The Effects of *Clinacanthus nutans* (Burm. f.) Lindau Extract for Improving Insulin Resistance in Type 2 Diabetic Rats

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Abstract

Diabetes is one of the most significant global public health issues, and it tends to increase annually, particularly type 2 diabetes (T2D). T2D is commonly linked with insulin resistance, which leads to hyperglycemia and dyslipidemia. Although diabetes is usually treated with many conventional drugs, the side effects still exist. Nowadays, various plant extracts with antidiabetic effects have been extensively reported. One of the most promising natural extracts is Phaya Yo leaf (*Clinacanthus nutans* (Burm.f.) Lindau), which contains crucial substances. This research aims to investigate the effect of Phaya Yo leaf extract on control T2D. Rats were divided into six groups (n = 3/group): normal rats (ND), diabetic rats (DM), diabetic rats treated with Phaya Yo leaf extract 100 mg/kg/day (CN100), diabetic rats treated with 200 mg/kg/day of Phava Yo leaf extract (CN200), diabetic rats treated with combination of 100 mg/kg/day of Phava Yo leaf extract and metformin 100 mg/kg/day (CN100+Met) and diabetic rats treated with metformin 100 mg/kg/day (Met). To evaluate hyperglycemia and dyslipidemia, body weight, energy intake, glucose, cholesterol, triglycerides, and visceral fat weight were measured. The results showed that all groups of rats that received CN had low body weight, energy intake, glucose, cholesterol, triglyceride, and visceral fat as compared to a group of DM. Moreover, the treatment with CN100+Met showed the best result. In summary, Phaya Yo leaf extract helps explicitly reduce hyperglycemia and dyslipidemia in T2D rats. It may be a potential alternative herb or complementary use in improving the insulin resistance associated with diabetes mellitus in the future.

Keywords: *Type 2 Diabetes, Insulin Resistance, Phaya Yo Leaf (Clinacanthus Nutans (Burm.f.) Lindau)*

1. Introduction

Diabetes mellitus (DM) is a lifelong illness in which there is a high level of blood glucose (hyperglycemia). This is due to abnormalities in the function of insulin. The defects in insulin action and hyperglycemia could lead to high levels of cholesterol and triglycerides (dyslipidemia). Diabetes is a prevalent health issue globally, including in Thailand. International Diabetes Federation (2021) reported that there are 537 million people worldwide, accounting for 10.5 percent of the world's population diagnosed with diabetes, and the number is expected to increase to 783 million in 2045 (International Diabetes Federation, 2021).

According to the World Health Organization's 1980 definition, two main types of diabetes account for the great majority of instances of the disease. Type 1 diabetes (T1D) is the first group in which there is a complete lack of insulin production, which results in hyperglycemia. The second category, referred to as Type 2 diabetes, develops due to the combined action of both insulin resistance and inadequate insulin secretion (World Health Organization, 2022; Kalsi, Singh, Taneja, Kukal, & Mani, 2015). The most prevalent is type 2 diabetes. Over the past three decades, the incidence of type 2 diabetes (T2D) has experienced a significant and widespread increase across all nations. Long-term serious complications for T2D patients include

cardiovascular disease, stroke, renal dysfunction, chronic kidney disease (CKD), etc. (Ozougwu, Obimba, Belonwu, & Unakalamba, 2013). Hence, it is imperative to administer appropriate treatment to impede the progression of the disease.

Currently, it is common to manage diabetes by controlling blood sugar levels through a combination of diet, physical activity, and medication. Although there are many pharmacological agents for the treatment of T2D including metformin (MET), sulfonylureas (SUF), thiazolidinediones (TZDs), glucagon like peptide 1 (GLP-1), dipeptidyl peptidase 4 (DPP-4) inhibitors and sodium-glucose cotransporter-2 (SGLT-2) inhibitors, and side effects are still present (Lee, Noh, Lim, & Kim, 2021). Some of these drugs can lead to side effects on the body, such as lactic acidosis and weight gain. It is also expensive, and some contraindications and precautions may limit the use of the drug (Heine, Diamant, Mbanya, & Nathan, 2006).

