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Original Article

Effect of *Cnidoscolus aconitifolius* Leaf Extract in Lowering Triglyceride Levels and Body Weight of Wistar Rats with Metabolic Syndrome

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Abstract

Background: *Cnidoscolus aconitifolius* has the potential to be antidyslipidemic which has the effect of lowering triglyceride levels. This study aimed to explore the effect of *Cnidoscolus aconitifolius* leaf extract on triglyceride levels and body weight of Wistar rats with metabolic syndrome.

Methods: This randomized controlled trial with a pre- and posttest design was conducted in 2021 at the Center for Food and Nutrition Studies, Yogyakarta, Indonesia. Male Wistar rats were subjected into metabolic syndrome state with a high-fat and high-fructose diet (HFHFD) for 21 days, then injected with Streptozotocin (STZ, 45 mg/kg) and Nicotinamide (NA, 110 mg/kg), and designated as positive control group. The intervention group was given 150 mg/kg, 300 mg/kg, and 450 mg/kg dose of leaf extract for 28 days. Triglyceride, glucose, high-density lipoprotein or HDL, and cholesterol were measured before and after intervention, using serum samples taken from the retro-orbital vein and analyzed using an enzymatic colorimetric method. The Wistar weight was measured every week. Data was analyzed by paired T-test and One-way ANOVA with Post-hoc Bonferroni and Games-Howell.

Results: There was a significant decrease in triglyceride levels after the intervention ($p<0.005$). Interestingly, there was also a significant increase in weight gain in all groups after the intervention ($p=0.000$), both were dose dependent.

Conclusion: *Cnidoscolus aconitifolius* leaf extract is significantly lowering triglyceride levels in the Wistar rats model. In addition, weight gain has also been observed after intervention. Clinical studies are needed to further explore the potential of *Cnidoscolus aconitifolius* leaves as anti-dyslipidemia.

Keywords: *Cnidoscolus aconitifolius*, metabolic syndrome, triglyceride, weight, Wistar rats

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Introduction

Metabolic syndrome is a cluster of metabolic symptoms including obesity (predominantly central obesity), hyperglycemia, hypertension, and dyslipidemia that occur progressively. The progression of metabolic syndrome can reduce the quality of life and increase the risk of cardiovascular diseases, type 2 diabetes, myocardial infarction, and stroke.[1] The Indonesian Basic Health Survey in 2018 showed that 7.6% of people had cholesterol levels of more than 200 mg/dl, 37.3% had LDL levels of more than 130 mg/dl, 24.3% had HDL levels of less than 40 mg/dl, and 27.9% had triglyceride levels of more than 150 mg/dl.[2] The diagnosis of metabolic syndrome from several international organizations includes triglyceride levels ≥150 mg/dl, HDL levels <40 mg/dl for male and <50 mg/dl

for female, glucose levels ≥100 mg/dl, blood pressure >130/85 mg/dl, and elevated waist circumference based on the population (for Asians, ≥90 cm for males and ≥80 cm for females).[3]

Recently, there has been growing interest in research on the correlation between triglyceride and the risk of cardiovascular diseases, diabetes, acute pancreatitis, and metabolic syndrome.[4] Many studies have shown that *Cnidoscolus aconitifolius* leaf extract has anti-dyslipidemia effects because it is rich in phenolic compounds such as flavonoid, tannin, saponin, coumarin, alkaloid, and terpenoid.[5–7] Phenolic compounds are able to induce the production of intracellular antioxidants (superoxide dismutase, catalase, and peroxidase) to counter the effects of oxidized LDL in adipose cells. Flavonoid can also activate AMP-activated protein kinase (AMPK) to increase glucose uptake into intracellular. Saponins create anti-dyslipidemia effects by inhibiting the production of lipase enzymes and reducing the rate of lipid metabolism in the liver so that free fatty acids in the blood decrease.[8]

Cnidoscolus aconitifolius has anti-dyslipidemia properties at doses of 250 mg/kg and 500 mg/kg.[6] Improvements in the lipid profiles of rats were shown at doses of 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg.[7] On the contrary, there is also an experimental study stating that leaf extract of Cnidoscolus aconitifolius, both at low (200 mg/kg) and high (400 mg/kg) doses, have no ability to reduce total cholesterol, LDL, and triglyceride levels or increase HDL levels.[9] Some studies also suggest that leaf extract of *Cnidoscolus aconitifolius* can increase the body weight of rats.[10,11]

Due to differences in previous studies, further study on the effects of *Cnidoscolus aconitifolius* leaf extract on metabolic syndrome is still needed. In fact, the Indonesian Ministry of Health has not yet included *Cnidoscolus aconitifolius* in the Indonesian Herbal Medicine Formulary because there has been insufficient study on *Cnidoscolus aconitifolius* in Indonesia. This study aimed to understand the potential of *Cnidoscolus aconitifolius* leaf extracts in lowering the risk of metabolic syndrome by decreasing triglyceride levels and body weight.

