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Effect of leunca (*Solanum nigrum*) on inflammatory status in rats induced by high-fat and high-sucrose diet

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ABSTRACT

Background: Consumption of foods that are high in calories over a long period of time is a cause of obesity and can increase proinflammatory cytokines. Leunca fruit contains various phytochemicals that act as anti-inflammatory, antioxidant, and antiobesity but not many people utilize leunca fruit as daily food.

Objective: To analyze the effect of leunca fruit on TNF-α and IL-6 levels in rats induced by high fat and sucrose diet (HFSD).

Methods: The research design was Randomized Post Test Only Control Group Design. The sample was 30 male Sparague Dawley rats and divided into 5 groups, namely group K1 which was given standard feed, group K2 which was induced by HFSD, group P1 which was induced by HFSD and leunca 0.8g/200gBW, group P2 which was induced by HFSD and leunca 1.6g/200gBW, and group P3 which was induced by HFSD and leunca 2.4g/200gBW. The research was conducted at the Nutrition Laboratory of the Center for Food and Nutrition Studies of Gadjah Mada University (PSPG PAU UGM) Yogyakarta in January-March 2024. Testing TNF-α and IL-6 levels through rat blood serum using the ELISA method. Data were analyzed by ANOVA and Post Hoc test.

Results: There was a significant difference in TNF-α and IL-6 levels between experimental groups (p=0.00). The levels of TNF-α and IL-6 in the P1, P2, and P3 groups that received HFSD + leunca were lower than in the K2 group that received HFSD only.

Conclusion: Leunca fruit can reduce proinflammatory cytokine levels (TNF-α and IL-6) in HFSD-induced rats.

Keywords *: antiinflammation; leunca; IL-6; TNF-α*

BACKGROUND

Obesity is a health problem in many countries including Indonesia. Obesity is a condition of excess fat accumulation caused by an imbalance between the energy that enters the body and the energy released by the body.[1] The prevalence of obesity has been increasing every year. Based on Riset Kesehatan Dasar (Riskesdas) 2018 data, the prevalence of obesity in Indonesia is 21.8%, an increase of 7% from 2013.[2] According to the latest data from Survei Kesehatan Indonesia (SKI) 2023, the prevalence of obesity has increased to 23.4%. Meanwhile, based on data from the World Health Organization, in 2022 more than 2.5 billion adults were overweight and 890 million were obese.[3] Obesity can lead to an increased risk of metabolic diseases such as cardiovascular disease, dyslipidemia, hypertension, diabetes mellitus, stroke, atherosclerosis, and death.[4]

Obesity is associated with chronic low-grade inflammation caused by an increase in proinflammatory cytokines and chemokines.[5] Inflammation occurs due to hypertrophy and hyperplasia of adipose tissue in obese conditions. This causes phenotypic changes in immune cells in adipose tissue so that these cells stop secreting anti-inflammatory adipokines and start secreting proinflammatory adipokines such as TNF-α, IL-6, leptin, resistin, and vistafin.[6]

Inflammation that occurs in obese conditions can cause various complications from metabolic diseases such as hypertension, diabetes mellitus, dyslipidemia, cardiovascular disease, stroke, as well as atherosclerosis and myocardial damage.[4] In addition to its impact on physical health, obesity also affects mental health, as well as social and economic conditions.[7] Therefore, obesity should be prevented and treated through lifestyle changes, dietary intake regulation, increased physical activity, pharmacological therapy, and surgery.[8]

Consumption of foods with high phytochemical content is starting to be applied because of its potential as an alternative prevention or supplement to overcome certain diseases, one of which is leunca (*Solanum nigrum*) which belongs to the Solanaceae family.[9] Leunca is commonly served as fresh vegetables in West Java or stir-fried in Sumatra, but not many people consume leunca as a daily food because they are not used to the bitter and astringent taste of leunca fruit.

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Leunca has complete nutritional content such as energy, carbohydrates, protein, fat, fiber, and various vitamins and minerals. Leunca also contains about 180 types of phytochemicals such as solanine, steroids, alkaloids, flavonoids, phytic acid, saponins, tannins, anthocyanins, hydrocyanic acid, phenylpropanoids, and glycosides. These phytochemicals can provide health effects as anti-inflammatory, antioxidant, antitumor, antibacterial, neuroprotective, hypotensive, immunomodulatory, and hepatoprotective.[10] Among the various phytochemicals in leuca, phytochemicals such as solanine, flavonoids, dietary fibre and steroids have anti-inflammatory activity.[11] In addition, flavonoids, gallic acid, tannins, alkaloids and saponins have anti-obesity activity.[12]

A study showed that leunca fruit extract has anti-inflammatory effects by increasing the production of SCFA, specifically acetic acid, and reducing the inflammatory cytokines TNF-α and IL-6 through inhibition of lipopolysaccharide (LPS) and Toll-like receptor 4 (TLR4) in alcoholic liver injury rats.[13] Another study showed that leunca extract can reduce Body Mass Index (BMI), Low Density Lipoprotein (LDL), triglycerides, and blood glucose in obese rats.[14] Previous studies used leunca extract and were conducted on obese rats. However, in this study, leunca fruit was given as a juice rather than an extract, and leunca was given at the same time as obesity inducers.