Alternative herbal medicines with low side effects tend to be more popular. Several plant extracts have been extensively reported with antidiabetic effects including *Piper sarmentosum* Roxb (Soraksa, & Luangpirom, 2014), *Houttuynia cordata* Thunb. (Tunkamnerdthai, Auvichayapat, & Chaiwiriyakul, 2019), *Scoparia dulcis* (Latha, & Pari, 2004). One of potential candidate plant is Phaya Yo (*Clinacanthus nutans* (Burm.f.) Lindau), which is a Thai herb that garnered attention and gained recognition as a medicinal herb, being included in the National List of Essential Medicines in 1999 (Nam *et al*., 2011). It is traditionally used for the management of many medical conditions. Its extracts have been reported blood glucose-lowering, antioxidant, anticancer, and anti-inflammatory effects (Umar Imam, Ismail, George, Chinnappan, & Yusof, 2019). Moreover, its extract contains significant groups of substances, such as steroids which exert the effect of decreasing cholesterol absorption in the small intestine (Wanikiat *et al*., 2008; Kahn, Buse, Ferrannini, & Stern, 2005). Triterpenoid, phenolic, and flavonoid groups have been reported to exhibit diverse biological activities including anti-inflammatory effects. Additionally, substances like phenolics and flavonoids possess antioxidant properties (Karim, Suleiman, Rahmat, & Bakar, 2014). In this study, PhayaYo leaf extract may be a promising alternative treatment that can be used in combination with diabetes medicine to reduce use and relieve danger from the side effects of drugs. However, exploring its potential utilization in treating individuals with insulin resistance (IR) is still required.

2. Objectives

To investigate the effect of Phaya Yo leaf (*Clinacanthus nutans* (Burm.f.) Lindau) extract on preventing weight gain, hyperglycemia, and dyslipidemia in type 2 diabetes rats.

3. Materials and Methods

3.1 Preparation of plant extract

Phaya Yo leaf (*Clinacanthus nutans* (Burm.f.) Lindau) extract was obtained from the Department of Biology, Faculty of Science, Chiang Mai University, Thailand. The leaves were purchased from the Lampang herb conservation group in Lampang, Thailand, and extracted with 70% ethanol for 3 hours. The solution extract was filtered with Whatman® filter paper. The solvent was completely removed using a rotary evaporator and then dried under a laboratory fume hood. The crude extract was subsequently stored in an amber bottle at -20°C until use. The extract yield from the fresh leaves was about 12.71%.

3.2 Animals

Male Wistar rats weighing 180 – 200 g were obtained from Nomura Siam International Co., Ltd., Pathumwan, Bangkok, Thailand. The animal experiments were conducted at Laboratory Animal Center, Chiang Mai University, Thailand and approved by the Laboratory Animal Care and Use Committees under permit number 2566/RT-009. A pair of animals were housed in separate cages under controlled temperature

at 25 ± 1°C (12 h light/dark cycles) with food and water *ad libitum*. The rats remained for 7 days in acclimatization before the beginning of the experiment.