Methods

This was an experimental study using a pre-test and post-test design with a control group

Figure 1 Wistar rat (*Rattus norvegicus*)

held at the Laboratory of the Central for Food and Nutrition Studies (CFNS), Gadjah Mada University, Yogyakarta, Indonesia from March to May 2021 for 60 days. This study was approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital, Surakarta (132/II/ HREC/2021 dated 15th February 2021).

This study used 35 male albino Wistar rats (*Rattus norvegicus*) selected using purposive random sampling and treated in conformity with the guidelines from the Institutional Animal Care and Use Committee (IACUC) (Figure 1). Wistar rats were chosen because they were more sensitive to high fat diets so they achieved insulin resistance, gained weight, and increase triglyceride levels earlier than other rats.[12] The inclusion criteria were albino Rattus norvegicus from Wistar strain, aged 8–12 weeks, weighing 150–200 grams, and male. Physically sick rats (dehydrated, loose skin, chromodacryorrhea, watery feces, etc.) and psychologically sick rats (aggressive, disturbed sleeping behavior, making loud noises) were excluded.

Furthermore, the rats were divided randomly into five groups. In the negative control group (NCG), subjects were not induced into metabolic syndrome and only received water and a standard diet ad libitum. Subjects in the positive control group (PCG) were induced into metabolic syndrome and received water, standard diet ad libitum, HFHFD, STZ, and NA. Subjects in the first intervention group (IG1), the second intervention group (IG2), and the third intervention group (IG3) had the same treatment as PCG but were given Cnidoscolus aconitifolius leaf of 150 mg/kg, 300 mg/kg,

Figure 2 *Cnidoscolus aconitifolius*, also known as Pepaya Jepang or Chaya Leaves

and 450 mg/kg extracts respectively (Figure 2).

Cnidoscolus aconitifolius leaves, also known as Pepaya Jepang leaves or Chaya leaves, were taken from Surakarta, Central Java, Indonesia. Thise plant originated from Mexico and has been cultivated in tropical and subtropical countries, so it could be easily found anywhere. In this study, the leaves taken were only leaves from the same planting area in Surakarta to avoid differences caused by soil, humidity, microbiome, and others that could potentially distort the results. Older leaves were chosen. The leaves were washed and dried at room temperature for two weeks to get rid of all the dirt and excess water before being extracted using a three-times maceration technique in 70% ethanol for 72 hours. Ethanol 70% was chosen because it was a semi-polar solvent that can bind more plant metabolites effectively without including toxic cyanogenic glycosides.[7,13] Phytochemical analysis was not done in this study.

All subjects received water and a 10%-of-weight standard diet (containing protein and fiber) ad libitum every day. After seven days of adaptation with only a standard diet, all groups except NCG were modeled into metabolic syndrome by giving them oral HFHFD for 21 days (days 8 to 28) and a one-time STZ (45 mg/kg) and NA (110 mg/kg) intraperitoneal injection on the 28th day.[14,15] High-fat diet contains 4 g/200 g of cholesterol and 2 g/200 g of fatty acids, while a high-fructose diet contains 0.36 g/200 g of fructose. The number was obtained from the daily maximum intake of lipids and carbohydrates for humans by the Indonesian Health Ministry that had been converted into rats' doses.[16,17] Giving a high-fat diet for three weeks and injection of STZ (40 mg/kg) on the last day of the high fat diet was enough to induce stable metabolic syndrome in Wistar rats.[18]

Streptozotocin is stable at pH 4.5 so it was dissolved in the citrate buffer. NA was injected 15 minutes earlier.[15,19] Rats were in a 8–10 hours fasting-state before STZ-NA injection. Streptozotocin and Nicotinamide were given in those doses to destroy beta cells in the pancreas, but not completely, so that the rats did not need exogenous insulin to live. Along with HFHFD, this model could better represent metabolic syndrome conditions.[15]

One study stated that the best way to examine the STZ effects was to obtain pancreas insulin content 24 hours after STZ injection, but in most studies, increases in serum or plasma glucose levels could be examined from 72 hours to 8 weeks after STZ injection.[15] Due to time constraints in this study, blood glucose and other metabolic syndrome parameters were measured 72 hours after STZ-NA injection. Thus, on the 31st day, serum glucose, triglyceride, HDL, and total cholesterol levels of all groups were analyzed to ensure subjects fulfilled the criteria for metabolic syndrome (pretest).

