1
3	6.44	98%	126	Ethane, 1-bromo-2-fluoro-, 2	C2H4BrF CAS:762-49-2
4	6.44	98%	172	Butenedioic acid (e)-, diethyl ester	C8H12O4 CAS:623-91-6
5	10.051	100%	395	2-(((carbobenzyloxy) amino) methyl)-4-benzyl-5-((carbomethoxy)-amino) oxazole	C21H21N3O5 CAS:0-00-0
6	10.051	100%	530	Ditridecyl Ester Of Phthalic Acid. Phthalic acid	C34H58O4 CAS:0-00-0

Convirmation based on resting time and mass spectrum using Wiley8.Lib. RT: Resting Time, SI: similarly Index, MW : Molecular Weight.

The results of identification of EADS active compounds using GCMS obtained 48 peaks, with 5 of them having the largest area. Based on confirmation of resting time and spectrum mass using Wiley8.Lib from the five peaks identified several active compounds having Similirity Index (SI) of more than 94% with Benzene, 1,2,3,4-tetramethyl, # 2-Oxazolidinone, 3,3 ' -ethylidenebis [5-methyl, # Ethane, 1-bromo-2-fluoro-, 2- # Butenedioic acid (e)-, diethyl ester, # 2 - ((Carbobenzyloxy) amino) methyl) -4-benzyl-5 - ((carbomethoxy) -(amino) oxazole, # Ditridecyl Ester Of Phthalic Acid. Benzene, 1, 2, 3, 4-tetramethyl, is one class of aromatic hydrocarbon compounds.

The formation of benzeneoid compounds by plants occurs when the plant experiences stress resulting in secondary metabolites. One of the secondary metabolites produced by plants is benzenoid compounds. Benzoid compounds are intermediate compounds of benzoic acid, salicilic acid, lignins, flavonoids, xanthones, phenolics, and other products [31].

2-Oxazolidinone, 3, 3'-ethylidenebis [5-methyl.Oxazolidinone is an organic compound containing a heterocyclic ring, which has potential as an antibacterial, especially gram-positive bacterium such as methicillin-resistant Staphylococcus aureus (MRSA), penicillin-resistant streptococci, and vancomycin-resistant enterococci. Oxalidinone contains a 2-oxazolidine group.

As an antibacterial, Oxazolidinones works by inhibiting protein synthesis through bonding with P site on ribosomal 50S sub-units thereby inhibiting the formation of functional protein initiation in 70S [32].Ethane, 1-Bromo-2-Fluoro-. Ethane is a hydrocarbon organic compound with the chemical formula C2H6. Ethane in large quantities will be produced by plants when stressed plants such as ultra violet rays.

Ethane which causes cell death due to cell membrane damage. This mechanism can be inhibited by the presence of glutathione (GSH) contained in the chloroplast. GSH production increases in the presence of ultaviolet light. Thus ultraviolet light causes the formation of ethane but at the same time can trigger the chloroplast to form antioxidants for the repair process [33]. 2-Butenedioic Acid (E)-, Dimethyl Ester, Fumaric Acid, Dimethyl Fumarat (DMF), has the effect of being an immunomodulator to increase cytokine production which plays a role in Thelper 2 (TH2) activity.

Besides that DMF has the effect of being an antioxidant that increases the transcription of nuclear factor genes (erythroid derived 2)-like2 (NRF2). This activates the transcription of antioxidant genes such as hemoxygenase-1 (HMOX1), nicotinamide adenine dinucleotide phosphate (NADPH), and quinoline oxidoreductase-1 (NQO1) [34].2-((carbobenzyloxy) amino) methyl) - 4 - benzyl - 5-((carbomethoxy)-amino) oxazole, is a heterocyclic compound, modified by post translation of residues of peptide serine and threonin. Oxazole compounds contain nitrogen and oxygen components in aromatic ring groups which are capable of binding to various enzymes and receptors in biological

systems. Oxazole is known to have an anti-fungal effect, anti-virus, anti-tuberculosism anti-cancer, anti-inflammatory and analgesic, antidiabetic, antiparasitic, anti-obesity, antineuropathy and antioxidants [35].Ditridecyl Ester of Phthalic Acid. Phthalic acid is a class of phenol compounds.

