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Antimetabolic Syndrome Effect of 70% Ethanol Leaves Extract *Ficus carica* Linn. in Streptozotocin-Induced and High-Fat Diet Rats

(Efek Sindrom Antimetabolik Ekstrak Daun Etanol 70% *Ficus carica* Linn. pada Tikus yang Diinduksi Streptozotocin dan Diet Tinggi Lemak)

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Abstract: Diabetes and obesity are risk factors for metabolic syndrome. Fig (*Ficus carica* L.) one of the plants is well known in traditional medicine system for their medicinal and therapeutic potentials. Fig leaves contain flavonoid and triterpenoid compounds which have antimetabolic effect. Streptozotocin is used to make diabetogenic rats to produce hyperglycemia conditions in test animals and high-fat diet to make rats model obesity. This study aimed to test the antidiabetic and antiobesity effect of 70% ethanol extract of fig leaves based on the parameters of measuring blood glucose levels with glucometer and Cholesterol strips. In this study, rats were divided into 6 groups, namely normal, negative, positive control, and 3 control groups at 70% ethanol extract dose level (250, 500, and 600 mg/kgBW) per oral. The measurement results in normal, negative, and positive control dose of 250, 500, 600 mg/kgBW at blood glucose levels were 105.8, 192, 134.2, 161, 157.75, 145.2 mg/dL. The conclusion of the results obtained was that 70% ethanol extract of fig leaves with a dose of 600 mg/kgBW can have an antidiabetic and obesity effect by lowering blood glucose levels as measured using glucometer, cholesterol strips and decreased body weight rats.

Keywords: Anti diabetic, antiobesity, fig leaves, streptozotocin

Abstrak: Diabetes dan obesitas merupakan faktor risiko sindrom metabolik. Tin (*Ficus carica* L.) salah satu tumbuhan yang dikenal dalam sistem pengobatan tradisional karena potensi pengobatan dan terapinya. Daun tin mengandung senyawa flavonoid dan triterpenoid yang memiliki efek antimetabolik. Streptozotocin digunakan untuk membuat tikus diabetes untuk menghasilkan kondisi hiperglikemia pada hewan uji dan *high-fat diet* untuk membuat tikus model obesitas. Penelitian ini bertujuan untuk menguji efek antidiabetes dan antiobesitas ekstrak etanol 70% daun tin berdasarkan parameter pengukuran kadar glukosa darah dengan strip glukometer dan kolesterol. Pada penelitian ini tikus dibagi menjadi 6 kelompok yaitu kontrol normal, kontrol negatif, kontrol positif, dan 3 kelompok kontrol pada taraf dosis ekstrak etanol 70% (250, 500, dan 600 mg/kgBB) per oral. Hasil pengukuran pada kontrol normal, kontrol negatif, kontrol positif dosis 250 mg/kgBB, 500 mg/kgBB, 600 mg/kgBB pada kadar glukosa darah adalah 105,8 mg/dL; 192 mg/dL; 134,2 mg/dL; 161 mg/dL; 157,75 mg/dL; 145,2 mg/dL. Kesimpulan dari hasil yang diperoleh adalah ekstrak etanol daun tin 70% dengan dosis 600 mg/kgBB dapat memberikan efek antidiabetes dan obesitas dengan cara menurunkan kadar glukosa darah yang diukur menggunakan strip glukometer, kolesterol, dan penurunan berat badan tikus.

Kata kunci: Antidiabetes, antiobesitas, daun tin, streptozotocin

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INTRODUCTION

INCREASED waist circumference, elevated blood triglyceride levels, decreased levels of high-density lipoprotein (HDL)-blood cholesterol, high blood pressure, and glucose intolerance are among the signs of the metabolic syndrome. According to the World Health Organization (WHO), metabolic syndrome can already be identified in someone who exhibits 3 out of 5 of these symptoms[1,2]. Numerous plants, including the fig tree *Ficus carica*, a deciduous tree in the Moraceae family, are well known for their medicinal and therapeutic properties in traditional medicine systems around the world. One of the earliest fruit trees to be cultivated, it is a significant crop grown both for dry and fresh consumption around the world. FC leaves are consumed as tea or used as a medication. According to reports, FC leaves have positive benefits on cardiovascular illness, pulmonary conditions, and anti-diabetic conditions[3]. Different FC leaf extracts have been shown to exhibit anti-diabetic, anti-hyperlipidemic, and anti-oxidative effects in rats. The use of FC in the treatment of anemia, cancer, diabetes, liver illnesses, paralysis, skin diseases, and ulcers was also described in a recent comprehensive study, which further suggested that it may have clinical applications[4,5]. Based on the findings of previous research, it was reported that aqueous and 70% ethanol extract leaves fig decrase blood glucose. FC leaves contain alkaloid, saponin, β-setosterols, polifenol, flavonoids, β-sitosterol, and polyphenol that reported as a anti diabetic[6].