Blood samples were taken from the posterior orbital veins of anesthetized rats and then analyzed using colorimetric enzymatic methods. Ketamine 10% at a dose of 70 mg/kg was used as anesthetics intraperitoneally. Rats were in a fasting-state 8–10 hours before samples collection.[15,19] The weight of rats was measured every week from the beginning to the end of the study. Lee index and weight gain percentage was used as parameter of obesity in rats.[18] Lee index was the cube root of rats' body weight in grams divided by the length of nasal to anal in millimeters. Lee index of more than 300 indicated obesity in rats.[20] Analysis of the metabolic syndrome indicators levels was based on the harmonized metabolic syndrome criteria of several international organizations as stated by other studies.[3,18]

Previous study showed that 28-day intervention of ethanolic *Cnidoscolus aconitifolius* leaf extract could improve lipid profile in rats.[7] In this study, groups IG1, IG2, and IG3 were given 150 mg/kg, 300 mg/kg, and 450 mg/kg of ethanolic *Cnidoscolus aconitifolius* leaf extract orally per day for 28 days (days 32 to 59) respectively. The extract was dissolved in CMC-Na 0.5% in a volume of 2 ml/200 g before being given to rats. After 28

Table 1 Metabolic syndrome Indicator Levels

Indicator	Metabolic syndrome	NCG	PCG	IG1	IG2	IG3
Fasting glucose level (mg/dl)	> 200	62.3	258.9	263.3	265.5	259.4
Triglyceride level (mg/dl)	>150	76.5	136.2	134.1	133.6	133.6
HDL level (mg/dl)	< 35	79.4	26.2	27.2	25.1	26.5
Total cholesterol level (mg/dl)	> 110	86.6	182.9	182.3	181.6	182.0
Lee index	> 300	291.9	322.6	321.1	318.2	322.1
Weight gain (%)	> 8%	8.5%	21.4%	22.3%	22.0%	22.2%

Note: NCG: negative control group (subjects were not induced into metabolic syndrome and only received water and a standard diet ad libitum), PCG: positive control group (subjects were induced into metabolic syndrome and received water, standard diet ad libitum, HFHFD, STZ, and NA), IG1: first intervention group (subjects were given Cnidoscolus aconitifolius leaf of 150 mg/kg), IG2: second intervention group (subjects were given Cnidoscolus aconitifolius leaf of 300 mg/kg), IG3: third intervention group (Subjects were given Cnidoscolus aconitifolius leaf of 450 mg/kg), HDL: high-density lipoprotein, mg: milligram, dl: deciliter. Lee index is the cube root of rats' body weight in grams divided by the length of nasal to anal in milimeters

days of intervention (on the morning of 60th day), triglycerides levels (posttest) in serum samples of all groups were analyzed using the same method as pretest (day 31). The weight of rats were also measured.

Data was analyzed using IBM Statistical Package for the Social Sciences (SPSS) Statistics Version 28. The Shapiro-Wilk test was used to analyze the data distribution. A P-value of more than 0.05 showed that the data was normally distributed. The paired T-test was done to analyze the difference between pretest and posttest data, so that the effect of the intervention could be understood. One-Way ANOVA, Post-Hoc Games-Howell, and Post-Hoc Bonferroni test were done to determine the effects of different doses *Cnidoscolus aconitifolius* leaf extract on posttest triglyceride levels and body weight of Wistar rats. The results were considered significant statistically if the P-value was less than 0.05.[21]

Results

The result showed a significant decrease in triglyceride levels and weight gain after the intervention. Higher dose showed more decrease in triglyceride levels, but more weight gain. These results did not entirely correlate with the hypothesis of anti-metabolic syndrome capability in Cnidoscolus aconitifolius leaf extract. All groups except NCG fulfilled the criteria for metabolic syndrome after modelling for 21 days (day 8 to 28) as shown in Table 1. Subjects from the PCG and intervention groups could be diagnosed as metabolic syndrome because they had met three of the five criteria for metabolic syndrome, namely obesity, hyperglycemia, and

Table 2 Triglyceride Levels Before and After Intervention of *Cnidoscolus aconitifolius* Leaf Extract

Group	Triglyceride Levels (mg/dl)		P¬-value (paired T-test)
	Before	After	
NCG	76.53	78.68	0.002
PCG	136.19	138.27	0.000
IG1	134.07	118.55	0.000
IG2	133.57	93.60	0.000
IG3	133.57	86.67	0.000