The main structure of the phenol compound is the presence of an aromatic ring containing one or two –OH groups. The main classification of phenol compounds consists of phenolic acids, flavonoids, stilbene, and lignans. Phenolic acid consists of 2 types, namely derivatives of hydroxybenzoic acid and hdrioxycinamic acid derivative.

Based on the similarity of the chemical structure, phthalic acid belongs to the class of penolic acid derived from hydroxybenzoic acid. In the intestine and colon, polyphenol compounds have an antioxidant effect [36]. Aside from being an antioxidant, polyphenol compounds also have an anti-inflammatory effect. The mechanism of polyphenols as anti-inflammatory, among others, is through the activities of free radical scavenger [37].

SLWE Increase Food Intake and Slowdown Body Weight Gain

The effects of SLWE on mice given a HFHF diet on food intake and weight gain can be seen in Table (3) below.

Table 3: Average dietary intake, initial weight and final body weight and weight gain in all groups

Description	N group	P group	T1group	T2 group	T3 group
Average of food intake (g) at 10 weeks	25,63±5,08[a]	24,53±4,50 [a]	26,71± 3,35[b]	26,51±3,55[b]	26,44 ± 4,26 [b]
Average pretreatment weight (g)	186,6±18,34	211,0 ± 14,46	192,0±16,84	217,4±22,23	191,8 ± 20,94
Average Post treatment weight (g)	288,6±26,84	329,8 ± 35,47	348,0±65,81	364,8±50,32	336,2 ± 33,80
Weight gain analysis	y = 6,017x + 224,23 R^2 = 0,4329	y = 11,164x + 232,12 R^2 = 0,8034	y = 16,21x + 203,95 R^2 = 0,9093	y= 18,318x + 212,49 R^2 = 0,9165	y = 14,851x + 217,68 R^2 = 0,8144

Data is the Mean ± SD value. The numbers of experimental animals (n) were 5 animals / group. Data analysis used One Way ANOVA with significance p <0.05. Different symbols indicate a significant difference. The result of weight gain analysis was shown that N group had the lowest weight gain with a value of 6.017 per increment of 1 unit (weeks).

T2 group had the highest weight gain compared to the other groups with a value of 18.318 per increment of 1 unit (weeks). N: normal group, P: positive group, T1: Treatment of HFHF diet + SLWE at dose of 100 mg / kgbw, T2: Treatment of HFHF diet +SLWE at dose of 200mg / kgbw, T3: Treatment of HFHF + SLWE at dose of 400mg / kgbw. Different symbols indicate a significant difference. Food intake was not significantly different between N group and P group (p> 0.05). Giving SLWE in T1, T2 and T3 groups caused an increase in food consumption compared to P group (p <0.05).

The average pretreatment and post treatment body weight of the study did not differ significantly in all groups. The smallest weight gain in N group and the highest weight gain in T2 group. Giving SLWE at dose of 400 mg/kgbw causes a slowdown in weight gain. The induction of the HFHF diet is often used as an obesity model but the results obtained differ. The study conducted by Tillman et al., reported that given HFHF diet in mice aged 3-4 weeks was not able to increase body weight [38]. The results of other studies showed the opposite results. Given HFHF diet for 8 weeks in Wistar rats aged 8-10 weeks can increase body weight [39].

Hall et al reported HFHF diet can affect the center of hunger and satiety and thus induce obesity [40].The ability of fat to affect the center of hunger or the center of satiety depends on the length of the short chain fatty acids, the degree of saturation and the degree of esterification of fatty acids. Double chain unsaturated fatty acids have the ability to hold the hungry center stronger than single chain unsaturated fatty acids and saturated fatty acids.

A high fructose (HF) diet also increases the risk of obesity and its complications trough accumulation of triglycerides in hepatocyte cells, changes lipid profiles, and induces insulin resistance [41]. The response of experimental animals to diet induction to

cause an increase in body weight was influenced by several factors such as diet composition used, duration of administration of diet, age and sex of experimental animals, and breeding location [42]. Giving HFHF diet is more effective in increasing body weight than if only given a HF diet [39]. Fructose is more effective in increasing body weight when mixed in drinking water [43]. The age of 8-10 weeks of Wistar rats is an effective age to produce diet-induced obese mouse models compared to 3-4 weeks of age [38].