3.3 Experimental design

After acclimatization, the Wistar rats were divided into two dietary groups including a normal diet (ND) and high-fat diet (HFD) groups. The ND group (n = 3) was fed a standard chow diet (C.P. Mice Feed Food No. 082) throughout the experiment. The HFD group (n=15) was fed with HFD for 4 weeks before diabetes induction. Diabetes was induced with an intraperitoneal injection of 40 mg/kg/day body weight of streptozotocin (STZ) and 100 mg/kg/day body weight of nicotinamide (NA). After inducing the T2D, the Wistar rats were divided into six groups (n = 3/group) as follows in Figure 1. The sample size was calculated using the formula according to Parks (2009) and Bowonsomsarita *et al*. (2021). There were ND-fed rats (ND), diabetic rats (DM), diabetic rats treated with Phaya Yo leaf extract 100 mg/kg/day (CN100), diabetic rats treated with 200 mg/kg/day of Phava Yo leaf extract (CN200), diabetic rats treated with the combination of 100 mg/kg/day of Phava Yo leaf extract and metformin 100 mg/kg/day (CN100+Met) and Diabetic rats treated with metformin 100 mg/kg/day (Met). All experimental groups were treated by oral gavage feeding for 4 weeks together with free access to water and food which was freshly provided every day. The body weight was recorded weekly throughout the experiment, and energy intake, which was calculated according to Gong *et al*. (2016), was recorded at weeks 7 and 12.

Blood parameters were measured at week 7 (pre-treatments) and week 12 (post-treatments). Blood collections were done via tail tipping after 12 hours of fasting for plasma glucose, triglyceride and cholesterol levels analysis which was determined using a colorimetric assay kit (Erba Diagnostics Mannheim GmbH, Mannheim, Germany).

After 13 weeks, the rats were 12 hours fasted on the night before sacrifice. In the morning, euthanasia was done under deep anesthesia with isoflurane inhalation which was maintained throughout the surgical procedure. The abdominal cavity was opened, and the blood was taken by the abdominal aorta to determine metabolic parameters, and the visceral fat weight was recorded.

3.4 Statistical analysis

All statistical analyses were carried out using SPSS version 29.0. software package. The data were checked variances that were analyzed by one-way ANOVA and the comparison of means was made using Duncan's new multiple range test. A value of $p < 0.05$ was considered statistically significant.

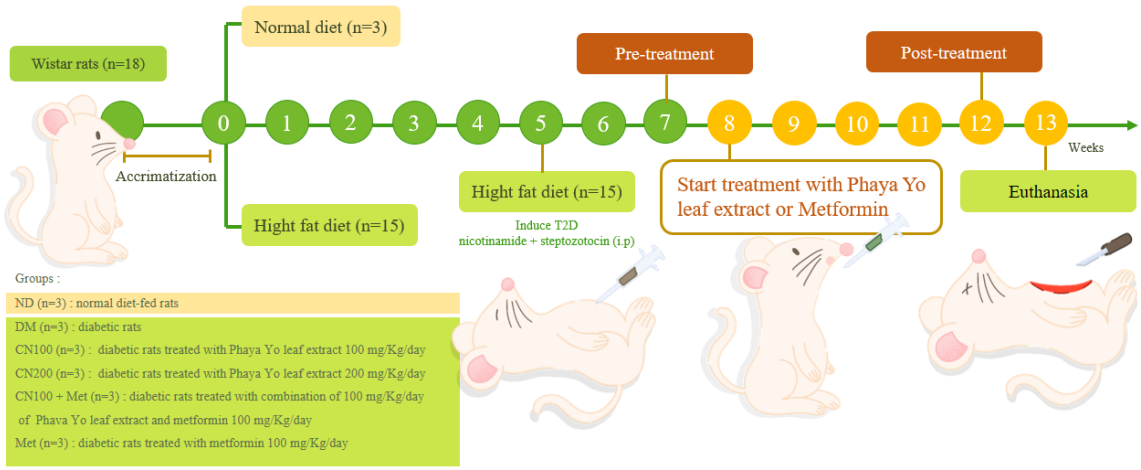

Figure 1 Experimental design

4. Results

4.1 Effects of CN on body weight and energy intake

The effect of CN on body weight and amount of energy intake is shown in Figure 2 and Figure 3. In the pre-treatment before starting CN extract, the body weight was significantly high in DM-fed with HFD. Furthermore, energy intake in the DM-fed with HFD groups was significantly higher than in the ND group. After treatment, the body weight was notably delayed after receiving the vehicle, especially in CN200 and CN100+Met. However, energy intake did not change in the DM-fed with HFD.