Diabetogenic, which is often used in research to produce hyperglycemia conditions in test animals is streptozotocin[7]. Streptozotocin is an unstable hydrophilic compound that is known to rapidly disrupt pancreatic function through the mechanism of reactive oxygen formation and is a major factor in cell damage. The formation of reactive oxygen begins with the reduction process of streptozotocin in Langerhans β cells, in addition to the formation of reactive oxygen streptozotocin also causes disturbances in intracellular calcium homeostasis. Streptozotocin can increase the concentration of cytosolic free calcium ions in pancreatic Langerhans β cells. This condition then causes insulin concentrations to increase very rapidly, and significantly results in disturbances in peripheral insulin sensitivity[8].

Male Wistar rats were given a high-energy diet for 4 weeks before receiving a low-dose injection of streptozotocin (STZ) to create a diabetes model. The rats were then given an astaxanthin-containing diet for an additional 3 weeks to further demonstrate the anti-diabetic effects of astaxanthin. In STZ-induced diabetic mice, astaxanthin dose-dependently reduced blood glucose and total cholesterol (TC) levels and elevated blood levels of high-density lipoprotein cholesterol (HDL-C). These findings reduced the severity of STZ-induced diabetes by increasing the expression in vivo of genes related to insulin sensitivity (adiponectin, adipoR1, and adipoR2)[9]. Therefore, in order to determine the scientific basis for using this plant in the treatment of diabetes and to examine the blood glucose profile for 28 days of treatment using diabetic mouse models with STZ inductors, our current study examined the effects of 70% ethanol extract of Fig (*Ficus carica*) leaves in the STZ-induced diabetic rats.

MATERIALS AND METHODS

MATERIAL. 70% extract ethanol fig leaves (*Ficus carica*), male rats (*Mus muculus*) weighed 20-40g, Farnsworth reagent for phytochemical screening.

Equipment. Ultrasonic(LC 30 H, Elma), Triple Beam Balance (Ohaus), Autocheck® 3in1, Kit Glocouse, and cholesterol (Autocheck), syringe 1 mL (OneMed).

METHODS. Collection of Plant Material. The leaves of fig (*Ficus carica*) were collected during July 2021 from the Khazanah Fig Garden, Cikarang, West Java, Indonesia. The leaves determination at the BRIN (Plant Conservation Research Center and Botanical Gardens), Bogor, west Java Indonesia.

Extraction of *Ficus carica* Leaves. Ultrasonic extraction procedure: 50 g of fig leaves simplicia powder were placed in a glass beaker, to which was added 70% ethanol. Ultrasonic waves at a frequency of 50 kHz were then used to extract the powder for 15 minutes, after which it was filtered with Whatman paper 42. To create a thick extract, the liquid extract was evaporated in a rotating vacuum evaporator at 40 °C, 60 rpm, and 200 mBar of pressure.

Phytochemical Screening. According to Farnsworth and Harborne's method, phytochemical screening tests were carried out to identify the compounds of the samples, including alkaloids by Dragendorff's reagent/Mayer's reagent, flavonoids by the reduction test (Mg-HCl/amyl alcohol), saponins by the foam formation test, tannins by the iron (III) chloride reagent, quinone by the NaOH reagent, steroids/triterpenoids by the Liebe.

Preparation of Experimental Animals. The experimental animals used were male white mice (*Mus musculus*) DDY strain aged 2-3 months with an average weight of 20-30 g of 30 mice. Before testing, the mice were acclimatized for one week in the experimental animal cage.

RESULTS AND DISCUSSION

Plant Determination. At the Center for Plant Conservation and Botanical Gardens-BRIN, Cibinong, the determination was made. Based on reference number B-481/IV/D1.01/7/2022, the findings of this determination suggest that the material under study corresponds to the *Ficus carica* Linn species. *Ficus carica* Linn. leaf are presented in Figure 1.

Extraction. The ultrasonic technique was retrieved using an ultrasonicator that resonated three times for 15 minutes at a frequency of 15 kHz. The extracted material weighed 50.13 g, with a yield of 20.05%, and had a native DER of 4.98. Using a rotary evaporator, the produced macerate was collected and concentrated.

The ultrasonic extraction method utilizes ultrasonic waves which can speed up the contact time between the sample and the solvent so that it can destroy leaf cells and speed up the process of movingbioactive compounds from inside the cell to the solvent.