Differences in breeding locations and treatments can lead to different results even though given the same dietary induction treatment [42].This study uses pork oil as a source of cholesterol. The fatty acid content of 100g lard is composed of 45.1% single chain unsaturated fatty acids (oleic acid), 11.2% double chain unsaturated fatty acids (linolenic acid and linoleic acid) and 39.2% saturated fatty acids (palmitic acid, stearic acid , miristic acid and lauric acid) [44, 45]. In addition to using a high-fat diet, this study also uses a high-fructose diet as fructose 10%.

Addition of fructose 10% refers to the consumption of fructose in humans is called high if more than 10% of total calories or around 55g / day [30]. The presence of carbohydrates and fat in the intestine increased secretion of CCK and GLP-1, resulting in inhibition of gastric emptying [8].Based on the description above, it can be concluded that the absence of differences in body weight between N group and P group is thought to be due to the effect of the high content of fatty acids and fructose in intestine.

HFHF inducing increased secretion of CCK and GLP-1. Both of these hormones cause decreased gastric emptying so that appetite decreases. Besides that, there are other factors that are thought to contribute to the failure of increasing body weight in the P group, including how to administer fructose and the duration of HFHF diet in this study.

The results showed that SLWE dosages of 100 mg / kgbw, 200 mg / kgbw and 400 mg / kgbw increased the amount of food consumption by 9%, 8% and 7.7% compared to P group, but at the end of the study the weight of all groups did not differ significantly. Emphasis on increasing body weight due to administration of SLWE at a dose of 400 mg / kgbw was thought to be caused by the effects of the active compounds contained in SLWE.

These active compounds work synergistically to reduce weight gain. The active compounds in the intestine act as antioxidants, antibacterial and anti-inflammatory. All three cause improvement in the function of the intestinal microbiota and improve fat metabolism. The high ratios of acetate to propionate decrease the synthesis of colesterol de novo by the liver. Improving intestinal microbiota function will increase intestinal hormone secretion in response to carbohydrate and fat.

SLWE Elevated Leptin Plasma Levels

The effects of SLWE on mice given a TLTF diet on Leptin plasma levels can be seen in Figures (2).

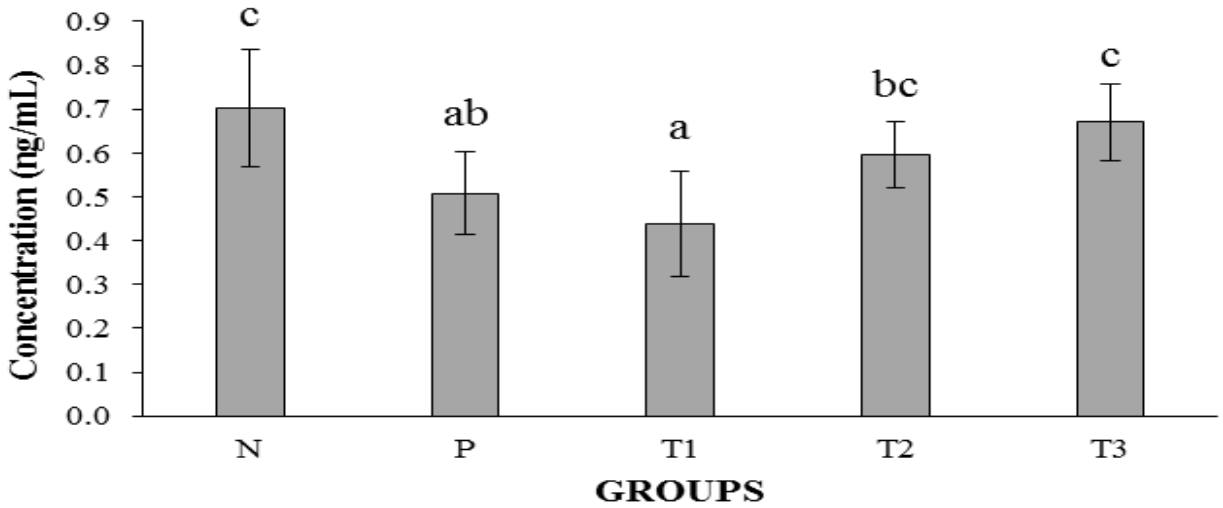

Figure 2: Leptin Plasma Levels In All Study Groups

Data is the result of calculating Mean ± SD. The numbers of samples were 5 / groups. Data analysis using ANOVA followed by Duncan test with significance p <0.05Leptin levels in the P group were lower than N group. Giving SLWE dose 400 mg / kgbw in HFHF diet mice caused an increase in leptin levels close to normal group. N: normal group, P: positive group, T1: Treatment of HFHF diet + SLWE at dose of 100 mg / kgbw, T2: Treatment of HFHF diet +SLWE at dose of 200mg / kgbw, T3: Treatment of HFHF + SLWE at dose of 400mg / kgbw. Different symbols indicate a significant difference.