Based on the potential of leunca (*Solanum nigrum*) as anti-inflammatory but not many people use leunca fruit, it is the basis for researchers to analyze the effect of leunca fruit on TNF-α and IL-6 levels in rats induced by high fat and sucrose diet (HFSD).

MATERIALS AND METHODS

Research Design, Place, and Time

The research design used was Randomized Post Test Only Control Group Design. This study was a preventive study, both HFSD and leunca were given simultaneously so that all rats were healthy and had normal body weights at the start of the study. The study started with the rats acclimatised for 7 days (week 1) and then continued with the treatment (HFSD and leunca) for 42 days (week 2 to week 7). This study was conducted in January-March 2024. Treatment of experimental animals and testing of TNF-α and IL-6 levels were carried out at the Nutrition Laboratory of the Center for Food and Nutrition Studies at Gadjah Mada University (PSPG PAU UGM) Yogyakarta.

The analysis of leunca fruit content was carried out at the Food Laboratory of Gadjah Mada University, Yogyakarta. The analysis was performed by laboratory technician. Each analytical method was repeated twice (duplo). Proximate analysis was conducted by gravimetric, protein by Kjeldhal, carbohydrates by difference, vitamin C by iodimetric, and vitamin A, flavonoids, and tannins by spectrophotometric.

Animal Experimental Design

This study used Sprague Dawley male rats obtained from the Nutrition Laboratory of the Center for Food and Nutrition Studies of Gadjah Mada University (PSPG PAU UGM) Yogyakarta. The sample amounted to 30 with the inclusion criteria of male Rattus Norvegicus Sprague Dawley white rats, 2-3 months of age, body weight 150-200 grams, and healthy conditions and active movements. Samples were divided into 5 groups, namely:

Table 1. Anti-inflammatory test treatment groups

Group	Treatment
Negative control group (K1)	Healthy rats were given standard diet ad libitum for 28 days.
Positive control group (K2)	Rats were given HFSD but not intervened with leunca for 28 days.
Treatment group 1 (P1)	Rats were given HFSD and leunca 0.8g/200gBW for 28 days.
Treatment group 2 (P2)	Rats were given HFSD and leunca 1.6g/200gBW for 28 days
Treatment group 3 (P3)	Rats were given HFSD and leunca 2.4g/200gBW for 28 days

The standard diet was Comfeed AD II composed of 51% carbohydrates, 15% protein, 7% fat, and 6% fiber. High Fat Sucrose Diet (HFSD) composed of 21% lard and 34% sucrose.

Preparation of Leunca Fruit

In this study, the dose determination was obtained from the daily consumption of leunca in humans and converted to rats using the Evaluation of Drug Activities Pharmacometics conversion table.[15] Thus, the doses of leunca given to rats were 0.8 g/200gBW (dose 1), 1.6 g/200gBW (dose 2), and 2.4 g/200gBW

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(dose 3). Leunca fruit was washed and pulverized using a blender with the addition of water until the weight was 5 g. The leunca fruit was given to rats using a gastric sonde once a day in the morning.

Body Weight Weighing and Measurement of TNF-α and IL-6 Levels

Body weight was weighed using a digital scale and was carried out every week from the first day of acclimatization to the last day of the study. TNF-α and IL-6 levels were measured on the last day after the intervention and analyzed through blood serum using the ELISA method.

Rat blood was collected through the periorbital area and placed in plain venoject. The blood was then centrifuged at 4000 rpm for 15 minutes to separate serum and plasma. ELISA assays were performed on blood serum using mouse TNF-α and IL-6 antibodies loaded onto 96-well plates. Standards and samples were added to the wells. TNF-α and IL-6 in the samples were bound by the detection antibodies. The standards, samples and detection antibodies present in the wells were then washed with wash buffer to remove unbound components. Tetramethylbenzidine (TMB) substrate solution is added to the wells to visualise the incubated Horseradish Peroxidase (HRP) enzyme reaction. TMB is catalysed by HRP to produce a blue colour and turns yellow after the addition of stop solution and quantified using an ELISA reader to read the absorbance at a wavelength of 450 nm.