Phytochemical Screening. The results of the phytochemical screening of 70% ethanol extract fig leaves (*Ficus carica* L.) are contains alkaloids, tannins, flavonoids, saponins, steroids, triterpenoids, essential oils and coumarins. The results can be seen in Table 1.

Animals and Treatments. This study uses experimental animals and was approved. KET-1156/UN2. F1/ETIK/PPM.00.02/2021 is the reference number provided by the Faculty of Medicine, Universitas Indonesia's Health Research Ethics Committee. Experiment was conducted Healthy adult albino ratsRats were acclimated for a week at 25°C 2°C, 65% 10% humidity, 11–13 air changes per hour, and 12 hours of illumination per day (07:00–19:00). Rats were fed with typical pellets and allowed unlimited access to water.

Test animals were grouped into several groups with a total of 5 animals per group. Test animals are grouped into several groups as follows: a) The normal control group (normal test animals) was given standard feed and given a test preparation carrier which is inert and does not affect blood glucose levels. b) The positive control group (hyperglycemia test animals) was given standard feed and streptozotocin which can increase blood glucose levels in test animals carrying inert test preparations until the end of the test and given standard drugs (conventional drugs) to reduce levels. c) The negative control group (hyperglycemia test animals) was given standard feed and streptozotocin which can increase the blood glucose level of the test animals in inert test preparation carriers until the end of the test and given test preparation carriers which are inert and do not affect blood glucose levels. d) Dosage group I, namely diabetic mice, was given orally daily at 250 mg/kg of fig leaf ethanol. e) Dosage group II, namely diabetic mice, was given orally daily 500 mg/kg of fig leaf ethanol. f) Dosage group III, namely diabetic mice, was given orally daily at 600 mg/kg of fig leaf ethanol.

Table 1. Phytochemical screening.

No.	Group of chemical compounds	Result
1	Alkaloid	-
2	Flavonoid	+
3	Saponin	+
4	Tanin	+
5	Steroid	-
6	Triterpenoid	+
7	Kuinon	-
8	Minyak atsiri	-
9	Kumarin	-

Note: (+) indicates present, (-) indicates absent/not detected

Changes in Body Weight. The body weight of diabetic control rats decreased dramatically after the course of treatment, as shown in Table 2, whereas the body weight of normal rats, treated with aqueous extract and standard medication, significantly rose. Weight gain can be caused Storage of fatty acids in the form of compounds chemical form of triacylglycerol contained in adipocytes can protect the body

Table 2. Effect of aqueous extract of *Ficus carica* leaves on body weight.

Group treatment (n=5)		Body weight (gram)					
		0	3	7	14	21	28
I	Normal control	32.78±1.10	32.62 ±1.11	32.68±1.23	33.16±1.26	33.12±1.31	33.34±1.12
II	Negative control	32.72± 0.88	31.24±0.72	30.36±1.07	29.60±1.18	29.36±1.21	19.36±1.11
III	Positive control (glibenclamid)	31.32±0.63	29.76±0.57	30.24±1.22	30.58±1.18	30.92±1.16	31.32±1.27
IV	Diabetic + 70% ethanol extract (250 mg/kgBW)	31.48±0.76	29.92±1.40	29.78±1.56	29.7±1.28	29.8±0.98	30.1±0.90
V	Diabetic + 70% ethanol extract (500 mg/kgBW)	32.4±0.96	30.62±1.49	30.78±0.98	30.54±1.10	30.72±0.88	31.0±0.80
VI	Diabetic + 70% ethanol extract (600 mg/kgBW)	32.2±1.01	30.84±1.27	29.88±1.28	30.48±1.04	31.04±0.82	31.9±0.91

of the toxic effects of fatty acids. Fatty acid in free form can circulate deep blood vessels throughout the body and cause oxidative stress as we know it with lipotoxicity. The emergence of lipotoxic effects caused by a number of acids Free fat released by triacylglycerols in compensation for destruction excessive fat storage effect on adipose and nonadipose tissue, and play a role in pathophysiology weight gain[12].

Effect of 70% Ethanol Extract on Oral Glucose Tolerance Test in STZ-Induced Diabetic Rats (OGTT). When compared to diabetic control, the study's findings showed that the 70% extract of *Ficus carica* leaves at 250, 500, and 600 mg/kg significantly lowered blood sugar levels (hyperglycemia caused by glucose load of 2 g/kg p.o.) and glibenclamide (0.25 mg/kg) after 60 minutes of oral administration (Table 3). The increase in blood cholesterol and glucose is due to the action of hormone sensitive lipase, which promotes lipolysis and subsequently increases the level of plasma free fatty acids and triglycerides. These free fatty- acids are catabolized to acetyl CoA which is further channeled to cholesterol synthesis, thus increasing blood cholesterol level[13].