The results showed that there was a decrease in leptin hormone levels in P group compared to N group (p <0.05). The administration of SLWE at dose of 400 mg / kgbw increased leptin levels compared to P group (p<0.05) and was equivalent to N group (p> 0.05). The long term regulation of the mechanism of satiety and hunger is more regulated by Leptin. Leptin is a hormone secreted by adipose tissue to control energy balance.

The role of leptin in controlling energy balance through the inhibitory effect of the center of hunger and increased satiety center activity [46]. Leptin works in hypotamalus especially in the arcuate nucleus, the ventromedial and lateromedial nucleus hypothalamus. In the arcuata nucleus, leptin will inhibit the orixogenic nucleus Agouti Related Peptide (AgRP) so that it inhibits appetite, and increases nucleus activity at the Proopiomelanocortin (POMC).

POMC activity causes an increase in Tyroid Stimulating Hormone (TSH) and adrenaline secretion in paraventromedial. Both of these hormones cause an increase in energy expenditure and thermogenesis [22, 46, 47]. Increasing leptin secretion is positively correlated with the size of adipocyte tissue. Increasing the size of adipocyte tissue is related to the amount of triglyceride accumulation in adipocyte tissue [47, 48]. The effects of leptin are related to its ability to bind to its receptors in the hypothalamus. There are 6 leptin receptor isoforms, namely LEPRa, LEPRb, LEPRc, LEPRd, LEPRe and LEPRf. Leptin has the ability to bind to LEPRb better than other types of leptin receptors.

The loss of the ability of leptin to bind to its receptors causes disruption of leptin signaling and induces obesity [47, 49].The results of the joint study indicate that the administration that the HFHF diet provides is capable of causing changes in lipid profiles. HDL serum levels in P group decreased by 53% compared to N group (P <0.05). Giving SLWE at dose of 400mg/kgbw increased HDL levels by 71% compared to P group (p <0.05). Giving SLWE at dose of 200mg/kgbw causes a decrease of LDL serum level by 45% compared to P group [50]. Cholesterol serum levels of P group increased 3-fold compared to N group.

However, giving SLWE ad dose of 100mg/kgbw and 400mg/kgbw increased cholesterol levels by 11% and 16% compared to P group (p <0.05). Trigliseride serum (TG) levels did not differ significantly between N, P, T1, T2 and T3 group (p> 0.05) [51]. Based on the facts above, an increase leptin plasma levels is thought to be due to the effect of SLWE increasing Leptin syntesis and secretion from adipocyte tissue.

SLWE have Tendency Reduce TNF α Plasma Levels

The effects of SLWE on mice given a TLTF diet on TNF α plasma levels can be seen in Figures (3).