Data Analysis

The data obtained will be analyzed for normality using the Saphiro Wilk test. Bivariate analysis uses ANOVA if the data is normally distributed and if the test results obtained are significant, then proceed with the Post-Hoc test to determine the most effective dose. Data analysis using SPSS software.

Ethical Clearance

This study has been declared feasible and passed the ethical test by the Ethics Commission of the Faculty of Medicine, Diponegoro University with number 007/EC-H/KEPK/FK-UNDIP/I/2024.

RESULTS

Content of Leunca Fruit (*Solanum nigrum*)

Analysis of proximate content (water, ash, total fat, protein, carbohydrate), dietary fiber, and antioxidants of leunca fruit was conducted at the Food Laboratory of Gadjah Mada University, Yogyakarta. The results of the leunca fruit content analysis are in Table 2.

Table 2. Nutrient and Phytochemical Content of Leunca Fruit (per 100g)

Component of Leunca Fruit	Analysis Result
Water (%)	92.46 ± 0.07
Ash (%)	0.60 ± 0.01
Fat (%)	-
Protein (%)	1.75 ± 0.09
Carbohydrate (%)	5.17 ± 0.03
Antioxidant (%)	28.58 ± 0.09
Dietary Fiber (cal/g)	2.28 ± 0.07
Vitamin C (mg/100g)	25.180 ± 1.54
Vitamin A (mg/100g)	2.242 ± 0.28
Flavonoids (%)	0.091 ± 0.00
Tannins (%)	0.059 ± 0.00

Effect of Leunca on Body Weight

The study started with the rats acclimatised for 7 days (week 1) and then continued with the treatment (HFSD and leunca) for 42 days (week 2 to week 7). Both HFSD and leunca were given simultaneously so that all rats were healthy and had normal body weights at the start of the study. The highest mean body weight was found in group K2 (322.16 ± 2.92), which received HFSD without leunca. The lowest body weight was found in group K1 (229.66 ± 2.16), which received standard diet. The increase in body weight in the leunca intervention groups (P1, P2, P3) can be suppressed as the dose of leunca given increases (dose-dependent), so that the lowest body weight in the intervention group is group P3 (233.00 ± 2.89). However, the body weight of group P3 was not able to reach the body weight of group K1, the healthy rats fed standard diet.

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Effects Leunca on Inflammation

Based on the results of univariate analysis of the average in Table 3, the group with the lowest TNF-α and IL-6 levels was group K1 which was given a standard diet without leunca. While the group with the highest TNF-α and IL-6 levels was group K2 which was given HFSD but not leunca intervention. Table 3 also shows a decrease in TNF-α and IL-6 levels along with an increase in the dose of leunca given. Group P3, which was given leunca intervention at a dose of 2.4 grams, was the treatment group with the lowest TNF-α and IL-6 levels compared to other treatment groups, but could not be equal to the levels of group K1.

Table 3. Levels of Inflammatory Cytokines TNF-α and IL-6 in All Research Groups

Cytokines (pg/ml)	K1 (Normal)	K2 (HFSD)	P1 (HFSD + 0.8 g Leunca)	P2 (HFSD + 1.6 g Leunca)	P3 (HFSD + 2.4 g Leunca)	p^*
TNF-α	6.0 ± 0.34^a	19.1 ± 0.34^b	9.7 ± 0.33^c	7.8 ± 0.22^d	6.9 ± 0.40^e	0.000
IL-6	64.0 ± 2.4^a	123.9 ± 4.69^b	88.0 ± 2.29^c	77.3 ± 2.44^d	68.7 ± 2.87^a	0.000

(p^*) :ANOVA, (^{a,b,c,d,e}) :post hoc

TNF-α and IL-6 levels were highest in group K2, which received HFSD without leunca and lowest in group K1, which received a standard diet. The levels of TNF-α and IL-6 in the intervention groups decreased as the dose of leunca administered increased. In the intervention group, the lowest levels of TNF-α and IL-6 were found in the P3 group. However, the levels were not equivalent to the K1 control group. The results of the ANOVA test showed a significant difference in TNF-α and IL-6 between the study groups, which amounted to 0.000 ($p < 0.05$) (Table 3). Post Hoc test results for TNF-α and IL-6 levels in Table 3 were marked with a,b,c,d,e notation, the difference in notation indicates that there were significant differences in TNF-α and IL-6 levels between groups. There were significant differences in TNF and IL-6 levels between group K2 (HFSD) and groups K1, P1, P2, and P3. However, there was no significant difference for IL-6 levels between group K1 (normal) and P3 (HFSD + leunca 2.4g). This showed that leunca (*Solanum nigrum*) with the highest dose of 2.4g/200gBW was the most effective dose in reducing IL-6 in HFSD-induced rats.