Note: NCG: negative control group (subjects were not induced into metabolic syndrome and only received water and a standard diet ad libitum), PCG: positive control group (subjects were induced into metabolic syndrome and received water, standard diet ad libitum, HFHFD, STZ, and NA), IG1: first intervention group (subjects were given Cnidoscolus aconitifolius leaf of 150 mg/kg), IG2: second intervention group (subjects were given Cnidoscolus aconitifolius leaf of 300 mg/kg), IG3: third intervention group (Subjects were given Cnidoscolus aconitifolius leaf of 450 mg/kg),

Table 3 Mean Weight Measurement During the Study

	Group	NCG	PCG	IG1	IG2	IG3
Weight (gram)	Week-1 (Adaptation)	180	181	180	178	181
	Week-2 (MS modelling)	185	192	193	191	194
	Week-3 (MS modelling)	190	207	206	204	208
	Week-4 (MS modelling, after STZ-NA)	198	216	215	213	217
	Week-5 (Intervention)	204	210	218	219	221
	Week-6 (Intervention)	210	204	220	222	227
	Week-7 (Intervention)	217	198	223	228	235
	Week-8 (Intervention)	224	191	226	232	242
P-value of Week-4 (after STZ-NA) and Week-8		0.000	0.000	0.000	0.000	0.000

Note: NCG: negative control group (subjects were not induced into metabolic syndrome and only received water and a standard diet ad libitum), PCG: positive control group (subjects were induced into metabolic syndrome and received water, standard diet ad libitum, HFHFD, STZ, and NA), IG1: first intervention group (subjects were given Cnidoscolus aconitifolius leaf of 150 mg/kg), IG2: second intervention group (subjects were given Cnidoscolus aconitifolius leaf of 300 mg/kg), IG3: third intervention group (Subjects were given Cnidoscolus aconitifolius leaf of 450 mg/kg), MS: metabolic syndrome, STZ: Streptozotocin, NA: Nicotinamide

dyslipidemia, although the triglyceride levels were still considered normal (Table 1).

Analysis of serum triglyceride levels on day 31 was considered as pretest. Posttest triglyceride levels were obtained on day 60, after 28 days of intervention with *Cnidoscolus aconitifolius*. Paired T-test showed a significant decrease (p=0.000) in the mean triglyceride levels after 28 days of Cnidoscolus aconitifolius leaf extract intervention as shown in Table 2.

Subjects in NCG and PCG showed a significant increase in posttest triglyceride levels. Both were not given any intervention, thus proving that the anti-dyslipidemia effect of *Cnidoscolus aconitifolius* leaf extract in the intervention group was not just a random coincidence. One-Way ANOVA with Post-Hoc Games-Howell test showed significant differences (p=0.000 and p=0.001) in posttest triglyceride levels among groups, except for the second and third intervention groups (p=0.066). This could mean that the anti-dyslipidemia effect of *Cnidoscolus aconitifolius* leaf extract was dose dependent, but there was no significant difference at of 300 mg/kg and 450 mg/kg dose.

The weight of the rats was observed every week since the beginning of the study. The average weekly weight of each group can bee seen in Table 3.

Paired T-test showed that subjects significantly gained (p=0.000) more weight after the intervention of *Cnidoscolus aconitifolius* leaf extract of all doses (Table 3). Subjects in NCG and PCG also showed significant weight gain. Possible reasons for the weight gain in all groups should be analyzed, especially to determine whether it was really a side effect of *Cnidoscolus aconitifolius* leaf extract or other factors. One-Way ANOVA with Post-Hoc Bonferroni analysis showed significance difference in post-intervention weight among groups (p=0.000, p=0.002, and p=0.026), except for NCG and IG1 (p=1.000). This could indicate that *Cnidoscolus aconitifolius* leaf extract could cause weight gain in a dose-dependent manner, due to the significant differences between IG1, IG2, and IG3. The NCG group gained weight every week, unlike the PCG group. This could be caused by the destructive effects of STZ in the PCG group which had not been countered by the effects of *Cnidoscolus aconitifolius* leaf extract. The NCG did not experience this, so that the weight gain in NCG was steady and almost the same as the intervention group, especially IG1 which caused the difference between the two to be insignificant (p=0.066).

Discussion

The study findings showed that *Cnidoscolus aconitifolius* leaf extract can significantly lower triglyceride levels, but can also significantly increase body weight. This is interesting because dyslipidemia and obesity are components of metabolic syndrome. Both occur in various overlapping pathways .