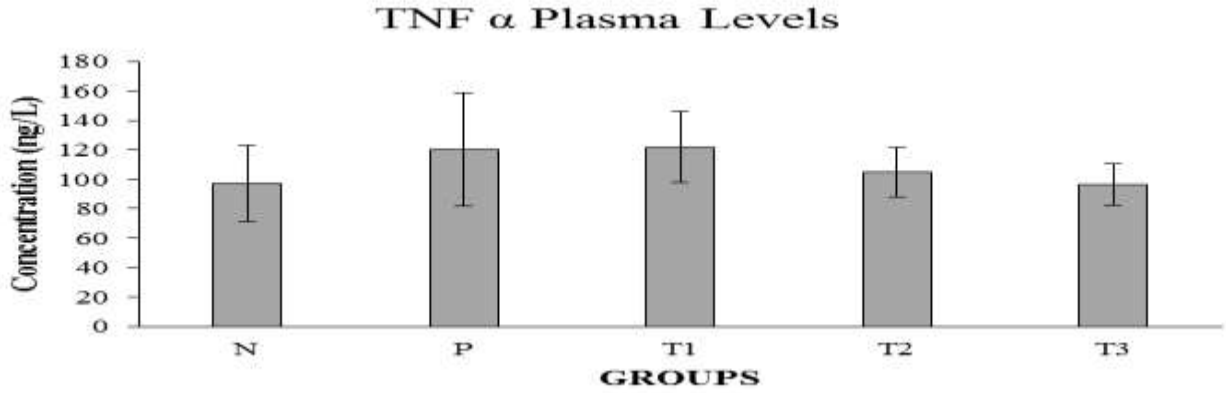

Figure 3: Levels of TNF α plasma in all study groups

Plasma levels of TNF α were not significantly different between the study groups, although there was a tendency to decrease serum TNF α levels due to administration of SLWE compared to P Group. The higher the dose of SLWE given the greater the decrease in TNFα plasma levels. N: normal group, P: positive group, T1: Treatment of HFHF diet + SLWE at dose of 100 mg / kgbw, T2: Treatment of HFHF diet +SLWE at dose of 200mg / kgbw, T3: Treatment of HFHF + SLWE at dose of 400mg / kgbw. Different symbols indicate a significant difference.

The results of statistical analysis showed that TNF α levels in all groups did not differ significantly. However, there was a tendency to decreased TNF α level due to SLWE at dose 100 mg/kgbw, 200mg/kgbw, and 400 mg/kgbw compared to P group by 0.8%, 13%, and 20%. The higher dose of SLWE caused a greater decrease in TNF α plasma levels. Long term HFHF diets can trigger inflammation due to changes in intestinal microbiota composition [52]. Disbiosis in the intestine triggers the growth of pathogenic microbes that express lipopolysaccharide (LPS). This condition triggers a moderate inflammatory process in the intestine characterized by an increase in inflammatory mediators TNF α and IL-1ß [53].

Increased inflammatory mediators reduce claudin-1 protein, thereby damaged tigh juction between intestine cells, increased free radicals in the intestine, and increased the number of endotoxins which all damage the barrier system and intestine cell integrity [10].Tumor necrosis factor-α (TNFα) was included in the TNF family.

TNF α binds to TNFR1 and TNFR2 transmembrane receptors.TNF α functions as a regulator of several cell functions such as proliferation, defense, differentiation and cell apoptosis. Macrophages were the main producer of TNF α. Increasing production of TNFα associated with the occurrence of various chronic diseases such as atherosclerosis, Rematoid arthritis, obesity, diabetes, Crohn's disease, psoriasis and sepsis. The involvement of TNF α in various pathogenesis of the disease, caused TNF α to be called the main regulator of the production of other inflammatory mediators [54].From the facts above, it can be concluded that the absence of differences in levels of TNF α plasma between the N and P groups were thought to be due to the induction of the HFHF diet in this study which has not caused a massive inflammatory process. The administration of SLWE, especially at dose 400 mg/kgbw, was thought to be able to reduced the inflammatory process through its effects as an antioxidant, anti-inflammatory and antibacterial.

Conclusion

SLWE contains active compounds that function as antioxidants, anti-inflammatory, and antibacterial. Giving SLWE in mice with HFHF diet caused an increase in appetite, suppressed weight gain, increased Leptin secretion, and a tendency to inhibit the inflammatory process.

Emphasis on weight gain was thought to be due to decreased gastric emptying, improved of intestinal microbiota, controlled of inflammation in the intestine and increased intestinal hormones. There was important to do further research on the effects of SLWE on animals models with the variation diet such as high fructose diet, high fat diet , and high fat- high fructose diet on the disbiosis of intestinal microbiota and its impact on fat metabolism to prove this suspicion.

Acknowledgement

Thank you to the dean of Medicine Faculty of Univesitas Islam Malang (UNISMA) which has provided financial assistance, Prof. Dr. Achmad Rudijanto, Sp. PD (KEMD) for his input in improved the writing of this article, and Obesity Research Groups of Medicine Faculty of UNISMA, which have helped implement this research.