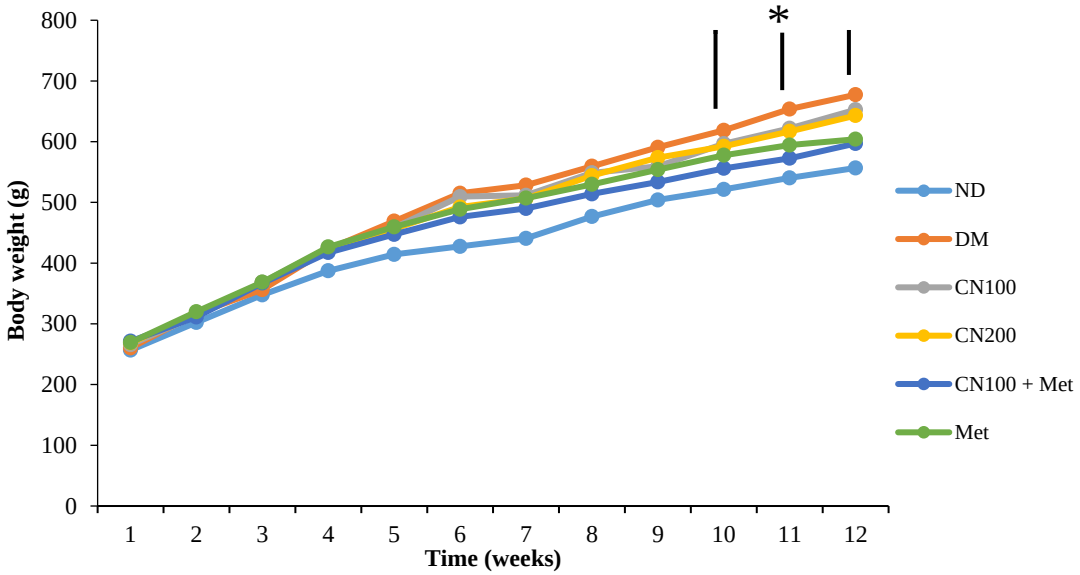

Figure 2 Effect of CN on body weight in T2D-induced rats during 12 weeks. *Significant difference from the ND group ($P<0.05$) using Duncan's new multiple-range test. (ND = normal diet-fed rats; DM = diabetic rats; CN100 = diabetic rats treated with Phaya Yo leaf extract 100 mg/kg/day; CN200 = diabetic rats treated with Phaya Yo leaf extract 200 mg/kg/day; CN100+Met = diabetic rats treated with a combination of 100 mg/kg/day of Phava Yo leaf extract and metformin 100 mg/kg/day; Met = diabetic rats treated with metformin 100 mg/kg/day)