DISCUSSION

Consumption of foods with high calories, including high fat and carbohydrates over a long period of time can lead to weight gain which results in obesity.[16] This study is in line with previous research which states that *Solanum nigrum* can provide antiobesity effects. The average body weight in HFSD + leunca groups was not as high as in K2 group which only received HFSD without leunca. The content of fiber, flavonoids, gallic acid, tannins, alkaloids, and saponins in *Solanum nigrum* can provide anti-obesity effects by reducing body fat and body weight by inhibiting lipogenesis through a decrease in the Fatty Acid Synthase (FAS) enzyme. Lipogenesis is the process of triglyceride formation from acetyl CoA. In the process of lipogenesis, the AMP-activated protein kinase (AMPK) enzyme acts as a metabolic regulator that can stimulate catabolism and inhibit anabolism, so that the activation of the AMPK enzyme can reduce the Fatty Acid Synthase (FAS) enzyme and triglyceride levels will also decrease.[12] Another study showed that *Solanum nigrum* is antiobesity by inhibiting peroxisome proliferator-activated receptor-γ (PPARγ) and adiponectin. PPARγ and adiponectin are factors that cause adipogenesis. Adipogenesis is the process of preadipocytes differentiating into mature adipocytes in adipose tissue. Thus, the administration of *Solanum nigrum* can reduce body weight and inhibit adipogenesis.[17]

The administration of HFSD to rats causes changes in gut microbiota and an increase of free fatty acids (FFA). This condition can lead to inflammation and an increase in pro-inflammatory cytokines, including TNF-α and IL-6.[18] Based on the results of the study, the administration of leunca fruit can suppress the increase of TNF-α and IL-6 levels in HFSD-induced rats. This is because there are several phytochemicals in leunca that may have anti-inflammatory effects. Leunca (*Solanum nigrum*) contains fiber that can act as an anti-inflammatory through various mechanisms. Fiber can reduce leptin levels in obese conditions so that it will reduce chronic inflammation.[19] In addition, fiber can also help improve the balance of gut microbiota. Obesity causes an imbalance in the gut microbiota where the number of pathogenic bacteria tends to be high. Fiber consumption can increase short chain fatty acids (SCFA) which will help improve the balance of gut microbiota by increasing the growth of good bacteria and reducing the number of pathogenic bacteria so as to suppress inflammatory cytokines including TNF-α and IL-6.[20]

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Solanin A is a phytochemical found in plants with the genus solanum that has potential as an anti-inflammatory. Solanin A can reduce inflammation through inhibition of the NFκB pathway by inhibiting the degradation of kappa B kinase (IκBα).[21] The imbalance of gut microbiota in obese conditions can cause an increase in Lipopolysaccharide (LPS) levels or so-called endotoxaemia. This is because the gut microbiota secrete endotoxins. Increased LPS can stimulate the Toll Like Receptor (TLR) which will activate the inhibitor of nuclear factor kappa-β kinase (IKK). Inhibitor of nuclear factor kappa-β kinase (IKK) will cause degradation of kappa B kinase (IκBα) resulting in the release of NF-κB to the nucleus and stimulate the release of inflammatory cytokines.[22]

Obesity is also associated with changes in the number of macrophages, where there will be an increase in proinflammatory M1 macrophages and a decrease in anti-inflammatory M2 macrophages. This is due to adipocyte hypertrophy, causing hypoxia and adipose cell death. The dead cells will secrete proinflammatory cytokines and these cytokines will attract M1 macrophages around the dead adipocytes to form a "crown-like" structure. This causes an increase in the number of M1 macrophages that can secrete proinflammatory cytokines such as TNF-α, IL-6, IL-1β, IL-12, and IL-23.[23] There are various antioxidant compounds in leunca, including flavonoids. Flavonoids can provide anti-inflammatory effects through increasing M2 macrophages and decreasing M1 macrophages so that inflammatory cytokines including IL-6 and TNF-α can be suppressed. Flavonoids can activate AMP-activated protein kinase (AMPK).[24] Activated AMPK will inhibit M1 macrophage polarization through suppression of the NF-κB pathway.[25] NF-κB is a major transcription factor that can cause macrophage M1 polarization.[26]

CONCLUSIONS

There is effect of leunca fruit on TNF-α and IL-6 levels in rats induced by HFSD. TNF-α and IL-6 levels in rats given leunca with doses of 0.8 g/200gBW, 1.6 g/200gBW, and 2.4 g/200gBW were lower than rats that were only induced by HFSD. The number of doses affects TNF-α and IL-6 levels, the higher the dose, the lower the TNF-α and IL-6 levels of rats. If these doses are converted to humans, the dose that can be given are 45g/70kgBW, 90g/70kgBW, and 135g/70kgBW.

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