References

1. CL Ogden, Susan Z Yanovski, Margaret D, Carroll A, Flegal KM (2007) The Epidemiology of Obesity. Gastroenterology, 132: 2087-2102.
2. Chooi YC, Ding C, Magkos F (2019) The epidemiology of obesity. Metabolism: Clinical and Experimental, 92: 6-10.
3. Hruby A, Hu FB (2016) HHS Public Access The Epidemiology of obesity; A big picture. Pharmacoeconomics, 33(7): 673-689.
4. Abdelaal M, le Roux CW, Docherty NG (2017) Morbidity and mortality associated with obesity. Annals of Translational Medicine, 5(7): 161-161.

5. Hariri N, Thibault L (2010) High-fat diet-induced obesity in animal models. Nutrition Research Reviews, 23(2): 270-299.
6. Ekmen N, Helvaci A, Gunaldi M, Sasani H, Yildirmak ST (2016) Leptin as an important link between obesity and cardiovascular risk factors in men with acute myocardial infarction. Indian Heart Journal, 68(2): 132-137.
7. Pereira RM, Botezelli JD, Da Cruz, Rodrigues KC, Mekary RA, Cintra DE, Pauli JR, De Moura LP (2017) Fructose consumption in the development of obesity and the effects of different protocols of physical exercise on the hepatic metabolism. Nutrients, 9(4): 1-21.
8. Guyenet SJ, Schwartz MW (2012) Regulation of Food Intake, Energy Balance, and Body Fat Mass: Implications for the Pathogenesis and Treatment of Obesity. The Journal of Clinical Endocrinology & Metabolism, 97(3): 745-755.
9. Lecomte V, Kaakoush NO, Maloney CA, Raipuria M, Huinao KD, Mitchell HM, Morris MJ (2015) Changes in gut microbiota in rats fed a high fat diet correlate with obesity-associated metabolic parameters. PLoS ONE, 10(5): 1-22.
10. Gulhane M, Murray L, Lourie R, Tong H, Sheng YH, Wang R, Hasnain SZ (2016) High Fat Diets Induce Colonic Epithelial Cell Stress and Inflammation that is Reversed by IL-22. Scientific Reports, 6 (June): 1-17.
11. Duan Y, Zeng L, Zheng C, Song B, Li F, Kong X, Xu K (2018) Inflammatory links between high fat diets and diseases. Frontiers in Immunology, 9 (Nov): 1-10.
12. WHO (2013) 2 0 1 4-2 0 2 3 WHO Traditional Medicine Strategy. Retrieved from www.who.int
13. Elfahmi Woerdenbag HJ, Kayser O (2014) Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. Journal of Herbal Medicine, 4(2): 51-73.
14. Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, Kadir HA (2015). Annona muricata (Annonaceae): A review of its traditional uses, isolated acetogenins and biological activities. International Journal of Molecular Sciences, 16(7), 15625–15658.
15. Nik Mat, Daud NNN, Ya'akob H, Mohamad Rosdi MN (2016) Acetogenins of Annona muricata leaves: Characterization and potential anticancer study. Integrative Cancer Science and Therapeutics, 3(4): 543-551.
16. Adeyemi DO, Komolafe OA, Adewole OS, Martins EM, Kehinde AT (2009) Anti hyperglycemic activities of Annona muricata (Linn). African Journal of Traditional, Complementary and Alternative Medicines, 6(1): 62-69.
17. Nwokocha CR, Owu DU, Gordon A, Thaxter K, Mccalla G, Ozolua RI, Young L (2012) Possible mechanisms of action of the hypotensive effect of Annona muricata (soursop) in normotensive SpragueDawley rats. Pharmaceutical Biology, 50(11): 1436-1441.
18. George VC, Kumar DRN, Suresh PK, Kumar RA (2015) Antioxidant, DNA protective efficacy and HPLC analysis of Annona muricata (soursop) extracts. Journal of Food Science and Technology, 52(4): 2328-2335.
19. Mithun Pai BH, Rajesh G, Shenoy R, Rao A (2016) Anti-microbial efficacy of Soursop leaf extract (Annona muricata) on oral pathogens: An in-vitro study. Journal of Clinical and Diagnostic Research, 10(11): ZC01-ZC04.
20. Sovia E, Ratwita W, Wijayanti D, Novianty DR (2017) Hypoglycemic and Hypolipidemic Effects of Annona Muricata L. Leaf Ethanol Extract. International Journal of Pharmacy and Pharmaceutical Sciences, 9(3): 170.
21. Ishola IO. Awodele O, Olusayero AM, Ochieng CO (2014) Mechanisms of Analgesic and Anti-Inflammatory Properties of Annona muricata Linn. (Annonaceae) Fruit Extract in Rodents. Journal of Medicinal Food, 17(12): 1375-1382.
22. Gavamukulya Y, Wamunyokoli F, El-Shemy HA (2017) Annona muricata: Is the natural therapy to most disease conditions including cancer growing in our backyard? A systematic review of its research history and future prospects. Asian Pacific Journal of Tropical Medicine, 10(9): 835-848.
23. Yuniarti L, Dewi MK, Lantika UA (2013) Inhibition body weight gain and blood cholesterol level by soursop leaves aqueous