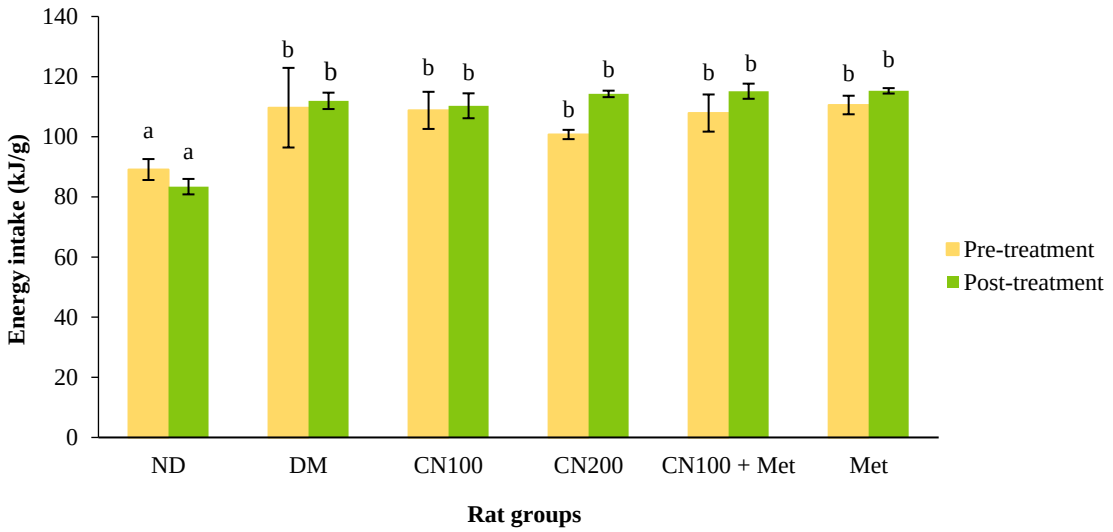

Figure 3 Effect of CN on energy intake in T2D-induced rats at week 7 (pre-treatment) and week 12 (post-treatment). Bars with the same letter within each cluster in pre-treatment or post-treatment are not significantly different (P<0.05) using Duncan's new multiple-range test. (ND = normal diet-fed rats; DM = diabetic rats; CN100 = diabetic rats treated with Phaya Yo leaf extract 100 mg/kg/day; CN200 = diabetic rats treated with Phaya Yo leaf extract 200 mg/kg/day; CN100+Met = diabetic rats treated with a combination of 100 mg/kg/day of Phava Yo leaf extract and metformin 100 mg/kg/day; Met = diabetic rats treated with metformin 100 mg/kg/day)

4.2 Effect of CN on blood sugar and lipid profiles

As presented in Table 1, showed the results of blood parameters before treatment (Week 7). All groups of the DM-fed with HFD rats (DM, CN100, CN200, CN100+Met, and Met) significantly increased in level of glucose when compared to ND rats. Additionally, noticeable hyperlipidemia was observed, as reflected by significantly increased levels of cholesterol and triglycerides in the plasma of the DM-fed with HFD group when compared to the ND group.

Table 2 shows the results of blood parameters after treatment (Week 12). All groups of diabetic rats treated with Phaya yo leaf extract (CN100, CN200, and CN100+Met) had significantly lower levels of glucose than the group of diabetic rats not receiving the extracts (DM). Also, the levels of cholesterol and triglyceride showed a significant decrease in all groups of diabetic rats treated with Phaya yo leaf extract when compared to the DM groups. Interestingly, the most favorable results were observed in the CN100+Met group.

Table 1 Blood parameters in male Wistar rats before treatment

Blood parameters	Rat groups					
	ND	DM	CN100	CN200	CN100 + Met	Met
glucose	162.86± 4.72^{a}	284.29± 35.58^{b}	259.29± 22.30^{b}	245.60± 31.05^{b}	266.43± 16.37^{b}	314.64± 42.86^{b}
triglyceride	135.67± 25.81^{a}	176.65± 17.68^{a}	160.05± 23.53^{a}	179.51± 25.00^{a}	176.65± 22.68^{a}	168.61± 14.71^{a}
cholesterol	46.84± 7.40^{a}	95.90± 7.25^{b}	86.15± 0.84^{b}	88.03± 10.93^{b}	80.34± 5.88^{b}	93.68± 28.94^{b}

Data are expressed as mean ± SD. ND = normal diet-fed rats; DM = diabetic rats; CN100 = diabetic rats treated with Phaya Yo leaf extract 100 mg/kg/day; CN200 = diabetic rats treated with Phaya Yo leaf extract 200 mg/kg/day;

CN100+Met = diabetic rats treated with a combination of 100 mg/kg/day of Phava Yo leaf extract and metformin 100 mg/kg/day; Met = diabetic rats treated with metformin 100 mg/kg/day. The different letters indicate significant differences (P<0.05), and a comparison of means in rat groups was performed using Duncan's new multiple-range test.