Subjects were designed to have metabolic syndrome by giving injections of HFHFD and STZ-NA. STZ can destruct beta cells in the pancreas so that insulin production will decrease, but not in a lethal state because NA has the opposite effect. HFHFD causes insulin resistance through several pathways such as the reduced expression of glucose transporter-4, changes in membrane cell fluidity, increased cytokines expression, and many more.[22] Insulin deficiency and resistance will occur together and resemble the complex pathophysiology of metabolic syndrome.

After 21 days of metabolic syndrome modeling, subjects in the PCG and intervention groups experienced weight gain of at least 8% and Lee index scores of more than 300. The group also achieved fasting glucose levels >200 mg/dl, HDL levels <35 mg/dl, total cholesterol levels >110 mg/dl, but triglyceride levels <150 mg/dl. This might be caused by enhancement of lipid metabolism that could change triglycerides into different form of lipids such as free fatty acids and cholesterol rapidly. Increased lipolysis could also escalate fat accumulation in adipose cells causing weight gain. Based on these results, the subjects have met the criteria for metabolic syndrome.[3,18]

After four weeks of intervention with Cnidoscolus aconitifolius leaf extract, triglyceride levels showed a significant decrease in the intervention groups. This shows the potential of *Cnidoscolus aconitifolius* leaf extract in the management of dyslipidemia in line with previous studies.[5–7] Higher doses resulted in a greater decrease in triglyceride levels, but the decrease was not statistically significant between IG2 (300 mg/kg) and IG3 (450 mg/dl). This indicates that 300 mg/kg is sufficient to provide a significant decrease in triglyceride levels and also to minimize the side effect of weight gain. Further research is needed to confirm this.

The anti-dyslipidemia effect of *Cnidoscolus aconitifolius* leaf extracts is mainly caused by secondary metabolites. Secondary metabolites in *Cnidoscolus aconitifolius* such as alkaloids, flavonoids, and saponins have anti-oxidant and anti-inflammatory effects.[23] Flavonoids can inhibit the production of reactive oxygen species (ROS), reactive nitrogen species (RNS), and cyclooxygenase enzyme in the inflammation process.[8,24] Insulin resistance in metabolic syndrome causes low glucose uptake. Glucose uptake can be enhanced by flavonoids and alkaloids through activation of AMP-Activated Protein Kinase (AMPK), therefore blood glucose levels will decrease. Free fatty acid uptake can also be increased by saponins through AMPK. Furthermore, insulin deficiency can be managed by flavonoids through Glucose-Dependent Insulinotropic Polypeptide (GIP) and Glucagon-Like Polypeptide-1 (GLP-1) pathways. By stimulating insulin production, lipolysis can be slowed down. Saponins also contribute in reducing the rate of lipolysis.[25]

The results showed a significant increase in body weight of rats in all groups at week 8. In the intervention group, the higher the dose given, the greater the increase in body weight. Previous studies showed a significant increase in rats' body weight after intervention with *Cnidoscolus aconitifolius* leaf extracts.[5,7,11] This suggests that the ethanolic leaf extract can reduce almost all cyanogenic glycosides so it is not toxic to rats. Various nutrition contained in *Cnidoscolus aconitifolius* leaves may play a role in increasing body weight of rats. Another reason is that better glycemic control after intervention can lead to increased tissue proteins synthesis.[5,7]

There was also significant weight gain in the control group, although there was no intervention given. Diet was given as supposed to the design of this study. Therefore, it could be caused by better adaptation of the rats over time of the study that led to increased eating. More weight gain means more standard diet was provided (10% of body weight). This shows that giving fixed amount of standard diet should be preferred to avoid bias. The NCG group showed more increase in weight than the PCG group, this could be due to the destructive effect of STZ on PCG which has not been countered by the anti-dyslipidemia, anti-oxidative, and nutritional effects of *Cnidoscolus aconitifolius* leaves extract.

As for the limitations of this study, only three doses variations of *Cnidoscolus aconitifolius* leaf extracts were used. A wider dose range is needed to find a therapeutic dose for metabolic syndrome. A longer duration of study is also needed to understand the potential of *Cnidoscolus aconitifolius* leaf extracts in improving metabolic syndrome. It is recommended to conduct more studies to better understand the therapeutic dosage and side effects of *Cnidoscolus aconitifolius* leaf extract in treating metabolic syndrome, especially weight gain. Human studies may also be considered.

In conclusion, *Cnidoscolus aconitifolius* leaf extract can significantly lower triglyceride level. Increased weight gain can be observed

after *Cnidoscolus aconitifolius* intervention. Higher doses give more decrease in triglyceride level and more increases in body weight gain. This study shows that a dose of 300 mg/kg is enough to give significant decrease in triglyceride levels.

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