extract (Annona muricata l.). Obesity Research & Clinical Practice, 7(October): 34.

24. Tugiyanti E, Mawarti N, Rosidi R, Harisulistyawan I (2017) The Effect of Soursop (Announa Muricata L.) Leaves Powder on Diameter of Muscle Fiber, Lipid Cell, Body Weight Gain and Carcass Percentage of Tegal Duck. Animal Production, 19(1): 47.
25. Bikomo E, Ebuehi O, Magbagbeola O (2017) Antidepressant Activity of Ethanol Leaf Extract of Annona muricata L., in Sprague-Dawley Rats. American Journal of Biochemistry, 7(1): 1-5.
26. Coria-Téllez AV, Montalvo-Gónzalez E, Yahia EM, Obledo-Vázquez EN (2018) Annona muricata: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arabian Journal of Chemistry, 11(5): 662-691.
27. Félix-Silva J, Souza T, Camara RB, Arro G, Cabral B, Silva-Júnior AA, Rebecchi IM, Arin M, Fernandes-Pedrosa M, de F (2014) In vitro anticoagulant and antioxidant activities of Jatropha gossypiifolia L. (Euphorbiaceae) leaves aiming therapeutical applications. BMC Complementary and Alternative Medicine, 14(October): 405.
28. Ezhilan BP, Neelamegam R (2012) GC-MS analysis of phytocomponents in the ethanol extract of Polygonum chinense L. Pharmacognosy Research, 4(1): 11-14.
29. S Murwani, Mulyohadi Ali KM (2006) Diet Aterogenik Pada Tikus Putih (Rattus novergicus strain Wistar) Sebagai Model Hewan Aterosklerosis. Jurnal Kedokteran Brawijaya, 22(1): 6-9.
30. Bantle JP (2009) The Journal of Nutrition Supplement: The State of the Science on Dietary Sweeteners Containing Fructose Dietary Fructose and Metabolic Syndrome and Diabetes 1-3. J. Nutr., 139(5): 1263-1268.
31. Misztal PK, Hewitt CN, Wildt J, Blande JD, Eller ASD, Fares S, Goldstein AH (2015) Atmospheric benzenoid emissions from plants rival those from fossil fuels. Scientific Reports, 5(July): 1-10.
32. Pandit N, Singla RK, Shrivastava B (2012) Current Updates on Oxazolidinone and Its Significance. International Journal of Medicinal Chemistry, 1-24.
33. Peiser GD, Yang SF (1979) Ethylene and Ethane Production from Sulfur Dioxide-injured Plants. Plant Physiology, 63(1): 142-145.
34. Bomprezzi R (2015) Dimethyl fumarate in the treatment of relapsing-remitting multiple sclerosis: An overview. Therapeutic Advances in Neurological Disorders, 8(1): 20-30.
35. Von Nussbaum F (2006) Natural Products. Drug Discovery and Therapeutic Medicine. Herausgegeben von Lixin Zhang und Arnold L. Demain. In Angewandte Chemie 118.
36. KB Pandey, SI Rizvi (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity, 2(5): 270-278.
37. Nijveldt RJ, Nood E, Van Hoorn, DE Van, Boelens PG, Klaske van, Norren A, Leeuwen PA Van (2001) Flavonoids : a review of probable mechanisms of action and. American Journal of Clinical Nutrition, 74(4): 418-425.
38. Tillman EJ, Morgan DA, Rahmouni K, Swoap SJ (2014) Three months of high-fructose feeding fails to induce excessive weight gain or leptin resistance in mice. PLoS ONE, 9(9): 1-8.
39. Lozano I, Van Der Werf R, Bietiger W, Seyfritz E, Peronet C, Pinget M, Dal S (2016) High-fructose and high-fat diet-induced disorders in rats: Impact on diabetes risk, hepatic and vascular complications. Nutrition and Metabolism, 13(1): 1-13.
40. Hall KD, Heymsfield SB, Kemnitz JW, Klein S, Schoeller DA, Speakman JR (2012) Energy balance and its components: Implications for body weight regulation. American Journal of Clinical Nutrition, 95(4): 989-994.
41. Rosas-Villegas A, Sánchez-Tapia M, Avila-Nava A, Ramírez V, Tovar AR, Torres N (2017) Differential effect of sucrose and fructose in combination with a high fat diet on intestinal microbiota and kidney oxidative stress. Nutrients, 9(4): 1-13.