Table 2 Blood parameters in male Wistar rats after treatment.

Blood parameters	Rat groups					
	ND	DM	CN100	CN200	CN100 + Met	Met
glucose	142.62± 35.77^{a}	310.48± 21.15^{c}	231.31± 12.92^{b}	245.00± 35.67^{b}	158.75± 54.17^{a}	149.38± 18.07^{a}
triglyceride	127.20± 51.62^{a}	209.84± 31.16^{b}	168.43± 10.82^{ab}	118.85± 25.05^{a}	102.14± 31.82^{a}	109.75± 72.27^{a}
cholesterol	50.15± 5.83^{a}	113.31± 15.63^{c}	72.24± 16.16^{b}	62.34± 2.23^{ab}	54.90± 8.67^{ab}	59.24± 11.46^{ab}

Data are expressed as mean ± SD. ND = normal diet-fed rats; DM = diabetic rats; CN100 = diabetic rats treated with Phaya Yo leaf extract 100 mg/kg/day; CN200 = diabetic rats treated with Phaya Yo leaf extract 200 mg/kg/day; CN100+Met = diabetic rats treated with a combination of 100 mg/kg/day of Phava Yo leaf extract and metformin 100 mg/kg/day; Met = diabetic rats treated with metformin 100 mg/kg/day. The different letters indicate significant differences (P<0.05), and a comparison of means in rat groups was performed using Duncan's new multiple-range test.

4.3 Effect of CN on visceral fat weight

The results of Phaya Yo leaf extract on visceral fat weight are depicted in Figure 4. The accumulation of visceral fat in diabetic rats was significantly alleviated in those that received Phaya Yo leaf extract. The most notable improvements were observed in the CN100+Met group (Figure 4).

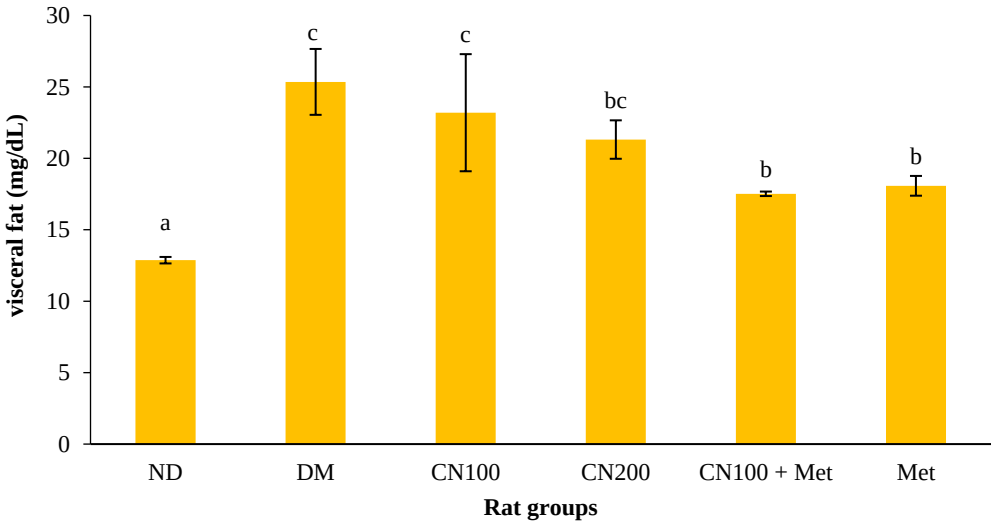

Figure 4 Effect of CN on visceral fat weight in T2D-induced rats. Bars with different letters within each cluster indicate significant differences (P<0.05) using Duncan's new multiple-range test. (ND = normal diet-fed rats; DM = diabetic rats; CN100 = diabetic rats treated with Phaya Yo leaf extract 100 mg/kg/day; CN200 = diabetic rats treated with Phaya Yo leaf extract 200 mg/kg/day; CN100+Met = diabetic rats treated with a combination of 100 mg/kg/day of Phava Yo leaf extract and metformin 100 mg/kg/day; Met = diabetic rats treated with metformin 100 mg/kg/day)