Effect of 70% Ethanol Extract on Total Cholesterol Tolerance Test in STZ-Induced. The study's findings showed that, when compared to diabetic controls, the 70% extract of *Ficus carica* leaves at 250, 500, and 600 mg/kg significantly lowered blood sugar levels (hyperglycemia caused by glucose load of 2 g/kg p.o.) and glibenclamide (0.25 mg/kg) after 60 minutes of oral administration in Table 4. *Ficus carica* was extracted with ethanol, therefore polar extract contains an extract of *Ficus carica*. The primary enzymes in the synthesis and metabolism of cholesterol are found in polar extract. By encouraging the excretion of the cholesterol-containing metabolite of bile acid, known as CYP7A1, the polar extract may increase the activity and mRNA expression level of CYP7A1. HMG-CoA reductase activity and mRNA expression levels were simultaneously suppressed to reduce cholesterol production.The most common positive risk factor for the development of atherosclerosis and coronary heart disease is thought to be hypercholesterolemia[18].

Since polyphenols can inhibit HMG-CoA reductase, they can covalently bond to sugar residue to mimic the effects of statins and prevent HMG-CoA

Table 3. Effect of aqueous extract of *Ficus carica* leaves on serum glucose level.

	Group treatment (n=5)	Fasting plasma glucose level (mg/dL)/days					
		0	3	7	14	21	28
I	Normal control	105.4±2.07	105.2 ±2.77	105.0±2.45	104.4±2.79	104.8±3.63	105.8±2.59
II	Negative control	106.6±3.44	230.0±2.35	242.2±1.92	265.0±3.16	288.6±2.30	299.4±2.07
III	Positive control (glibenclamid)	102.4±1.14	208.6±2.30	178.4±1.14	170.4±2.79	148.0±2.00	134.2±2.28
IV	Diabetic + 70% ethanol extract (250 mg/kgBW)	102.0±1.00	216.4±1.52	213.4±3.21	197.8±2.05	178.4±1.14	168.4±2.41
V	Diabetic + 70% ethanol extract (500 mg/kgBW)	102.6±4.39	213.6±2.41	211.2±1.92	191.0±3.36	171.8±1.92	157.6±1.67
VI	Diabetic + 70% ethanol extract (600 mg/kgBW)	114.4±5.32	210.2±1.30	203.6±1.67	184.4±3.00	160.2±1.64	144.6±3.65

Table 4. Effect of aqueous extract of *Ficus carica* leaves on serum cholesterol level.

	Group treatment (n=5)	Hypercholesterolemia level (mg/dL)/days					
		0	3	7	14	21	28
I	Normal control	185.4±1.07	185.2±1.77	186.0±1.33	184.4±1.09	184.8±1.23	185.4±1.19
II	Negative control	186.6±1.14	200.0±1.15	212.2±1.32	235.0±1.06	258.5±1.39	269.4±1.07
III	Positive control (glibenclamid)	186.4±1.04	203.6±1.11	198.4±1.02	191.4±1.09	188.0±1.12	184.2±1.02
IV	hypercholesterolemia + 70% ethanol extract (250mg/kg BW)	187.0±1.06	206.4±1.22	213.9±1.21	201.8±1.05	198.4±1.14	195.4±1.43
V	hypercholesterolemia + 70% ethanol extract (500mg/kg BW)	187.6±1.09	203.1±1.21	211.1±1.12	199.0±1.16	191.8±1.22	193.6±1.67
VI	hypercholesterolemia + 70% ethanol extract (600mg/kg BW)	184.4±1.32	200.2±1.31	203.6±1.17	194.4±1.10	190.2±1.34	184.6±1.15

reductase from working[19]. The rate of triglyceride (TG) hydrolysis (lipolysis) is positively regulated by an increase in intracellular cAMP. Cyclic nucleotide phosphodiesterases (PDEs) are cyclic nucleotide phosphodiesterases (PDEs) that catalyze cAMP (cyclic adenosine monophosphate) hydrolysis. By encouraging its phosphorylation, hormone-sensitive lipase (HSL), one of the major enzymes regulating lipolysis, is stimulated by cAMP via activating a cAMP-dependent protein kinase called PKA. Flavonoids can cause lipolysis in adipose tissue, perhaps via inhibiting PDEs and preventing the breakdown of cAMP, which could lower triglyceride levels.

CONCLUSION

The 70% ethanol extract of fig leaves (*Ficus carica* L.) with a dose of 600 mg/kgBW can have an antidiabetic and obesity effect by lowering blood glucose levels as measured using glucometer strips and decreasing body weight rats. Based on the phytochemical screening, it is suspected that the compounds that play a role are the flavonoids group.

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