42. Assaad H (2014) Analysis of energy expenditure in diet-induced obese rats. *Frontiers in Bioscience*, 19(6): 967.

43. Haring SJ, Harris RBS (2011) The relation between dietary fructose, dietary fat and leptin responsiveness in rats. Physiology and Behavior, 104(5): 914-922.

44. Rohman A, Triyana K, Sismindari, Erwanto Y (2012) Differentiation of lard and other animal fats based on triacylglycerols composition and principal component analysis. International Food Research Journal, 19(2): 475-479.

45. Ahmad Nizar NN, Nazrim Marikkar JM, Hashim DM (2013) Differentiation of Lard, Chicken Fat, Beef Fat and Mutton Fat by GCMS and EA-IRMS Techniques. Journal of Oleo Science, 62(7): 459-464.

46. Gautron L, Elmquist JK (2011) Sixteen years and counting: an update on leptin in energy balance. Journal of Clinical Investigation, 121(6): 2087-2093.

47. Wasim M (2015) Role of leptin in obesity. Journal of Obesity & Weight Loss Therapy, 5(2): 1-3.

48. Singh P, Peterson TE, H F Sert-Kuniyoshi, Glenn JA, Davison DE, Somers VK (2012) Leptin signaling in adipose tissue: Role in lipid accumulation and weight gain. Journal of Circulation Research, 111(5): 599-603.

49. Nanjappa1 V, Raju1 R, Babylakshmi Muthusamy, Jyoti Sharma, Thomas JK, Nidhina PA H, Prasad TSK (2011) A Comprehensive Curated Reaction Map of Leptin Signaling Pathway. Journal Proteomics Bioinformatic, 04(09): 184-189.

50. Maulana A, Lestari RD, Damayanti DS (2019) Efek Infusa Daun Sirsak (Annona muricata) terhadap Kadar LDL dan HDL Serum Tikus Wistar (Rattus norvegicus) Induksi Diet Tinggi Lemak Dan Tinggi Fruktosa, SKRIPSI, Fakultas Kedokteran Universitas Islam Malang, (Unpublish)

51. Indriyani, Dewi Fitri, Hidayah Fenti Damayanti, Dini Sri (2019) Efek Pemberian infusa Daun Sirsak (Annona muricata) terhadap Kadar Kolesterol Total dam Trigliserida Serum Tikus yang diinduksi Diet Tinggi Lemak tinggi Fruktosa. SKRIPSI, FK UNISMA, Unpublish)

52. Schulz MD, Çigdem Atay, Jessica Heringer, Franziska K Romrig, Sarah Schwitalla, Begüm Aydin, Paul K Ziegler, Julia Varga, Wolfgang Reind, Claudia Pommerenke, Gabriela Salinas-Riester, Andreas Böck, Carl Alpert, Micha, MCA (2014) High-fat diet-mediated dysbiosis promotes intestinal carcinogenesis independent of obesity. Nature, 514(7523): 508-512.

53. Ding S, Chi MM, Scull BP, Rigby R, Schwerbrock NMJ, Magness S, Lund PK (2010) High-fat diet: Bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. PLoS ONE, 5: 8.

54. Parameswaran Narayan, Patial S (2010) Tumor Necrosis Factor-α Signaling in Macrophages. Critical reviews in eukaryotic gene expression, 20(2): 87-103.