5. Discussion

Diabetes is widely recognized, and interventions for both types of diabetes are imperative. The prevalence of this disease is anticipated to surge globally, exerting a substantial impact on the population (Marx, 2002). The metabolic syndrome is characterized prominently by alterations in glucose and lipid metabolism, leading to sustained elevation of blood glucose levels. Persistent hyperglycemia is a prerequisite

for the onset of associated complications. For example, nephropathy (Pirart, 1977; Mauer *et al*., 1983), endothelial dysfunction in vascular complications (Funk, Yurdagul, & Orr, 2012; Sharma, Bernatchez, & De Haan, 2012), congestive heart failure (Vijan, 2010). which has significant adverse effects on the body.

The findings of this study demonstrated that the groups of rats that fed on a high-fat diet and received NA and STZ (i.p.) at pre-treatment developed T2D after 2 weeks. Their body weight, energy intake, glucose, cholesterol and triglyceride were significantly higher than the control group. It was consistent with Alenzi (2009) and Bayrasheva *et al.* (2016), who reported that STZ ruins pancreatic β cells, leading to hypoinsulinemia and hyperglycemia. NA can partially protect pancreatic β cells from STZ cytotoxicity, resulting in incomplete damage to β cells and therefore the development of non-insulin-dependent T2D (Yan, 2022).

In the post-treatment, the group of rats treated with CN had significantly low levels of glucose, cholesterol and triglycerides as well as visceral fat weight. As a result, CN effectively reduced insulin resistance by improving hyperglycemia and dyslipidemia. It has been reported that CN has several pharmacological properties, including antihyperglycemic activity (Alam *et al*., 2017), antioxidant activity (Sangkitporn *et al*., 1995) and antihyperlipidemic activity (Sarega *et al*., 2016). CN contains compounds of flavonoid, phenolics, terpenoids, and sulfur-containing glucosides categories (Alam *et al*., 2017; Khoo *et al*., 2018; Liao, Shi, Wang, Shao, & Tan, 2024). These constituents contribute to the α-glucosidase inhibitory activity. Inhibition of the α-glucosidase enzyme is an effective approach for the management of carbohydrate metabolic disorders, including T2D (Azemi, Mokhtar, & Rasool, 2020; Deka, Choudhury, & Dey, 2022). Moreover, it has been reported that flavonoids have strong antioxidant properties that can increase insulin release, regenerate pancreatic islets, and protect pancreatic β-cells against oxidative damage. It is a free radical scavenger and, therefore, inhibits peroxidation reactions. It can also inhibit enzymes such as cyclooxygenases and protein kinases involved in cell proliferation and apoptosis. (Vessal, Hemmati, & Vasei, 2003; Soraksa, & Luangpirom, 2014).

The treatment with CN100+Met showed the best result. As a good source of phytochemicals (Khoo *et al*., 2018), CN probably supports metformin to increase insulin sensitivity more. This study indicates the potential applicability of incorporating Phaya Yo leaf extract as an adjunct therapy with diabetes medication in future medical interventions. The mechanism of CN in reducing insulin resistance associated with T2D should be further investigated.

5. Conclusion

Phaya Yo leaf extract (*Clinacanthus nutans* (Burm. f.) Lindau) can reduce insulin resistance by improving weight gain, hyperglycemia, and dyslipidemia in type 2 diabetes rats. The treatment with CN100+Met was the best result in decreasing body weight, lowering levels of glucose, cholesterol, and triglycerides, and visceral fat accumulation. This finding suggests that Phaya Yo leaf extract may be a potential alternative herb or complementary use in improving the insulin resistance associated with diabetes mellitus in the future.

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